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Seed Legislation in Europe and Crop Genetic Diversity

Tone Winge

Abstract Crop genetic diversity has always been important for food production. With changing climatic conditions, the importance of crop genetic diversity is increasing as diversity is central to agriculture's ability to adapt to higher temperatures, precipitation changes and new pests and diseases. Maintenance of and access to this genetic diversity has become crucial. Legislation on the marketing of seed and plant propagating material, often referred to as 'seed legislation', specifies the requirements that seed and other propagating material must fulfil to be marketed legally, and how this marketing may be conducted. Such legislation can have a great impact on the composition of the seed market, as well as on cultivation and breeding, not least as it has the potential to restrict access to and maintenance of crop genetic diversity. In the European Union (EU) seed legislation is based on the principles of variety registration and certification of seed lots. Seed may be marketed only if it belongs to a variety that has been registered and the seed lot has been certified. A variety must satisfy distinctness, uniformity and stability requirements. For heterogeneous varieties this can be problematic, which in turn has potential consequences for the maintenance and further development of crop genetic diversity.

The introduction of derogations for the marketing of certain types of varieties and seed mixtures for conservation purposes provided greater legal space for the maintenance of crop genetic diversity in the EU. However, these derogations cover only some of the crop genetic diversity excluded from marketing by the main legislation. In addition, restrictions limit where and to what extent such varieties and seed mixtures can be marketed. In a preliminary ruling on the validity of current restrictions on the marketing of unregistered varieties, the Court of Justice of the EU in 2012 held that the legislation was valid. Many central stakeholders had expected the judgment to follow the opinion of Advocate General Kokott, who had reached the opposite conclusion. While the opinion had found that the disadvantages of the restrictions in question outweighed the benefits, the judgment concluded that the legislation was not manifestly inappropriate, given the objective of improved

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productivity. However, current legislation has been under review, and some changes are expected. During the review process various stakeholders voiced a wide range of differing views. Also in the literature, various suggestions for changing the EU seed legislation have been offered. As the details of seed legislation have received little attention outside a small circle of stakeholders and decision-makers, it is hoped that this article can help bring greater awareness of its importance and potential impact on the maintenance of crop genetic diversity.

Keywords Crop genetic diversity • Agriculture • Legislation • Seed • Propagating material • Marketing • Variety • Landrace • European Union • Directives • Derogations • Conservation variety • Review • Evaluation • Conservation • Maintenance • Kokopelli • Opinion • Judgment • Reform

Acronyms and Abbreviations

DG SANCO	Directorate General for Health and Consumers
DUS	Distinct, Uniform and Stable (of plant varieties)
EC	European Community
ESA	European Seed Association
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FCEC	Food Chain Evaluation Consortium

1 Introduction

Crop genetic diversity is central to solving the challenges facing agriculture, among them changing environmental conditions and growing populations. It is essential to both current and future food security. Utilisation of this diversity, through cultivation and breeding, is important to its maintenance and development, and such utilisation depends on users having access to a wide range of seed and other plant reproductive material. One determining factor here is international and national law.

The legislation on the marketing of seed and plant propagating material in the European Union (EU) specifies the requirements that seed and propagating material must fulfil to be marketed legally in the EU, and how this marketing may be conducted. This legislation, often referred to as seed legislation, has considerable impact on the composition of the European seed market, as well as on cultivation and breeding. It also affects the maintenance of crop genetic diversity and national implementation of the International Treaty on Plant Genetic Resources for Food and Agriculture of 3 November 2001 (the Plant Treaty). The impacts have not been altogether positive; and recent studies show that many stakeholders in Europe worry about the effects of this seed legislation on farmers' possibilities for maintaining crop genetic diversity, and want a new legal framework (Andersen and Winge 2011; Thommen et al. 2010).

Seed legislation was originally introduced in Europe against a backdrop of confusion surrounding variety names and varietal identity. It has been argued that the intention was to create clarity and transparency in the market (Louwaars 2002b), partly to ensure that the seed marketed had sufficient germination capacity, was disease-free and came from the claimed variety. Variety registration and certification became central; and in the 1940s many European countries passed seed laws (Louwaars 2002b).

The first European Economic Community directives regulating the marketing of seed and propagating material came in 1966. Between 1966 and 1970 altogether nine directives were introduced, and three further directives were issued in 1991 and 1992. When this legislation was first introduced, the aim was to increase competitiveness, create more open markets and harmonize national seed laws (DG SANCO 2011).

Increased productivity is now also regarded as a general objective; the specific stated objectives are to harmonize marketing standards, to ensure that *new* varieties cannot be marketed unless they are genuinely new and represent an improvement on already marketed varieties, and that the seed and propagating material is of high quality (FCEC 2008). However, the complexity, implementation costs and non-harmonized national implementation of current legislation, together with calls for adjusted and new objectives, like greater focus on sustainability, prompted a review of EU seed legislation (DG SANCO 2011).

Fig. 1 Norwegian apple varieties (Source: The Norwegian Genetic Resource Centre, Norwegian Forest and Landscape Institute. Photographer: Åsmund Asdal)

The various developments in the EU with regard to seed legislation did not take place in a vacuum. An important part of the context comes from international processes concerning the conservation and sustainable use of plant genetic resources for food and agriculture.

The Commission on Genetic Resources for Food and Agriculture was established in 1983,[1] and in the same year the International Undertaking on Plant Genetic Resources for Food and Agriculture was adopted.[2] Then followed the *Global Plan of Action for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture* in 1996,[3] and the final publication of the first *State of the World's Plant Genetic Resources for Food and Agriculture* in 1998.[4] The adoption of the legally binding Plant Treaty in November 2001 constituted a new milestone.[5] In 2010, a second *State of the World's Plant Genetic Resources for Food and Agriculture* was issued,[6] and an updated version of the *Global Plan of Action* was adopted in 2011.[7] In addition, the Convention on Biological Diversity of 5 June 1992 pertains to all biological diversity, including agricultural biodiversity. These treaties, international bodies and documents have contributed to placing crop genetic diversity, and how seed legislation can affect maintenance and sustainable use, on the agenda in Europe and elsewhere.

As the European Commission in 2013 adopted a proposal for a new regulation 'on the production and making available on the market of plant reproductive material'[8] it is timely to take a closer look at current EU seed legislation. This article aims to provide an accessible overview of this legislation, its key principles and its potential implications for the conservation and sustainable use of crop genetic diversity.[9]

The 12 basic directives and the three directives introduced to facilitate conservation efforts are reviewed. With the basic directives the focus is on key requirements, categories of seed/material used and how marketing is defined. For the three directives aimed at conservation of genetic resources, the derogations as well as the restrictions they contain are presented.

In 2012, the validity of the prohibition on the marketing of seed from non-registered varieties in the current legislation was considered by the Court of

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[1] For more information about the Commission on Genetic Resources for Food and Agriculture see http://www.fao.org/nr/cgrfa/en/

[2] For more information about the International Undertaking on Plant Genetic Resources for Food and Agriculture see http://www.fao.org/ag//CGRFA/iu.htm

[3] For more information about the Global Plan of Action see http://www.globalplanofaction.org/

[4] See http://www.fao.org/agriculture/crops/core-themes/theme/seeds-pgr/sow/en/

[5] For more information about the Plant Treaty see http://www.planttreaty.org/ or the website of the Farmers' Rights Project (www.farmersrights.org).

[6] See http://www.fao.org/agriculture/crops/core-themes/theme/seeds-pgr/sow/sow2/en/

[7] For more information, see http://www.fao.org/agriculture/crops/thematic-sitemap/theme/seeds-pgr/gpa/en/

[8] Proposal for a Regulation of the European Parliament and of the Council on the production and making available on the market of plant reproductive material (plant reproductive material law) of 6 May 2013.

[9] The article is a revised and updated version of a study published in 2012 as an FNI Report.

Justice of the European Union, following a reference for a preliminary ruling in connection with the French court case *Association Kokopelli vs. Graines Baumaux SAS*. The key aspects of this central process are presented here.

This article also offers a review of main academic contributions on the development of seed regulation in Europe and regulatory reform, the effects of seed legislation on agricultural biodiversity and sustainable agriculture, and the oldest of the three directives aimed at conservation efforts, Commission Directive 2008/62/EC of 20 June 2008.[10] In addition, a section is devoted to the review of EU seed legislation that led to the proposal adopted by the Commission, and the problems and options identified in this process. Central here are an external evaluation conducted by the Food Chain Evaluation Consortium (FCEC) and a paper outlining various scenarios for reform, together with the response the paper received as part of an on-line consultation organized by the Directorate General for Health and Consumers (DG SANCO). The article concludes with some thoughts on the way forward.

2 European Legislation on the Marketing of Seed and Plant Propagating Material

EU legislation on the marketing of seed and propagating material[11] is based on the two key principles of registration and certification, and currently consists of 12 basic Council Directives. One of these is a horizontal directive,[12] Council Directive 2002/53/EC of 13 June 2002 on the common catalogue of varieties of agricultural plant species, which specifies that for agricultural plant species (beet, fodder plant, cereal, potato and oil and fibre plants) a common catalogue of varieties should be compiled on the basis of national catalogues that have been drawn up in accordance with uniform rules.

The remaining 11 directives are vertical directives that regulate the marketing of seed and propagating material from specific types of crops: fodder-plant seed; cereal seed; beet seed; seed of oil and fibre plants; vegetable seed; vine propagating material; seed potatoes; vegetable reproductive material other than seed; fruit-plant propagating material; ornamental plants and forest reproductive material (DG

[10] Commission Directive 2008/62/EC of 20 June 2008 providing for certain derogations for acceptance of agricultural landraces and varieties which are naturally adapted to the local and regional conditions and threatened by genetic erosion and for marketing of seed and seed potatoes of those landraces and varieties

[11] This term, 'EU legislation on marketing of seed and propagating material', is what is used about the current legislation, among other cases in connection with the review process, and it has also been taken as the point of departure here. However, the proposal for a new regulation recently adopted by the European Commission introduces the term 'plant reproductive material law'.

[12] A directive is a legislative act that specifies results the EU countries must achieve, but which leaves the forms of methods of how this is to be done to the national authorities. A regulation, by contrast, is binding in its entirety in all member countries. See http://europa.eu/about-eu/basic-information/decision-making/legal-acts/.

SANCO 2011). Although they share similarities, each of these 11 directives comes with its own systems for production and marketing, and its own marketing categories.

Current EU legislation on marketing of seed and propagating material is fragmented and complex, with some 90 other legal acts (DG SANCO 2011) in addition to the 12 basic directives. Recent additions include three directives introduced to create greater legal space for the on-farm conservation and sustainable use of plant genetic resources for food and agriculture. The first of these encompasses agricultural landraces and varieties, the second vegetable landraces and varieties, and the third deals with fodder-plant seed mixtures.

In this section, the main principles of the 12 basic directives and the three later directives aimed at the conservation of genetic resources will be reviewed. In addition, the validity of the prohibition on the marketing of seed from non-registered varieties, which was considered by the Court of Justice of the European Union in connection with the French court case *Association Kokopelli vs. Graines Baumaux SAS*, will be discussed. Association Kokopelli[13] is a French non-governmental organization which produces and distributes seeds of old varieties, and Graines Baumaux[14] is a French seed company that specializes in vegetable seed. Many of the varieties distributed by Association Kokopelli have not been officially accepted and certified, and Graines Baumaux charged the organization with unfair competition. However, the details of the court case itself will be given less attention here than the process in the Court of Justice of the EU.

2.1 The 12 Basic Directives

2.1.1 Key Requirements

As noted, the two central requirements in the EU legislation on the marketing of seed and propagating material concern the registration of varieties[15] and the certification[16] of seed lots.

The registration requirement means that, in order to be marketed in the EU, a plant variety must be listed in a national catalogue[17] and, depending on the species,

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[13] See http://www.kokopelli-seeds.com/ and http://kokopelli-semences.fr/who_are_we

[14] See http://www.graines-baumaux.fr/presentation

[15] For some plant species the term ‘material’ is used, but for the sake of simplicity, only ‘variety’ is used here (as in the ‘Options and analysis’ paper published by the Directorate General for Health and Consumers).

[16] The term ‘certification’ as it is used here covers the inspection work conducted by the supplier, in addition to the intervention of official services in the form of visual inspections on the growing field and lots, including sampling and testing.

[17] The directive on ornamental plants is the only one of the eleven vertical directives that does not require some type of national list or catalogue to be established: Council Directive 98/56/EC of 20 July 1998.

in one of the EU Common Catalogues.[18] To qualify for registration, a variety must be demonstrated to be distinct, uniform and stable (DUS), and the rules for naming of varieties must be followed (DG SANCO 2011).

A variety is regarded as distinct if it is ‘clearly distinguishable on one or more important characteristics from any other variety known in the Community’ (see Council Directive 2002/53/EC, Article 5) and as stable if it ‘remains true to the descriptions of its essential characteristics’ after successive propagation or multiplications or at the end of each cycle (see Council Directive 2002/55/EC, Article 5). If, ‘apart from a very few aberrations, the plants of which it is composed are (account being taken of the distinctive features of the reproductive systems of the plants) similar or genetically identical as regards the characteristics, taken as a whole, which are considered for this purpose’, a variety is also regarded as sufficiently uniform (e.g. Council Directive 2002/55/EC, Article 5).

In addition, testing for value for cultivation and use is done for varieties of agricultural plant species[19] (DG SANCO 2011). According to the Directorate General for Health and Consumers, the values for cultivation and use are based on yield, resistance to harmful organisms, response to the environment and quality characteristics.[20] Council Directive 2002/53/EC specifies that a variety’s value for cultivation and use should be regarded as satisfactory if its qualities, ‘taken as a whole, offer, at least as far as production in any given region is concerned, a clear improvement either for cultivation or as regards the uses which can be made of the crops or the products derived therefrom’ (Article 5) compared to other registered varieties in the member state in question.

The principle of common catalogues was introduced in the European Community (EC) in 1966 (Chable et al. 2009). The horizontal directive on the common catalogue, Council Directive 2002/53/EC, applies only to varieties of agricultural plant species. Thus, the types of crops regulated by the other six vertical directives – ornamental plants, forest plants, fruit plants, vegetables (both seed and other types of propagating material) and grape vines – are not covered by this directive and its requirements on common catalogues based on national catalogues. Council Directive 2002/55/EC of 13 June 2002 on the marketing of vegetable seed

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[18] The common catalogue of varieties of agricultural plant species and the common catalogue of varieties of vegetable species are published in the *Official Journal* on the basis of information received from the member states; for an up-to-date account, see the EU database of registered plant varieties: http://ec.europa.eu/food/plant/propagation/catalogues/database/public/index.cfm?event=homepage) (see also http://ec.europa.eu/food/plant/propagation/catalogues/index_en.htm for further information).

[19] In EU legislation, the crops encompassed by this term are beet, fodder plants, cereal, potatoes and oil and fibre plants; value for cultivation and use requirements for these are specified in Council Directive 2002/53/EC on the common catalogue of varieties of agricultural plant species. However, Council Directive 2002/55/EC of 13 June 2002 on the marketing of vegetable seed specifies that varieties of industrial chicory also require a satisfactory value for cultivation and use.

[20] See http://ec.europa.eu/food/plant/plant_propagation_material/plant_variety_catalogues_databases/index_en.htm

establishes the other common catalogue: the common catalogue of varieties of vegetable species.[21]

The preambles of the basic directives of EU legislation on the marketing of seed and plant propagating material, represented by most of the vertical directives, emphasize improved productivity, the underlying assumption being that strict and uniform rules regulating seed marketing will promote productivity. The legislation therefore declares that it is desirable to establish a uniform certification scheme within the EC, based on member-state experiences.

Under this certification scheme, certification of seed lots and lots producing plant propagating material is carried out either by official bodies or under official supervision, and is mandatory for all seed-producers wishing to put their seed on the market.

In addition, accreditation[22] or registration[23] of suppliers is required for vegetable propagating and planting material other than seed, fruit-plant propagating material and fruit plants intended for fruit production, forest reproductive material and propagating material of ornamental plants.

2.1.2 Categories of Seed/Material

Most of the vertical directives operate with various categories of seed/material.[24] Eight of the 11 vertical directives distinguish between ‘basic’ material/seed and ‘certified’ material/seed. According to the oldest of these directives, Council Directive 66/401/EEC of 14 June 1966 on the marketing of fodder plant seed, ‘the

[21] As specified in article 9 of Council Directive 92/33/EEC of 28 April 1992 on the marketing of vegetable propagating and planting material, other than seed, the varieties officially accepted under this directive are also to be listed in this catalogue.

[22] Council Directive 92/33/EEC on the marketing of vegetable propagating and planting material, other than seed introduces accreditation of suppliers and laboratories: an official body must verify that the suppliers meet the requirements; accreditation must be renewed if their activities change.

[23] Under Council Directive 1999/105/EC of 22 December 1999 on the marketing of forest reproductive material and Council Directive 98/56/EC on the marketing of propagating material of ornamental plants, as well as Council Directive 2008/90/EC of 29 September 2008 on the marketing of fruit plant propagating material and fruit plants intended for fruit production (the recast version of Council Directive 92/34/EEC of 28 April 1998), suppliers must be officially registered (no exceptions are mentioned for forest reproductive material; for propagating material of ornamental plants suppliers only marketing to non-professionals is excepted, and for fruit-plant propagating material and fruit plants intended for fruit production member states may exempt suppliers marketing only to non-professional final consumers).

[24] Although the legislation defines the term ‘propagating material’ (and ‘forest reproductive material’), no definition is provided for the term ‘seed’. In Council Directive 92/33/EEC for example, propagating material is defined as ‘parts of plants and all plant material, including rootstocks intended for the propagation and production of vegetables’ (Article 3), and all five directives dealing with such material provide a definition of the term. The FCEC evaluation therefore recommends that an overall definition of ‘seed’ should be consistently introduced in all the relevant directives (FCEC 2008). The proposal for a regulation adopted by the Commission 6 May 2013, presumably as a solution to this problem, introduces the term ‘plant reproductive material’ and defines it as ‘plant(s) capable of, and intended for, producing entire plants’ (Article 3).

choice of the technical terms "basic seed" and "certified seed" is based on already existing international terminology' (Council Directive 66/401/EEC: preamble). Briefly put, the difference is that basic seed is intended for the production of certified seed, whereas certified seed in general is produced from basic seed and is intended for production of actual produce.

The only vertical directives where these categories are not used are Council Directive 1999/105/EC of 22 December 1999 on the marketing of forest reproductive material, which operates with four categories all derived from basic material, and Council Directive 92/33/EEC of 28 April 1992 on the marketing of vegetable propagating and planting material, other than seed and Council Directive 98/56/EC on the marketing of propagating material of ornamental plants, neither of which operate with such categories.

Of the eight directives that use the categories 'basic' material/seed and 'certified' material/seed, two also use the category 'commercial' seed. Both these – Council Directive 66/401/EEC and Council Directive 2002/57/EC of 13 June 2002 on the marketing of seed of oil and fibre plants – stipulate that seed of certain listed genera and species must be officially certified as 'basic' or 'certified' seed to be marketed, while seed of other than the listed genera and species can also be placed on the market if it 'is commercial seed' (Council Directive 66/401/EEC: Article 3 and Council Directive 2002/57/EC: Article 3).

'Commercial seed' is somewhat loosely defined as seed which is identifiable as belonging to a species and which has been found by official examination to satisfy the conditions laid down in Annex 2 of the respective directives regarding germination, analytical purity and content of seeds of other plant species. This means that the same requirements regarding varietal identity and varietal purity do not apply to 'commercial seed' as to 'basic' and 'certified' seed. In the preamble to Council Directive 66/401/EEC the explanation offered is that, with respect to certain genera and species, it is necessary to approve fodder plant seed that is not from a named variety, as not all genera and species of fodder plants important for cropping have produced the desired varieties or enough seed of the existing varieties to meet the needs of the European Community.

Another two of the eight directives using the categories 'basic' material/seed and 'certified' material/seed also employ the category 'standard' material/seed. These two – Council Directive 68/193/EEC of 9 April 1968 on the marketing of material for the vegetative propagation of the vine, and Council Directive 2002/55/EC on the marketing of vegetable seed – define 'standard' material/seed as material/seed of varietal identity and purity intended for the production of produce (vegetables in the case of Council Directive 2002/55/EC; grapes in the case of Council Directive 68/193/EEC) that satisfy the specific requirements laid down in the annexes of the respective directives. In addition, official examination is required, to check the varietal identify and purity (with vegetable seed) or that the requirements in general are met (with material for the vegetative propagation of the vine).

When Council Directive 68/193/EEC on the marketing of material for the vegetative propagation of the vine was amended by Council Directive 2002/11/EC of 14

February 2002,[25] the category 'initial' propagating material was added to the categories of vine propagating material. What distinguishes such material from the other categories of material in the directive is that it is to be used for the production of either basic or certified propagating material. After the amendments, basic propagating material must be obtained directly from initial material. To be put on the market, vine propagation material must be officially certified as 'initial', 'basic' or 'certified' material/seed or be officially checked standard material/seed.

Vegetable seed may be certified, verified as standard seed and marketed only if it is from a variety that has been officially accepted in at least one member state. The national catalogues of officially accepted varieties shall distinguish between varieties whose seed might be certified as either 'basic' or 'certified' seed or verified as 'standard seed', and varieties whose seed may be verified only as 'standard seed'. Council Directive 2002/55/EC further specifies that the seed of industrial chicory can be certified only as 'basic' or 'certified' seed.

Council Directive 2008/90/EC of 29 September 2008 on the marketing of fruit-plant propagating material and fruit plants intended for fruit production[26] deviates slightly, in that it also uses the category 'pre-basic' material: this is material intended for the production of 'basic' material or 'certified' material other than fruit plants (and thus quite similar to the category of 'initial' propagating material mentioned above). In addition comes a further category 'CAC (*Conformitas Agraria Communitatis*) material' – referring to propagating material and fruit plants which have varietal identity and adequate varietal purity and are intended for the production of propagating material, the production of fruit plants and/or the production of fruits, and which satisfy the specific requirements to be established for genus and species for such material. Propagating material may be marketed only if it has been officially certified as 'pre-basic', 'basic' or 'certified' material or if it qualifies as 'CAC material'; further, fruit plants may be marketed only if they are officially certified as 'certified' material or qualify as 'CAC material'.

2.1.3 Definitions of Marketing

One of the most central terms in the current EU legislation of the marketing of seed and plant propagating material, is, naturally, 'marketing'. Altogether, four slightly different definitions of this term are offered in the eleven vertical directives[27] that regulate the marketing of various categories of seed and plant propagating material.

[25] Council Directive 2002/11/EC of 14 February 2002 amending Directive 68/193/EEC on the marketing of material for the vegetative propagation of the vine and repealing Directive 74/649/EEC

[26] This is the recast version of Council Directive 92/34/EEC.

[27] Council Directive 2002/53/EC on the common catalogue of varieties of agricultural species does not contain any definition of this term. According to the evaluation conducted by the Food Chain Evaluation Consortium, this is because it is seen as a support directive and it was deemed unnecessary to include such a definition. However, the evaluation concludes that for the sake of thoroughness, clarity and consistency a definition should be added (FCEC 2008).

The four vertical directives from June 2002 and the two from 1966 as amended by Council Directive 98/95/EC of 14 December 1998,[28] as well as the one from 1968 as amended by Council Directive 2002/11/EC and the recast version of Council Directive 92/34/EEC of 28 April 1992 on the marketing of fruit-plant propagating material and fruit plants intended for fruit production (Council Directive 2008/90/EC) all use the same wording; the remaining one from 1992 provides another and those from 1998 and 1999 contain yet another two definitions.

The oldest of these definitions, the one used in Council Directive 92/33/EEC, defines marketing as ‘the holding available or in stock, displaying or offering for sale, selling and/or delivering to another person, in whatever form, of propagating or planting material/propagating material or fruit plants’ (Council Directive 92/33/EEC: Article 3).

Council Directive 98/56/EC on the marketing of propagating material of ornamental plants deviates slightly here, defining marketing as ‘sale or delivery by a supplier[29] to another person’ (Council Directive 98/56/EC: Article 2), with ‘sale’ defined as ‘holding available or in stock, display with a view to sale, offering for sale’ (Council Directive 98/56/EC: Article 2). Thus, the elements covered are the same although the organization of the definition is different, but the 1998 definition contains the limitation ‘by a supplier’. This is the only directive where such limitation has been included in the definition of ‘marketing’. As a result, the rules for marketing of propagating material of ornamental plants do not apply to the sale of such material by those not professionally engaged in the sale or import of such material.

The last directive from the 1990s, Council Directive 1999/105/EC on the marketing of forest reproductive material, defines marketing as ‘display with a view to sale, offering for sale, sale or delivery to another person including delivery under a service contract’ (Article 2). Here the element ‘holding available or in stock’ is not included, while the element ‘delivery under a service contract’ has been added.

The most recent definition of the term is offered in the four vertical directives from June 2002,[30] but it was also included in Council Directive 66/401/EEC and Council Directive 66/402/EEC of 14 June 1966 on the marketing of cereal seed following the amendments of Council Directive 98/95/EC,[31] Council Directive

[28] Council Directive 98/95/EC of 14 December 1998 amending, in respect of the consolidation of the internal market, genetically modified plant varieties and plant genetic resources, Directives 66/400//EEC, 66/401/EEC, 66/402/EEC, 66/403/EEC, 69/208/EEC, 70/457/EEC and 70/458/EEC on the marketing of beet seed, fodder plant seed, cereal seed, seed potatoes, seed of oil and fibre plants and vegetable seed and on the common catalogue of varieties of agricultural plant species

[29] This directive defines a supplier as ‘any natural or legal person engaged professionally in marketing or importing of propagating material’ (Council Directive 98/56/EC: Article 2).

[30] Council Directive 2002/54/EC of 13 June 2002 on the marketing of beet seed, Council Directive 2002/55/EC of 13 June 2002 on the marketing of vegetable seed, Council Directive 2002/56/EC of 13 June 2002 on the marketing of seed potatoes and Council Directive 2002/57/EC of 13 June 2002 on the marketing of seed of oil and fibre plants.

[31] With the addition of this definition to these two directives, it applies to all the vertical directives regulating the marketing of seed (as opposed to propagating material).

68/193/EEC following the amendments of Council Directive 2002/11/EC, and features in the recast version of Council Directive 92/34/EEC from 2008.[32] These eight directives define marketing as 'the sale, holding with a view to sale, offer for sale and any disposal, supply or transfer aimed at commercial exploitation of seed[33] to third parties, whether or not for consideration'[34] (see e.g. Council Directive 2002/54/EC: Article 2).

In addition, it is specified that trade in seed/propagating material 'not aimed at commercial exploitation of the variety' (e.g. in Council Directive 2002/54/EC: Article 2) should not be regarded as marketing. Supply of seed/propagating material to official testing and inspection bodies and to providers of services for processing or packaging are mentioned as examples of operations that are covered by this exemption. The supply of seed to service providers for industrial purposes is also mentioned as an activity that does not fall in the category 'marketing'.

This definition is more detailed as it also contains information about the types of activities that do not fall into the category 'marketing'. When it comes to the elements included in the definition of 'marketing' itself, this newest definition does not contain a reference to 'display', and where the other definitions refer to 'delivery', this definition uses the phrase 'any disposal, supply or transfer'. This definition has also substituted 'to another person' with 'to third parties'. Moreover, it is the only definition where the limitation 'aimed at commercial exploitation' has been included. However, as the term 'aimed at commercial exploitation (of the variety)' is not actually defined in the directives and the examples offered cannot be assumed to be exhaustive, it is not clear what limitations this places on the definition of 'marketing' as regards beet seed, vegetable seed, seed potatoes, fodder-plant seed, cereal seed, and seed of oil and fibre plants.

Moreover, none of these definitions refers specifically to import, although there is a general understanding that 'marketing' encompasses 'importing'. According to the Food Chain Evaluation Consortium evaluation, the explanation for this omission lies in the long history and evolution of EU seed legislation, as direct import of seed from other continents was unheard of when the first directives were drafted (FCEC 2008).

As all the directives in question are still in force, the various definitions offered are all equally valid. Although the different definitions apply to different crops, this situation does make it more difficult to navigate the complexities of current EU legislation in this area. By providing a single definition applicable to all types of

[32] Council Directive 2008/90/EC on the marketing of fruit plant propagating material and fruit plants intended for fruit production (recast version).

[33] Or, in the case of material for the vegetative propagation of the vine, 'propagating material', and fruit-plant propagating material and fruit plants intended for fruit production 'propagating material or fruit plants'.

[34] According to the Food Chain Evaluation Consortium evaluation, the rather confusing phrase 'whether or not for consideration' came about when the original French/German text was translated into English. In other EU legislation the phrase used is 'whether in return of payment or free of charge' (FCEC 2008).

plants, a new ‘plant reproductive material law’ might make EU seed legislation more easily understandable to stakeholders.[35]

2.1.4 Preliminary Conclusions: The 12 Basic Directives and Crop Genetic Diversity

As shown above, marketing of seed and propagating material in the EU may be conducted legally only if the seed/propagating material comes from a variety that has been registered and the seed lot has been certified. To qualify for registration, a variety must satisfy the distinctness, uniformity and stability requirements.

This section has demonstrated some of the complexity of today’s fragmented EU seed legislation. The legislation contains various different terms and definitions that apply to different crops – and a central term, ‘marketing’, is defined in four slightly different ways. Moreover, the legislation operates with different categories of seed/propagating material regarding different crops.

What is the impact on the maintenance of crop genetic diversity? As the cultivation of landraces and other heterogeneous populations and varieties is central to maintaining and further developing crop genetic diversity, the requirements for uniformity and stability may act as barriers. Fragmentation and complex details can make the legislation difficult to understand, also for stakeholders involved in the cultivation of heterogeneous populations and varieties. In addition, small-scale distributors of seed and propagating material are put at a certain disadvantage, because of the time and resources demanded in connection with registration and certification.

Another question is whether such obligatory registration and certification are necessary in order to achieve transparency in the market, high productivity and marketing of seed and propagating material of high quality. Perhaps a system based on optional registration and certification and clear labelling of uncertified seed lots and unregistered varieties would be sufficient.

The actual and potential impact of the EU seed legislation on the management of crop genetic diversity is also dealt with in the literature presented in Sect. 3.

[35] The Commission proposal from 2013 uses the term ‘production and making available on the market’ instead of ‘marketing’, and defines ‘making available on the market’ as ‘holding for the purpose of sale within the Union, including offering for sale or for any other form of transfer, and the sale, distribution, import into, and export out of, the Union and other forms of transfer, whether free of charge or not’. This might be changed if the regulation is adopted by the European Parliament and the Council.

Fig. 2 Wheat, *Triticum aestivum*, is one of the most commonly cultivated crops in Europe, and some traditional varieties are still being grown (Source: The Norwegian Genetic Resource Centre, Norwegian Forest and Landscape Institute. Photographer: Dan Aamlid)

2.2 Directives Aimed at the Conservation of Crop Genetic Resources

In addition to the twelve basic directives, the EU has introduced legislation aimed at the *in situ* conservation and sustainable use of plant genetic resources for food and agriculture. This dates back to 1998, when Council Directive 98/95/EC[36] established that 'it is essential to ensure that plant genetic resources are conserved' and that 'a legal basis to that end should be introduced to permit, within the framework of legislation on the seed trade, the conservation, by use *in situ*, of varieties threatened with genetic erosion' (Council Directive 98/95/EC: preambular paragraph 17).

[36] The full title is 'Council Directive 98/95/EC of 14 December 1998 amending, in respect of the consolidation of the internal market, genetically modified plant varieties and plant genetic resources, Directives 66/400//EEC, 66/401/EEC, 66/402/EEC, 66/403/EEC, 69/208/EEC, 70/457/EEC and 70/458/EEC on the marketing of beet seed, fodder plant seed, cereal seed, seed potatoes, seed of oil and fibre plants and vegetable seed and on the common catalogue of varieties of agricultural plant species'.

This directive amended the directives on the marketing of beet seed, fodder-plant seed, cereal seed, seed potatoes, seed of oil and fibre plants and vegetable seed, as well as the directive on the common catalogue of varieties.[37] For all the crop types mentioned it introduced the possibility of establishing specific conditions for the marketing of seed in relation to *in situ* conservation and the sustainable use of plant genetic resources. It was specified that included in the conditions for such marketing must be the requirement that 'the seed of these species shall be of a known provenance approved by the appropriate Authority in each Member State for the marketing the seed in defined areas', as well as 'appropriate quantitative restrictions' (both Council Directive 98/95/EC: Article 1, paragraph 24).[38]

Interestingly, the amendments of the directives on seed of agricultural species state that specific conditions *may* be established, whereas in the corresponding paragraph in the article amending the directive on vegetable seed the wording is that specific conditions *shall* be established. This wording is also used in the article amending the directive on the common catalogue.[39]

2.2.1 Derogations for Agricultural Species

After Council Directive 98/95/EC it would take another 10 years, and 12 drafts (Lorenzetti and Negri 2009), before the member countries further developed these principles and it was possible to promulgate Commission Directive 2008/62/EC of 20 June 2008 providing for certain derogations for acceptance of agricultural landraces and varieties which are naturally adapted to the local and regional conditions and threatened by genetic erosion and for marketing of seed and seed potatoes of those landraces and varieties.

This directive covers the agricultural species regulated by Directives 66/401/EEC, 66/402/EEC, 2002/54/EC, 2002/56/EC and 2002/57/EC: fodder plants, cereals, beets, potatoes, and oil and fibre plants, and 'lays down certain derogations in relation to the conservation *in situ* and the sustainable use of plant genetic resources through growing and marketing' (Commission Directive 2008/62/EC: Article 1). In this context the following derogations are mentioned: to accept for inclusion in the national catalogues of varieties of agricultural plants species, landraces and varieties which are naturally adapted to the local and regional conditions and are threatened by genetic erosion, and the marketing of seed and seed potatoes of such

[37] The amendments were made with regard to the consolidation of the internal market, genetically modified plant varieties and plant genetic resources.

[38] This is the article amending Directive 66/400/EEC (on beet seed), but the same phrasing is also used in the paragraphs amending Directive 66/401/EEC (on fodder plant seed), Directive 66/402/EEC (on cereal seed), Directive 66/403/EEC (on seed potatoes), Directive 69/208/EEC (on seed of oil and fibre plants) and Directive 70/458/EEC (on vegetable seed).

[39] Five of the directives amended by Council Directive 98/95/EC (the directives on beet seed, seed potatoes, seed of oil and fibre plants, vegetable seed and the one on the common catalogue) were updated in 2002.

landraces and varieties.[40] Such landraces and varieties are to be referred to as 'conservation varieties' in the common catalogue of varieties of agricultural plant species.

To be accepted as a conservation variety the landrace or variety in question must fulfil certain requirements. The first of these is that it must 'present an interest for the conservation of plant genetic resources' (Commission Directive 2008/62/EC: Article 4). And although the member states are free to adopt their own provisions regarding distinctness, uniformity and stability for conservation varieties, certain minimum standards apply. Member states are also obligated to carry out official post control of seed by random inspections for the purpose of verifying varietal identity and varietal purity.

In addition, procedural requirements must be met. If the information provided by the applicant is sufficient for determining whether the landrace or variety can be accepted as a conservation variety, no official examination is required. The necessary information consists of a description of the conservation variety and its denomination, results of unofficial tests, knowledge gained from practical experience and other relevant information (e.g. provided by the relevant authorities or organizations recognized for this purpose by the member state).

When a conservation variety is accepted, the member state must identify the region or regions where the variety has historically been grown and to which it is naturally adapted: this area shall be called the 'region of origin'.[41] This concept is central to implementation of the directive, as seed of a conservation variety, with some exceptions,[42] can be produced and marketed only in the region of origin. In addition, the directive specifies that 'Member States shall ensure that a conservation variety must be maintained in its region of origin' (Commission Directive 2008/62/EC: Article 9).

With regard to certification, Commission Directive 2008/62/EC refers to the vertical directives covering the various agricultural species[43] and their requirements

[40] Commission Directive 2008/62/EC defines 'conservation in situ' as 'the conservation of genetic material in its natural surroundings and, in the case of cultivated plant species, in the farmed environment where they have developed their distinctive properties', 'genetic erosion' as 'loss of genetic diversity between and within populations or varieties of the same species over time, or reduction of the genetic basis of a species due to human intervention or environmental change' and 'landrace' as 'a set of populations or clones of a plant species which are naturally adapted to the environmental conditions of their region' (Article 2).

[41] When the region of origin is located in more than one member state, the area shall be identified by all concerned member states by common accord. In both cases the Commission must be informed about the identified region.

[42] If the conditions for certification cannot be fulfilled in the region of origin due to a specific environmental problem, additional regions may be approved for seed production by the member state (seed produced in those regions must then be exclusively used in the region of origin); additional regions in a member state's own territory may be approved for marketing of seed if those regions are comparable to the region of origin as regards the natural and semi-natural habitats of the variety in question. However, a member state that makes use of the first exception (for seed production), cannot make use of the second exception (for seed marketing).

[43] Directives 66/401/EEC, 66/402/EEC, 2002/54/EC, 2002/56/EC and 2002/57/EC.

for certification of certified seed. The seed of a conservation variety[44] shall in general comply with these requirements, except those concerning varietal purity and official examination or examination under official supervision. Despite these exceptions, it is specified that the seed must have sufficient varietal purity, although what qualifies as 'sufficient' is not defined. The seed must also descend from seed produced in line with 'well defined practices for maintenance of the variety' (Article 10). For seed potatoes, it is further specified that member states may disregard the size requirements of Council Directive 2002/56/EC.

Although official examination or examination under official supervision is not required, member states must make sure that tests are carried out to ascertain compliance with the requirements. In this connection, samples must be drawn from homogeneous lots.

Commission Directive 2008/62/EC also imposes quantitative restrictions on the marketing of seed of conservation varieties. For each conservation variety, the amount of seed marketed may not exceed 0.5 %[45] of the seed of the same species used in the member state in question in one growing season, or the amount necessary to sow 100 ha, whichever is the greater amount. The marketing of seed of conservation varieties is further restricted by the specification that the total amount of seed of conservation varieties marketed in each member state may not exceed 10 % of the seed of the species in question used each year in the member state. If that should lead to an amount lower than what is required to sow 100 ha, the maximum amount of seed may be increased to reach the amount needed to sow 100 ha.

These quantitative restrictions also place administrative burdens on the stakeholders involved. Seed producers must notify the authorities in advance of each production season about the size and location of their area for seed production; and suppliers must report the amount of seed marketed of each conservation variety for each production season.

Further, although the term 'supplier' is used in the directive, for example in connection with the provisions concerning sealing and labelling of seed packages and the reporting of produced seed, no definition of this term is provided.[46] Due to the requirements that suppliers of seed must fulfil, some professionalism and resources are needed, but there is nothing in Commission Directive 2008/62/EC that otherwise restricts individuals, institutions or organizations from participating in the seed sector as suppliers.

[44] The only exception is seed of *Oryza sativa* (rice), which shall comply with the requirements of Directive 66/402/EEC for certification of 'certified seed, second generation' (with the exception of the requirements for minimum varietal purity and examination).

[45] The percentage is 0.3 % for some species (*Pisum sativum*, *Triticum* spp., *Hordeum vulgare*, *Zea mays*, *Solanum tuberosum*, *Brassica napus* and *Helianthus annuus*).

[46] This is also the case for Directives 66/401/EEC, 66/402/EEC, 2002/54/EC, 2002/56/EC and 2002/57/EC.

2.2.2 Derogations for Vegetable Species

Commission Directive 2008/62/EC was followed by Commission Directive 2009/145/EC of 26 November 2009 'providing for certain derogations, for acceptance of vegetable landraces and varieties which have been traditionally grown in particular localities and regions and are threatened by genetic erosion and of vegetable varieties with no intrinsic value for commercial crop production but developed for growing under particular conditions and for marketing of seed of those landraces and varieties'. Unlike Commission Directive 2008/62/EC, this directive provides derogations for two different categories of varieties.

With regard to the first category, the requirements put in place for vegetables with the promulgation of Directive 2009/145/EC are very similar to those of Directive 2008/62/EC. This is true with regard to definitions and substantive requirements, as well as procedural requirements, region of origin, and seed production and marketing. To be classified as 'conservation varieties', vegetable landraces or varieties must have a connection to a specific territory[47] and be threatened by genetic erosion, and must also 'present an interest for the conservation of plant genetic resources' (Commission Directive 2009/145/EC: Article 4).

Also for vegetables, member states are allowed to adopt their own rules regarding distinctness, uniformity and stability for conservation varieties, but certain minimum standards must be followed here as well. The term 'region of origin' is central also here: member states are required to identify one or more region (s) of origin for each accepted conservation variety, defined as a place where the variety has 'historically been grown and to which is it naturally adapted' (Directive 2009/145/EC: Article 8).

Conservation varieties of vegetables are also expected to be maintained in their respective region of origin and seed of these conservation varieties can be produced only in the respective region or regions of origin. In addition, marketing must take place in the region(s) of origin. However, member states may approve additional regions for marketing if such regions have habitats comparable to those of the region(s) of origin.

The quantitative requirements for vegetable conservation varieties of Commission Directive 2009/145/EC are slightly different from and somewhat simpler than those of Commission Directive 2008/62/EC. For each vegetable conservation variety, the amount of seed marketed per year in a member country is not to exceed the amount necessary to produce vegetables on 10, 20 or 40 ha, depending on the species.[48]

The second category of varieties for which Commission Directive 2009/145/EC provides derogations is 'varieties with no intrinsic value for commercial crop production but developed for growing under particular conditions' (Directive 2009/145/

[47] In Directive 2008/62/EC the phrase used is 'landraces and varieties which are naturally adapted to the local and regional conditions' (Article 1), while in Directive 2009/145/EC it is 'landraces and varieties which have been traditionally grown in particular localities and regions' (Article 1).

[48] See Annex 1 of Commission Directive 2009/145/EC for specification of which species belong in which group.

EC: Article 1). These varieties are referred to as 'varieties developed for growing under particular conditions'. The directive provides derogations for how such varieties can be accepted for inclusion in the national catalogues of varieties of vegetable species and marketed: the particular conditions in question are specified as being agro-technical, climatic or soil conditions.

For such varieties, production and marketing in a 'region of origin' is not mentioned, and the quantitative restrictions are based on maximum net weight and the requirement to market such seed in small packages – but otherwise the rules are quite similar to those regulating vegetable varieties classified as 'conservation varieties'.

2.2.3 Derogations for Fodder-Plant Seed Mixtures

The third of the directives aimed at the conservation of genetic resources is Commission Directive 2010/60/EU of 30 August 2010 providing for certain derogations for marketing of fodder plant seed mixtures intended for use in the preservation of the natural environment. This directive opens up for marketing of certain seed mixtures, in the directive called 'preservation mixtures', for the purpose of recreating the habitat type of authorized sites in connection with the conservation of genetic resources. In this sense it differs from Directive 2008/62/EC and Directive 2009/145/EC where, although the purpose is to ensure *in situ* conservation and sustainable use of plant genetic resources, the seed sold will often be used for the production of produce.

Certain requirements must be fulfilled by these preservation mixtures as well. In connection with the authorization of a preservation mixture, a region of origin must be identified, here defined as the region the mixture is naturally associated with, and it is in this region marketing may be authorized.

Various authorization measures are listed for the two types of preservation mixtures: directly harvested preservation mixtures, and crop-grown ones. Directly harvested preservation mixtures must be collected in their source area, defined as an area designated by the member state as a special area of conservation or an area that contributes to the conservation of plant genetic resources, in its region of origin; here the proportion of components and the germination rate should be as needed for recreating the habitat type in question.

For crop-grown mixtures the requirements are similar. The seed that the mixture seed is grown from must have been collected in the sources area in the region of origin, and the seed mixture should be of importance for the preservation of the natural environment. In addition, it is here specified that multiplication might take place for five generations.

For both types of preservation mixtures a time limit is set, in that the collection site cannot have been sown for 40 years at the time of application. Quantitative restrictions are imposed by this directive as well, along with requirements concerning sealing and labelling.

2.2.4 Preliminary Conclusions: The Derogations and Crop Genetic Diversity

The directives providing derogations for the marketing of certain types of varieties of agricultural and vegetable species for conservation purposes, as well as for the marketing of some fodder-plant seed mixtures, allowed increased legal space for the maintenance of crop genetic diversity in the EU. However, these derogations cover only some of the crop genetic diversity excluded from marketing by the basic directives.

For agricultural species and one category of vegetables, the favoured diversity is that which can be defined as being naturally adapted to local and regional conditions and threatened by genetic erosion. Varieties that qualify are to be called 'conservation varieties' and must be of interest for the conservation of genetic resources. However, as minimum standards regarding distinctness, uniformity and stability are required also for conservation varieties, varieties and populations that are too heterogeneous to be registered may still not be marketed.

In addition, the restrictions limit where and to what extent the marketing of conservation varieties can be conducted: a region of origin must be identified for all conservation varieties, and, with some exceptions, the production and marketing of seed/material from such a variety may take place only there. Quantitative limitations are also set. Similar restrictions apply to the fodder-plant seed mixtures termed 'preservation mixtures' and vegetable varieties developed for growing under particular conditions.

2.3 The Kokopelli Court Case and the Validity of Key Directives

In 2005 Association Kokopelli was brought to court by Graines Baumaux, on grounds of unfair competition, after Graines Baumaux had discovered that Association Kokopelli was distributing seeds from 461 varieties that had not been registered in the French national catalogue. The company claimed lump-sum damages of a total of EUR 50,000, and also sought to stop Association Kokopelli from advertising its varieties. In its decision the Nancy Regional Court awarded Graines Baumaux EUR 10,000 in damages, but dismissed the other claims (Advocate General Kokott 2012).

This decision was appealed by Association Kokopelli to the Nancy Court of Appeals, and during the appeal proceedings reference was made to the Court of Justice of the EU[49] for a preliminary ruling.[50] The question concerned Council

[49] The Court of Justice of the EU is made up of one judge per member country. Each judge is appointed for a term of six years, which can be renewed. The Court interprets EU law to ensure it is applied in the same way in all member countries. See http://europa.eu/about-eu/institutions-bodies/court-justice/index_en.htm

[50] This case falls in the category of a reference for a preliminary ruling, as a national court has requested the Court of Justice of the EU to check the validity of acts of EU law. When a national

Directives 98/95/EC, 2002/53/EC and 2002/55/EC and Commission Directive 2009/145/EC, and their validity 'in the light of the following fundamental rights and principles of the European Union, namely, freedom to pursue an economic activity, proportionality, equal treatment or non-discrimination and the free movement of goods, and also in the light of the commitments arising from the International Treaty on Plant Genetic Resources for Food and Agriculture, particularly in so far as they impose restrictions on the production and marketing of old seed and plants' (Advocate General Kokott 2012: paragraph 34).

Preliminary rulings are binding on all national courts of the member states of the EU.[51] As a result of the reference for a preliminary ruling, the national proceedings were stayed until the Court of Justice of the EU gave its ruling (Court of Justice of the European Union 2011: paragraph 26), which it did on 12 July 2012.

2.3.1 Opinion of Advocate General Kokott

In January 2012, before the Court of Justice of the EU announced its ruling, one of the eight advocates-general published an opinion.[52] Advocate General Kokott concluded that the prohibition on the marketing of seed of varieties that do not fulfil the distinctness, uniformity and stability criteria, and, where relevant, the value for cultivation and use criteria, as established in Council Directive 2002/55/EC on the marketing of vegetable seed,[53] is invalid because it infringes on the principle of proportionality, the freedom to conduct a business, the free movement of goods and the principle of equal treatment. The Advocate General argued that the disadvantages of this rule were disproportionate to its benefits, and held that this was the case also after the introduction of Directive 2009/145/EC (Advocate General Kokott 2012).

Proportionality is a general principle of EU law: any acts adopted by EU institutions are not to exceed what is necessary and appropriate to achieve the legitimate objectives of the legislation in question. In addition, of two or more possible measures,

court is in doubt about the validity or interpretation of an EU law it can, and is sometimes obliged to, refer the matter to the Court of Justice. In such cases the Court of Justice decision is called a 'preliminary ruling'. See http://europa.eu/about-eu/institutions-bodies/court-justice/index_en.htm#case1 and http://europa.eu/legislation_summaries/institutional_affairs/decisionmaking_process/l14552_en.htm. See also Article 267 (ex Article 234 TEC) of the Consolidated Version of the Treaty on the Functioning of the European Union (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2008:115:0047:0199:EN:PDF)

[51] See http://europa.eu/legislation_summaries/institutional_affairs/decisionmaking_process/l14552_en.htm

[52] The eight advocates general assist the Court of Justice of the EU by presenting opinions on the cases brought before it and are bound to do so impartially and publicly. Also the advocates general are appointed for six-year terms which can be renewed. See http://europa.eu/about-eu/institutions-bodies/court-justice/index_en.htm

[53] The Opinion states that 'the varieties at issue in the main proceedings are governed primarily or possibly exhaustively by' (Advocate General Kokott 2012: paragraph 10) this directive.

the least onerous is to be preferred, and the disadvantages are not to be disproportionate to the aims pursued. Advocate General Kokott underlined that the legality of a measure in this context would be affected only if it is 'manifestly inappropriate in terms of the objective which the competent institution is seeking to pursue' (Advocate General Kokott 2012: paragraph 60).

The rule in question is intended to provide protection against seed of varieties that do not satisfy the EU criteria and to ensure high levels of productivity, believed to be in the interest of many farmers. However, as underlined by Advocate General Kokott, in practice it serves to restrict seed producers, seed merchants and farmers whose focus is not primarily productivity, as well as consumer choice; moreover, genetic diversity in Europe is reduced. With regard to the latter, commercial use of varieties is a more robust and effective means of protecting agricultural biodiversity than for example seed banks, and the EU, as a party to the Convention on Biological Diversity and the Plant Treaty, has committed itself to maintain its biodiversity (Advocate General Kokott 2012).

Further, according to Advocate General Kokott, the main advantage of the prohibition is limited to 'preventing the mistaken use of seed that has not been accepted' (2012: paragraph 89), a risk that should be minimized by labelling requirements regarding clear warnings. The fear that European farmers will lose access to high-quality seed and any need for the seed industry to be protected from the competition from non-accepted varieties are dismissed with the argument that the listed varieties will still be available, as well as the existence of plant variety rights based on similar criteria to those for acceptance. The disadvantages of the prohibition are therefore seen as outweighing the advantages (Advocate General Kokott 2012).

Examining Council Directive 2009/145/EC with a view to establishing whether the introduction of this directive 'allows sufficient scope for the use of old varieties' (Advocate General Kokott 2012: paragraph 98), the Advocate General concluded that because of the directive's restrictions, 'disadvantages remain for operators and consumers whose access to old varieties that are not accepted is impeded' (Advocate General Kokott 2012: paragraph 103). The disadvantages of the prohibition are therefore disproportionate to its aims: as a result, the prohibition is invalid (Advocate General Kokott 2012).

In addition, the prohibition was also deemed to infringe on 'the freedom to conduct a business within the meaning of Article 16 of the Charter of Fundamental Rights of the European Union, the free movement of goods established in Article 34 TFEU[54] and the principle of equal treatment within the meaning of Article 20 of the Charter' (Advocate General Kokott 2012: paragraph 118).

It is on this background Advocate General Kokott proposed that the Court should rule that the prohibition on the marketing of seed from varieties that are not demonstrably distinct, stable and sufficiently uniform, and, in some cases, show satisfactory value for cultivation and use, is invalid.

[54] The Treaty on the Functioning of the European Union.

2.3.2 Reactions to the Opinion

The publication of Advocate General Kokott's opinion gave rise to cautious optimism among those hoping for less restrictive rules on the marketing of diverse varieties in the EU,[55] and a certain degree of consternation and dissatisfaction among European Seed Association (ESA) members.[56] Both sides seemed to expect the Court to reach the same conclusion as the Advocate General.

In January 2012, the European Seed Association warned its members that the Court tended to follow the argumentation of the Advocates-General in its final rulings[57] and that the ruling in this case would have an impact on the review of the EU seed legislation.[58] Then, in February 2012, the European Seed Association announced that together with Graines Baumaux it had sent a 'Friends of the Court' letter to the Court explaining what they saw as the rationale for the current legislation, to help the Court 'better grasp the wider picture and potential consequences' of following the opinion of Advocate General Kokott.[59]

In a cover letter to what is presumably the same statement mentioned in the February 2012 newsletter, the European Seed Association stated that it 'considered it its duty to express its legal and socioeconomic concerns'[60] about the opinion of Advocate General Kokott. In this statement the European Seed Association addressed what it called the 'alleged incompatibility with the principles of proportionality, freedom to conduct business, free movement of goods and non-discrimination' of the provisions in question, and argued that the Advocate General had not reached the right conclusion.

One element in the opinion stated by Advocate General Kokott that the European Seed Association took issue with were the statements about erosion of biodiversity and loss of traditional varieties. According to the European Seed Association, these statements about such 'alleged disappearance' were incorrect: thanks to EU seed legislation, European farmers now have access to a larger number of varieties than

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[55] See for example a posting on the matter by Arche Noah, an Austrian organisation devoted to the maintenance of heirloom varieties: http://www.arche-noah.at/discussion/viewtopic.php?f=2&t=1250

[56] See for example *ESA Newsletter,* January 2012, February 2012 and March 2012.

[57] The European Seed Association (ESA) does not provide any reference for this statement regarding the Court's rulings, but the presumption seems to be quite common among those following the Advocates-General's opinions and the preliminary rulings of the Court, and a figure of 'agreement' in 80 % of the cases has been used. However, although sources close to the Court say that in 'a majority' of the cases where an opinion has been written the Court agrees with the opinion, this 'majority' cannot be further specified. For more information see http://www.out-law.com/page-11458

[58] *ESA Newsletter,* January 2012. In June 2012 the organization reiterated that 'it must be expected that the Court will push for some form of 'liberalisation' of market access' (*ESA Newsletter*, June 2012, page 1).

[59] *ESA Newsletter*, February 2012, page 1.

[60] Letter from the European Seed Association to the Court of Justice of the European Union dated 27 February 2012, see http://www.kokopelli-semences.fr/medias/Letter-ESA.pdf

ever before, while the derogations of Directives 2008/62/EC and 2009/145/EC complement the choice made possible by the EU Common Catalogues. The European Seed Association also argued that conservation in gene banks is preferable to conservation *in situ* as regards the maintenance of identity and the genetic base of varieties.

However, most genetic resources experts would probably distance themselves somewhat from this dismissive attitude to the issue of genetic erosion in Europe: genetic erosion has been acknowledged as a substantial problem not only in Europe but globally (FAO 1998), and organizations like the Food and Agriculture Organization of the United Nations have underlined that European legislation 'discouraging the cultivation of farm landraces has had a strong negative impact on conservation' (FAO 1998: 38). The importance of *in situ* conservation and management of plant genetic resources for food and agriculture have also been recognized internationally (FAO 1998). *In situ* and *ex situ* conservation can indeed be seen as important complements.

The European Seed Association further argued that the Advocate General, in assessing the provisions in question, did not properly balance the interests and objectives at stake; and that the commercial interests of Kokopelli had been confused with the common-good concerns related to biodiversity. Interestingly, the European Seed Association also contested the extent to which the current system limits the choice of consumers, stating 'there are also various networks outside the commercial channels whose purpose is precisely to ensure that such varieties remain accessible and can still be freely cultivated'.[61]

Additionally, the European Seed Association rejected the view that what it called 'the limitations of the current system' were manifestly disproportionate and that a labelling system would be a viable alternative. In its opinion, particularly the small and medium-sized enterprises within the European seed sector would suffer if the Court came to the same conclusion as Advocate General Kokott.

Arche Noah, also known as 'the Austrian Seed Savers Association', also noted the importance of the ruling for the work of the Directorate General for Health and Consumers. Unlike the European Seed Association, it hoped the Court would follow the opinion of Advocate General Kokott.[62]

When the opinion was first published in January 2012, the preliminary ruling was mentioned as possibly being weeks away.[63] By early April, it was expected that the ruling would be announced towards the end of that month,[64] but the judgment was not handed down until 12 July 2012 (Court of Justice of the European Union 2012).

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[61] ESA letter, page 5, see http://www.kokopelli-semences.fr/medias/Letter-ESA.pdf

[62] See post by Arche Noah: http://www.arche-noah.at/discussion/viewtopic.php?f=2&t=1250

[63] In the *ESA Newsletter* from January 2012, the European Seed Association writes that it will provide more information 'once the final ruling of the ECJ is published which may still take several weeks' (page 8).

[64] See post by Arche Noah on 2 April 2012: http://www.arche-noah.at/discussion/viewtopic.php?f=2&t=1250

2.3.3 The Judgment of the Court of Justice of the EU

In its judgment from 12 July 2012, the Court ruled that Council Directive 2002/55/EC and Commission Directive 2009/145/EC were valid. The ruling stated that 'consideration of the question raised has disclosed no factor of such a kind as to affect the validity' (Court of Justice of the European Union 2012: paragraph 93) of these two directives. With regard to the two other directives mentioned in the question referred to the Court – Council Directive 98/95/EC and Council Directive 2002/53/EC – the Court did not deem it necessary to examine their validity, as the former is an amending act which *inter alia* amended an older directive on the marketing of vegetable seed now codified by Council Directive 2002/55/EC, and the latter concerns the common catalogue of varieties of agricultural plant species whereas the Kokopelli vs Graines Baumaux case concerns the marketing of vegetable seed (Court of Justice of the European Union 2012).

In its judgment the Court noted that 'in matters concerning the common agricultural policy the EU legislature has a broad discretion which corresponds to the political responsibilities given to it' and that the 'lawfulness of a measure adopted in that sphere can be affected only if the measure is manifestly inappropriate' (Court of Justice of the European Union 2012: paragraph 39). In its examination of whether the current system of acceptance of vegetable seed breaches the principle of proportionality by being manifestly inappropriate, the Court underlined that the primary objective of the rules on acceptance of vegetable seed is to improve the productivity of EU vegetable cultivation.

It further argued that the current acceptance regime, which is based on the distinctness, uniformity and stability criteria, allows for the increase of agricultural productivity 'on the basis of the reliability of the characteristics of the seed' (Court of Justice of the European Union 2012: paragraph 45), and that the 'derogating acceptance regime implemented by Directive 2009/145 (…) is capable of guaranteeing the conservation of plant genetic resources' (Court of Justice of the European Union 2012: paragraph 49). However, no further argumentation was offered as to how this regime is to ensure that plant genetic resources are satisfactory maintained.

The Court also found that the EU legislature was entitled to conclude that the current acceptance regime was necessary to achieve reliable and high productivity and to prefer this solution to less restrictive measures (like labelling). Therefore, the Court found that the legislation in question was not manifestly inappropriate in light of the objective of increased agricultural productivity, and that the principle of proportionality had not been breached.

As to the geographical, quantitative and packaging restrictions imposed on the seed of conservation varieties and of varieties developed for growing under particular conditions, the judgment states that these restrictions 'fall within the scope of the conservation of plant genetic resources' (Court of Justice of the European Union 2012: paragraph 64), but does not specify in what way and why. The judgement also seems to accept the view that 'preventing the emergence of a parallel market' (Court of Justice of the European Union 2012: paragraph 65) for seed of conservation varieties and varieties developed for growing under particular conditions was necessary, as such a market would have constituted ‘an impediment to the internal market for seed of vegetable varieties’ (ibid.). As the judgment notes, this was the argument used against liberalizing the marketing of seed, and for why it was desirable to ease only the rules of acceptance for the types of varieties in question.

Although this argument is accepted, the judgment does not explain why a ‘parallel market’ would be an impediment to the internal vegetable seed market. The judgment also notes that it is specified in Commission Directive 2009/145/EC that implementation is to be evaluated by the Commission by 31 December 2013 and that in particular the provisions on quantitative restrictions are to be assessed. Neither Council Directive 2002/55/EC nor Commission Directive 2009/145/EC is therefore seen as breaching the principle of proportionality.

The judgment also argues that these directives do not breach the principle of equal treatment because, by specifying particular conditions with regard to seed of conservation varieties, different situations are treated differently. Here it is noted that the specific cultivation and marketing conditions for seed of conservation varieties ‘fall within the scope of conservation in situ and the sustainable use of plant genetic resources’ (Court of Justice of the European Union 2012: paragraph 74).

With regard to the freedom to pursue an economic activity, the judgment states that the rules and measures of the directives in question cannot be said to be inappropriate to the attainment of the objectives of improved productivity of the EU vegetable cultivation, the establishment of an internal market and the conservation of plant genetic resources. Therefore, the obstacles represented by such rules and measures do not disproportionately impair the right to exercise the freedom to pursue and economic activity. In addition, the judgment argues that the current regime governing the marketing of vegetable seed promotes more than it restricts the free movement of goods.

Although the judgment of the Court differs on many points from the opinion of Advocate General Kokott, there is agreement on the issue of any non-compliance with the Plant Treaty: the judgment also concludes that none of the provisions of this treaty are unconditional or precise enough to challenge the validity of the directives in question. The judgment therefore argues that ‘no factor of such a kind as to affect the validity of Directives 2002/55 and 2009/145’ (Court of Justice of the European Union 2012: paragraph 93) had been disclosed.

2.3.4 Reception and Impact

Not surprisingly, the judgment was welcomed by the European Seed Association, whose Secretary General declared that ‘the European seed sector is very satisfied with the ruling’ (ESA 2012: 1). The EU Regional Group of the International Federation of Organic Agriculture Movements, on the other hand, declared that ‘for all those who want a wide diversity of colourful and tasty tomatoes and peppers on their plates’ the judgement was bad news, and that the Court had ‘failed to respond to the concerns of seed savers across the EU' (IFOAM 2012: 1). In its response, the International Federation of Organic Agriculture Movements also underlined that the EU, as part of the revision of EU seed legislation, must 'facilitate market access for traditional varieties and farm bred varieties' and create a framework that enables 'the marketing of open-pollinating varieties with a broader intra-varietal genetic diversity that are professionally bred' (ibid.). The organization emphasized that such varieties are crucial to meet challenges related to shifting environmental conditions.

If the preliminary ruling had declared invalid the prohibition on the marketing of seed from varieties that are not demonstrably distinct, stable and sufficiently uniform, or, in some cases, demonstrate satisfactory value for cultivation and use, it would have had far-reaching consequences for EU seed legislation. As noted, the preliminary rulings of the Court are, despite their name, binding on all national courts of EU member states. As they have the force of *res judicata* they are in fact final.

A ruling that followed the opinion of Advocate General Kokott would therefore have obliged the EU institutions to change the provisions in question. However, that the legislation was not deemed invalid does not mean that the contested provisions cannot be changed. The objective of improved productivity was a central factor in the Court's judgment. As the need to emphasize also other objectives has been brought up during the review process, as will be shown in Sect. 4, it could be argued that the new seed law should reflect that, by containing less restrictive provisions.

2.3.5 Preliminary Conclusions: The Validity Question, the Judgment and Crop Genetic Diversity

After the Court of Justice of the EU was asked to give a preliminary ruling on the validity of Council Directives 98/95/EC, 2002/53/EC and 2002/55/EC and Commission Directive 2009/145/EC, with a particular view to the restrictions these impose on the marketing of old varieties, the process was followed closely by many stakeholders. When the opinion of Advocate General Kokott was published, reactions and views followed the existing lines of conflict on the issue of seed regulation.

Advocate General Kokott had concluded that the prohibition on the marketing of seed from varieties that do not fulfil the distinctness, uniformity and stability criteria, and where relevant the requirements regarding value for cultivation and use, was invalid. The opinion had found that the disadvantages of the restrictions in question outweighed the benefits, and that these disadvantages remained also after the introduction of derogations.

However, although many expected the judgment to follow the conclusions of the opinion, the Court of Justice of the EU ruled that the directives in question were valid. In its judgment the Court gave weight to the objective of improved productivity and concluded that the legislation in question was not manifestly inappropriate in light of this objective. With this judgment the *status quo* was upheld and the legal space for the maintenance of crop genetic diversity could remain unchanged. It is now up to the European Parliament and the Council to determine how much space a potential new plant reproductive material law should provide for such maintenance.

3 European Seed Legislation and Crop Genetic Diversity in the Literature

3.1 Agriculture, Seed Use and Landraces in Europe

According to Negri et al. (2009) less than 4 % of the European population[65] is now involved in agriculture. Agriculture has to a large extent become industrialized and most of the input, including seed, comes from outside the farm. Agricultural production is heavily dominated by genetically uniform, commercially bred varieties, which have ousted the more genetically variable traditional varieties, often known as 'landraces', or 'local varieties' or 'farmer varieties' (Negri et al. 2009).

Europe (if Russia and other non-EU countries are included) is, according to Ceddia and Cerezo (2008), the world's largest market for commercial seed, accounting for an estimated 32 % of the total market in 2005. In Europe as a whole, Russia constitutes the biggest single market for commercial seed; within in the EU, France and Germany dominate. In 2005 the EU was a net exporter of seeds, but still had a seed trade deficit with the USA (Ceddia and Cerezo 2008).

Informal seed systems still exist: according to Bocci et al. (2010), in some countries in the south of Europe, such as Italy and Greece, as little as 10 % of the seed is purchased, whereas the figure is as high as approximately 90 % elsewhere (e.g. in Denmark and the Netherlands). However, they also note that there is little concrete information available, and it is difficult to determine the exact percentage of purchased seed, whether commercial varieties or landraces, in used various areas. It is also likely that the figures vary from crop to crop.

3.1.1 Landraces in Europe

Notwithstanding the dominance of commercial and uniform varieties, landraces are, as Negri et al. (2009) point out, still maintained in Europe. One factor that distinguishes landraces from modern varieties is the continuous development of diversity between and within the former that takes place when these are cultivated, due to natural and human selection pressures. Genetic diversity, rather than genetic uniformity is the result of such selection pressures; and while this diversity is central to the resilience of such crops, it is also part of the reason for difficulties

[65] Although not specifically defined, it can be assumed that in this publication 'Europe' refers to the continent, and not just the EU, as the edited volume it belongs to contains chapters on Russia and Switzerland.

with the maintenance and continued development of such varieties, when it comes to registration and seed certification (Negri et al. 2009).

As Negri et al. (2009) see it, apart from crop wild relatives, it is ecotypes and extant landraces that are most in need of active conservation in Europe. Both *in situ* and *ex situ* strategies play a part in this conservation work, but the authors stress that *in situ* conservation should be an important part of conservation efforts, as such an approach allows the evolutionary process to continue, as well as the preservation of different populations. Despite the difficulties in defining exactly what a 'landrace' is, Negri et al. (2009) maintain that such a definition is necessary for practical purposes, and highlight the definition proposed at the second meeting of the On-Farm Conservation and Management Taskforce of the European Cooperative Programme on Plant Genetic Resources:

> A landrace of a seed-propagated crop is a variable population, which is identifiable and usually has a local name. It lacks 'formal' crop improvement, is characterized by a specific adaptation to the environmental conditions of the area of cultivation (tolerant to the biotic and abiotic stresses of that area) and is closely associated with the uses, knowledge, habits, dialects, and celebrations of the people who developed and continue to grow it. (quoted in Negri et al. 2009: 9)

However, Negri et al. (2009) also acknowledge that this definition might prove problematic – for example, it excludes landraces that originated in one region but then were introduced to another and became adapted to the local environment there over time.

3.1.2 Genetic Erosion and Efforts to Stop It

The first modern varieties were developed in the early 1900s. Since then, similar breeding efforts have expanded to include all major crops, and advances in genetics have given plant breeders new tools. Important characteristics of modern varieties, according to Negri et al. (2009), include genetic uniformity and high yields. The latter factor is central in explaining why these varieties have replaced, and are still replacing, locally adapted but lower-yielding varieties. It is believed that this development has led to a considerable and still ongoing loss of genetic diversity (Negri et al. 2009)

Citing studies of loss of landraces in Southern Italy and Tuscany showing a genetic erosion of up to 70 %, Negri et al. (2009) argue that European landraces are very much threatened. While the previously mentioned diffusion of modern high-yielding uniform varieties is believed to have played a role, they also claim that landrace diversity in Europe is threatened by variety registration and seed certification systems.

However, many farmers still exchange farm-saved seed from unregistered varieties, and several European seed networks have found ways to circumvent the legislation for the purpose of conserving landraces and other unregistered varieties (Negri et al. 2009). A survey of European initiatives related to landraces conducted by the EU-funded research project Farm Seed Opportunities categorized 68 initiatives from 17 European countries. Among these were seed savers, initiatives promoting *in situ* conservation of landraces, producers of regional varieties, seed producers, farmer breeders, biodynamic breeders and/or supporting institutions (Osman and Chable 2009). According to Osman and Chable (2009) the current EU legislation on marketing of seed was seen by the initiatives as one of the barriers to scaling up the activities.

Another survey, conducted among stakeholders in the conservation varieties marketing chain on their expectations of bringing such varieties to the market, provided similar results. Among the various factors assessed by the stakeholders, seed laws received the worst rating. Respondents felt that the current seed legislation was overly restrictive and not adapted to the needs of their crops. This legislation was therefore seen as one of the main barriers to the development of markets for conservation varieties and other niche varieties (Thommen et al. 2010).

Table 1 Agriculture, seed use and landraces in Europe: main relevant points in the literature

Literature reference	Main relevant points
Bocci R, Chable V, Kastler G, Louwaars N (2010) Policy Recommendations. Farm Seed Opportunities and the French National Institute for Agricultural Research (INRA), Paris.	Informal seed market still important in Europe
Ceddia MG, Cerezo ER (2008) A Descriptive Analysis of Conventional, Organic and GM Crop and Certified Seed Production in the EU. Office for Official Publications of the European Communities, Luxembourg	Europe was world's largest market for commercial seed in 2005
	France and Germany dominate in EU
	Net exporter of seed in 2005
Negri V, Maxted N, Veteläinen M (2009) European landrace conservation: an introduction. In: European Landraces: On-farm Conservation, Management and Use. Bioversity Technical Bulletin No. 15. Bioversity International, Rome, Italy	Industrialization of European agriculture
	Genetically uniform commercially bred varieties dominate
	Landraces still maintained, but threatened
	Variety registration and seed certification also a threat
	Genetic diversity central to landraces
	Various definitions of 'landrace' used
Osman A, Chable V (2009) Inventory of initiatives on seeds of landraces in Europe. Journal of Agriculture and Environment for International Development 103: 95–130	Inventory of 68 initiatives from 17 European countries
	Different types: seed savers, initiatives promoting in situ conservation of landraces, producers of regional varieties, seed producers, farmer breeders, biodynamic breeders and supporting institutions
	Current EU seed legislation seen as barrier to expansion
Thommen A, Lammerts van Bueren ET, Serpolay E, Levillain T, Valero Infante T, Bocci R (2010) Characterisation of Stakeholder Expectations – An Expert Survey. Research Institute of Organic Agriculture (FIBL), Frick, Switzerland.	Stakeholder survey
	Seed legislation seen as obstacle

3.2 Development of Seed Regulation in Europe and Regulatory Reform

3.2.1 The History of Seed Regulation in Europe

Almekinders and Louwaars (2002) argue that seed laws came about in industrialised countries as a result of pressure from seed producers and farmers alike. Both groups wished to be protected against dishonest or speculative seed suppliers, as these were negative for farmers and for the integrity of serious seed producers (Almekinders and Louwaars 2002).

Louwaars (2002b) further details this argument, and states that the reason compulsory variety registration developed in Europe during the first half of the twentieth century was the lack of clarity with regard to names and varietal identity that had come about as a result of certain practices in the industry. Seed suppliers named varieties in an effort to create brands for their companies, and sometimes made unsubstantiated claims with regard to adaptation to distinguish their own product from that of a competitor. Varieties were also renamed after popular varieties, to increase sales (Louwaars 2002b).

According to Louwaars (2002b) both the industry and farmers called for transparency. The resultant solution was a registration system that linked one name to one variety, based on morphological descriptions and central agricultural characteristics. Such a variety register was first created in 1905 by the German Agricultural Society, and similar registers became mandatory in many European countries when national seed laws were enacted in the 1940s (Louwaars 2002b).

This argument is supported by Tripp (2002), who underlines that confusion over variety names as commercial seed markets developed in North America and Europe was part of the rationale for variety registration. Tripp argues that regulation can be seen as a response to information deficiencies, and that the main goal with respect to seed regulation is to provide information to farmers and to control negative externalities in farming. In addition, he notes, variety regulation is intended to prevent diseased seed from being sold (Tripp 2002).

As Louwaars (2002b) sees it, the purpose of current variety registration is still to identify varieties, and national registers are meant to ensure transparency in the market. In complex markets with many available varieties, such as the European, the requirements for registration tend to be stricter and more complicated than in markets with few available varieties. In the EU, procedures have been developed to establish distinctness, uniformity and stability for the purpose of variety registration (and the same requirements are used to determine whether a variety can be protected by plant breeders' rights). True identification of a variety is also seen as necessary for the certification of seed lots, as certification is about confirming the identity and varietal purity of the seed in question (Louwaars 2002b) and was introduced to ensure that the seed for sale actually is from the variety it is claimed to be (Tripp 2002).

As explained in Sect. 2, the EU seed legislation requires testing of the value for cultivation and use for agricultural plant species. Louwaars (2002b) claims that such testing has its origin in the testing systems created by farmers' associations to validate the claims made by seed suppliers, and that in its present form it usually focuses on a variety's adaptation to local conditions and product values.

However, according to Louwaars (2002b) the current variety control systems that emerged as a result of the mentioned developments also have some disadvantages. Here he mentions the widely recognized problem related to varietal change; a variety cannot change during its commercial life, because the registration system fixes it to a certain description. Problems noted with regard to performance testing include inappropriate site selection and poor trial management, and over-emphasis on yields during data collection and analysis (Louwaars 2002b). According to Tripp (2002), problems associated with seed regulation include regulatory capture, costs, relevance of regulations and standards and lack of transparency.

3.2.2 Approaches to Regulation

Tripp defines seed regulation as 'government control of the production and distribution of plant varieties and seeds through rules enacted to protect public welfare' (Tripp 1997: 43) and concludes that regulatory systems are formed by technical and economic conditions and political debate.

The political debate often centres on the role of government, and Louwaars (2002a) outlines three approaches to seed regulation based on differing philosophies as to the role government should play: control, competition and cooperation. The system adopted by most European[66] countries he describes as control-based, as new varieties must be registered and their value for cultivation and use tested before they can be formally released; control of seed production is conducted through certification systems, with the government playing an important role in these processes. The second approach is based on competition, where market forces – in this case, competition in the seed market – are seen as the only regulatory factor needed.

The third approach, on the other hand, is based on cooperation. According to Louwaars (2002a) this term can be attributed to the system in the United States. Under this approach, government shares tasks and responsibilities with the seed industry. In the USA this is practised in the sense that the suppliers of seed are responsible for the quality of the products they sell with regard to suitability and seed quality, while the government is involved in deciding the type of information seed dealers should include on labels and in checking the truth of labelling. Louwaars (2002a) also notes that US farmers' and seed growers' associations have given rise to many certification and quality control agencies that serve the same functions as their counterparts in Europe, although the legal basis is different.

[66] In Eastern Europe, as well as Central and Western Europe.

3.2.3 Comparisons with the USA

According to Tripp and Louwaars (1997) opportunism and lack of experience characterized the early development of the seed industry in both Europe and the United States, causing farmers to demand seed regulations. Such regulations developed in different directions on either side of the Atlantic: Tripp and Louwaars emphasize the differences between the seed regulatory system in the EU and in the United States.

While variety registration, performance testing and seed certification is voluntary in the United States (certification is conducted by independent agencies belonging to Association of Official Seed Certifying Agencies) and there is no national variety release authority (although there are National Variety Review Boards for many crops, the system is voluntary), all of the above are, as shown in Sect. 2, mandatory in the EU. All crop varieties must be registered (which means fulfilling the distinctness, uniformity and stability criteria) and performance tested (all agricultural varieties must have their value for cultivation and use tested) and all seed for sale must be certified. As the seed industries in both the EU and the USA seem to thrive under their respective regulatory regimes, Tripp and Louwaars (1997) conclude that effective regulation can be achieved by various tools.

One consequence of these differences is that uniformity is emphasized to a greater extent in the EU than in the USA, as illustrated by how some of the line mixtures produced by US public breeding programmes would not meet the distinctness, uniformity and stability criteria used for variety registration in the EU. Over-emphasis on uniformity will, according to Tripp and Louwaars (1997), interfere with plant breeding efforts that focus on utilizing diversity to cope with plant diseases or marginal growing conditions.

However, even though the EU system is mandatory and the US system is of a voluntary nature, they are quite similar in practice – many varieties in the USA are submitted voluntarily to National Variety Review Boards for evaluation, and the decision-making bodies in both systems receive input from seed companies and must answer to the farming communities through the democratic process (Tripp 2002).

3.2.4 Regulatory Reform

Although regulation sometimes is presented as a neutral tool, Tripp (2002) stresses that it is normally the result of compromise among various political interests and that seed regulatory reform will necessarily mean balancing competing interests.

This is supported by the arguments made by Tripp and Louwaars (1997) on the issue of seed regulatory reform in developing countries and the importance of such reform reflecting the development and change of national seed systems. This argumentation can be seen as relevant for developed countries as well, and two areas are discussed: variety regulation (registration, performance testing, and release) and seed quality control (certification and seed testing). As they see it, the process of seed regulatory reform will not necessarily be easy, and conflicts of interest are not unlikely.

The rather lengthy review process in the EU and the outspoken disagreement among the stakeholders regarding key principles of the current legislation supports this line of arguments.

According to Tripp (2002), the most useful way to approach regulatory reform is to separate between the standards, monitoring and enforcement. The various parts of the system may be mandatory or voluntary, and regulatory responsibility can be divided between public and private bodies. The EU and the United States have, as mentioned earlier, chosen different strategies in this respect.

Further, Tripp underlines that farmer education and empowerment and farmers' political power and organization are all central factors in ensuring that seed regulations are effective, and the two latter factors and farmer participation are especially important for systems based on voluntary testing. Regardless of the specific nature of the seed regulatory system, farmers and the seed industry must understand its operation and purpose if it is to be effective (Tripp 2002).

Possible approaches for dealing with the disadvantages of conventional variety controls and factors to consider in this context are discussed by Louwaars (2002b). One approach is to relax the regulations and let the market to a larger extent decide the level of voluntary control. He argues that such a voluntary system works in the United States because the country has competition in the seed industry, literate farmers and a network of universities and experimental stations that conduct variety trials. Another approach is to reform the existing systems by increasing participation and changing the performance standards.

Louwaars proposes changing the rule in many systems, saying that a new variety needs to perform better than the standard, to read that new varieties should not perform worse than the standard. He also underlines that governments should regulate only to the extent they are able to implement and that the objective should be to ensure farmers access to the best seed. One consequence is that governments should seek to control only the varieties that enter commercial seed trade, not those in farmers' seed systems. Neither should the system prevent genetically heterogeneous varieties, such as landraces, from becoming registered and entering the commercial market (Louwaars 2002b).

3.3 Effects of Seed Legislation on Crop Genetic Diversity

The potential effects of seed legislation on crop genetic diversity are increasingly being analysed and written about. For example, Visser (2002) has argued that seed regulations often have a negative impact on local seed systems and genetic diversity. He underlines that gene banks, although they conserve a substantial amount of crop genetic diversity, cannot single-handedly maintain the needed diversity and that on-farm conservation should be an important complementary strategy. As seed legislation has an impact on a wide range of breeding programmes, as well as on the number of varieties that are released and become available to farmers, he argues that seed policies and policies on agricultural biodiversity should be seen in connection (Visser 2002).

Table 2 Development of seed regulation in Europe and regulatory reform: main relevant points in the literature

Literature reference	Main relevant points
Almekinders CJM, Louwaars NP (2002) The importance of the farmers' seed systems in a functional national seed sector. In: Louwaars NP (ed.) Seed Policy, Legislation and Law: Widening a Narrow Focus. Food Products Press/The Haworth Press, Haworth.	Seed laws came about in Europe as result of pressure from both farmers and seed producers
	…who wanted protection from dishonest and speculative producers
Louwaars N (2002a) Seed policy, legislation and law. Journal of New Seeds 4(1): 1–14	Three approaches to regulation based on role of government: control, competition and cooperation
	Control systems common in Europe
	In competition systems, competition in seed market seen as only regulation needed
	Cooperation system in the USA: government shares tasks and responsibility with private sector
Louwaars N (2002b) Variety controls. Journal of New Seeds 4(1): 131–142	Compulsory variety registration developed in Europe during the first half of the 20th century
	Caused by lack of clarity with regard to names and varietal identity
	The resulting registration system linked one name to one variety based on morphological descriptions and central agricultural characteristics
	Distinctness, uniformity and stability (DUS) criteria for variety registration
	Testing of value for cultivation and use compulsory in the EU
	Value for cultivation and use testing originated from farmer-created testing systems for validation of supplier claims
	Now usually focuses on adaptation to local conditions and product values
	Varietal change not possible during commercial life of variety
	Rather than requiring a new variety to perform better than the standard it should be required not to perform worse
	Government should regulate commercial seed trade only, not farmers' seed systems
	Heterogeneous varieties should be allowed to be registered and enter commercial market
Tripp R, Louwaars NP (1997) Seed regulation: choices on the road to reform. Food Policy 22 (5): 433–446	Farmers in Europe and the USA demanded seed regulation as result of opportunism and inexperience during early stage of seed industry
	The two systems developed differently

(continued)

Table 2 (continued)

Literature reference	Main relevant points
	No national variety release authority in USA; variety registration, performance testing and seed certification is voluntary
	All the above are mandatory in the EU
	Uniformity is emphasized more in the EU than in the USA
Tripp R (1997) Regulation and regulatory reform. In: Tripp R (ed.) New Seed and Old Laws: Regulatory Reform and the Diversification of National Seed Systems. Intermediate Technology Publications and ODI, London, UK	Nature and rationale of regulation
	Regulation as political process
Tripp R (2002) Seed regulatory reform: an overview. Journal of New Seeds 4 (1/2): 103–115	Regulation as response to information deficiencies: seed regulation should provide information and control negative externalities
	Confusion over variety names as part of rationale for variety registration
	Also intended to prevent diseased seed from being sold
	Seed certification introduced to ensure seed for sale is from claimed variety
	Problems: regulatory capture, costs, relevance of regulations/standards and lack of transparency
	Regulation as result of compromise among various political interests
	Essential to separate between standards, monitoring and enforcement
	Systems in EU and USA quite similar in practice
	Farmer education and empowerment and farmers' political power and organization central to effective regulation, especially voluntary

The distinctness, uniformity and stability requirements are often highlighted in connection with seed legislation's effect on crop genetic diversity, and Pimbert (2011) argues that the current EU legislation regulating the sale of seeds acts as a barrier to on-farm conservation and participatory research, by restricting access to seeds from varieties that do not fulfil the requirements. These requirements have also been pointed to as potential barriers by Visser (2002), who underlines that legislation on variety registration and seed quality control can create problems for the maintenance and development of varieties that are not deemed sufficiently distinct, uniform and stable.

'Farmers' varieties' for example, defined as varieties developed through deliberate selection by one or more farmers, usually display a high degree of genetic heterogeneity and are adapted to the local environment under which they were developed. In addition, such varieties tend to be unstable and are not necessarily very distinct from each other. The legislation also constitutes a barrier, because those involved in such initiatives usually have only limited resources at their disposal for seed inspection and meeting the regulation requirements (Visser 2002).

Claiming that the demand for uniformity has reduced genetic diversity in the EU and the number of varieties available to farmers, Pimbert (2011) cites the threats from climate change as a reason for why it is necessary to change today's seed regulations so that they can allow for continued maintenance of heterogeneous crop varieties and in that way contribute to resilient food systems in the future. A similar argument is presented by Osman and Chable (2007), who claim that the scaling up of existing breeding initiatives on landraces and other heterogeneous varieties is limited by the EU seed legislation, because the farmers involved are not allowed to exchange or sell the seeds they produce. Osman and Chable therefore argue that adapted legislation is urgently needed to address this problem, so that such initiatives can flourish and expand. Also Bocci et al. (2009) see seed legislation as problematic: it interacts negatively with efforts related to protected geographical indications because EU seed legislation is not adapted to the type of seed relevant in such contexts, and restricts seed exchange.

Although the attention to seed legislation and crop genetic diversity might be growing, similar warnings have also been voiced before. In 1992, Vellvé argued that the laws regulating the marketing of seeds within the European Community[67] needed to be relaxed because of their adverse effects on agricultural biodiversity. The requirements that a variety had to fulfil to be registered and commercialized were seen as especially problematic: these were all 'geared towards uniformity' (Vellvé 1992: 130) and did not allow for legal marketing of diverse varieties like landraces. In addition, fee levels were viewed as a barrier to registration as it was believed that many interested organizations lacked the necessary resources to enter and maintain varieties on the lists (Vellvé 1992).

Seed legislation can also be seen as of importance for issues such as Farmers' Rights, as the concept is defined in the Plant Treaty, and the right to food, conceptualized as a human right. Seed laws are noted as a barrier to the realization of Farmers' Rights by Andersen (2009), who emphasizes this as a problem especially in the industrialized countries, as traditional varieties usually do not meet the requirements for registration and certification. These findings are highlighted in the report published in 2009 by the UN Special Rapporteur on the right to food, which recommends that all states ensure that their seed legislation does not cause the exclusion of farmers' varieties, and that these varieties should be included on national lists (United Nations 2009).

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[67] The European Economic Community was often referred to as the European Community also before it was officially renamed as such by the entry into force of the Maastricht Treaty in 1993.

Table 3 Effects of seed legislation on agricultural biodiversity: main relevant points in the literature

Literature reference	Main relevant points
Andersen R (2009) Information paper on Farmers' Rights submitted by the Fridtjof Nansen Institute, Norway, based on the Farmers' Rights Project. Input paper submitted to the Secretariat of the Plant Treaty, 19 May 2009 (IT/GB-3/09/ Inf. 6 Add. 3). Lysaker, Norway.	Seed laws as barrier to the realization of Farmers' Rights and the further development of crop genetic diversity Many varieties are excluded from the market as they do not fulfil criteria for variety release Farmers are not allowed to exchange and sell farm-saved seed
Bocci R, Levillain T, Kastler G, Serpolay E, Pino S, Nonne MF, Almekinders C, González JM, Valero T, Casado S (2009) National Survey on the Role of Innovative Market Mechanisms. Farm Seed Opportunities and the French National Institute for Agricultural Research (INRA), Paris.	Seed legislation problematic for protected geographical indications initiatives
Osman A, Chable V (2007) Breeding Initiatives of Seeds of Landraces, Amateur Varieties and Conservation Varieties. Farm Seed Opportunities and the French National Institute for Agricultural Research (INRA), Paris.	EU seed legislation hinders scaling up of initiatives for maintaining and developing diversity
Pimbert M (2011) Participatory Research and On-Farm Management of Agricultural Biodiversity in Europe. International Institute for Environment and Development, London, UK	EU seed legislation as barrier to conservation DUS (distinctness, uniformity and stability) requirement reduces diversity Changing the system necessary
United Nations (2009) Seed Policies and the Right to Food: Enhancing Agrobiodiversity and Encouraging Innovation. Report of the Special Rapporteur on the right to food. Sixty-fourth session of the General Assembly. A/64/170. United Nations, New York, USA	Recommends that states ensure that farmers'/traditional varieties are included in national lists/catalogues
Vellvé R (1992) Saving the Seed: Genetic Diversity and European Agriculture. Earthscan and GRAIN, London, UK	Genetic erosion widespread in Europe Increasing uniformity as contributing factor Seed registration requirements problematic for diverse varieties
Visser B (2002) An agrobiodiversity perspective on seed policies. Journal of New Seeds 4 (1): 231–245	Globalization main cause of genetic erosion Negative impact of seed regulations Agricultural biodiversity important for coping with e.g. climate change On-farm conservation and farmers' varieties central Seed legislation problematic for maintaining diversity: varieties not fulfilling requirements One solution: exclude traditional varieties from variety registration; or: voluntary registration Seed legislation should contribute to maintenance of diversity and food security

3.4 The Directive on Conservation Varieties of Agricultural Species

One way to approach the directive on conservation varieties of agricultural species, Commission Directive 2008/62/EC, is to note, as Louwaars (2007) does, that it represents an approach to farmers' seed systems that includes these systems in the regulatory framework. As opposed to leaving farmers' seed systems untouched by relaxing the regulatory system, this directive allows varieties defined as 'conservation varieties' to be marketed under somewhat different rules than other varieties. It can be argued that this system assumes that the farmers are well educated; further, that only interested farmers will be looking for seed from such varieties and that they will be familiar with the characteristics (Louwaars 2007).

3.4.1 Key Concepts

Lorenzetti and Negri (2009) consider three concepts used in the directive on conservation varieties of agricultural species to be of particular importance for its implementation: agricultural landraces and varieties, region of origin, and genetic erosion risk. As they see it, there is likely to be substantial variation in interpretation and implementation because not all the terms are defined in the directive, because of the lack of acceptance of some of the given definitions among some stakeholders, the use of different terms in different languages and the different meaning given to some terms in the English version compared to that generally accepted in scientific literature.

With regard to the definition of 'agricultural landraces' Lorenzetti and Negri (2009) recommend using the definition proposed and accepted at the Second Meeting of the On-farm Conservation and Management Task Force of the European Cooperative Programme on Plant Genetic Resources in 2006 (see Sect. 3.1.1), or the definition provided by the Working Group of the Italian Interregional Seed Project. These definitions see landraces as populations which have adapted to the local environment; considerable attention is paid to cultural heritage; and these landraces pave the way for recognition of farmers' rights. Further, they support the development of local economies based on cultivation of landraces, are somewhat restrictive, and better satisfy the requirement to indicate in which region the variety in question has been cultivated historically.

Regarding the use of the term 'region of origin' Lorenzetti and Negri (2009) point out that the context in which it is used in Directive 2008/62/EC, ('When a member state accepts a conservation variety, it shall identify the region or regions in which the variety has historically been grown and to which it is naturally adapted, hereinafter "region of origin"') seems to imply that the directive covers only populations currently under cultivation and that registration and commercialization of material from gene banks has not been foreseen. The registration of landraces and varieties from gene banks is also predicted to be problematic under the new directive because of difficulties in proving adaptation to the environment and their existence in historical records.

In addition, to be included in the national catalogues of conservation varieties, a conservation variety must be 'under threat of genetic erosion' as the term is defined in the directive. This makes an evaluation and assessment of the threat of genetic erosion becomes a necessary part of the national implementation of Directive 2008/62/EC. Lorenzetti and Negri (2009) argue that the first step when it comes to estimating the risk of losing a landrace should be to compile national inventories of landraces to be used as baselines. When it comes to the risk of losing diversity within the various landraces, an evaluation of this would require assessment of genetic diversity and population structure, as well as the socio-economic aspects involving in farmers' decision-making regarding cultivation.

Lorenzetti and Negri (2009) therefore conclude that active promotion and implementation of conservation activities related to crop genetic diversity will continue to be important after the implementation of Directive 2008/62/EC, and that implementation appears to be difficult due to the lack of data regarding the above-mentioned issues. In their opinion, the best way forward would be to use a bottom–up process involving regional authorities and agencies, *inter alia* for compiling and publishing data on the number of conservation varieties, their region of origin and the level of threat. In addition, these regional entities should 'listen to the requests of people interested in their commercialization' and 'prepare a list of conservation varieties that Member States will be called upon to register' (Lorenzetti and Negri 2009: 294).

Louwaars et al. (2010) note that, concerning the requirements for acceptance as a conservation variety given in Article 4 of Directive 2008/62/EC, the demand that a variety should present 'an interest for the conservation of plant genetic resources' can be interpreted in different ways – one being that any variety is of such interest, and another that only varieties falling outside the diversity expressed by modern varieties listed in the common and national catalogues qualify. It is the first solution, they hold, that best promotes conservation and sustainable use of crop diversity. They also argue that the focus with regard to implementation should be on the identifiability/distinctness of the landraces, not uniformity and stability, and that the most practical way to go about assessing distinctness would be to use descriptions of the distinguishing characters of the variety. Louwaars et al. also acknowledge the importance of Article 7 allowing member states to 'accept more than one name for a variety if the names concerned are historically known' (Directive 2008/62/EC: Art. 7, 1) because this might be important for maintaining the connection between variety and history.

As to the directive's 2-year exclusion of varieties that have been removed from the common catalogue, Louwaars et al. (2010) underline that there is no scientific reason why it should be necessary to wait two years before such varieties can be sold as conservation varieties, and that this limitation seems to be the result of a compromise between seed-industry interests and biodiversity concerns.

Louwaars et al. (2010) also discuss Articles 8 and 9 and the concept 'region of origin' as used in the directive, and argue that although concepts like regional identity, culture and history are important in relation to landraces, the decision to restrict the cultivation of a conservation variety to what is determined to be its region of origin according to the criteria of the directive seems to stem from fears that these varieties could be misappropriated, or could compete with regular varieties. They maintain that no evidence exists that indicates reason to fear either.

The directive leaves the interpretation of the term ‘region’ up to the member states, and in the opinion of Louwaars et al. (2010) it is important that it is interpreted widely. This is also of importance in relation to Article 11 on seed production, which limits seed production to the region of origin, except in cases where ‘a specific environmental problem’ (Directive 2008/62/EC: Article 11, 1) poses a barrier to certification in the region of origin. Part of the reason for why a narrowly defined region of origin may be harmful to the conservation and sustainable use of crop genetic resources, they note, is that the potential market for seed then might become too small for it to be possible to recover the costs associated with quality control, variety maintenance, seed production and marketing. Article 13 does open up for marketing of seed from conservation varieties in regions outside the region of origin, but only within the same country (Louwaars et al. 2010).

Another possibly problematic feature concerns the quantitative restrictions set for marketing of seed from each conservation variety. Louwaars et al. (2010) point out that, apart from avoiding too large areas being set aside for the cultivation of one conservation variety out of concerns for the biodiversity objective stated in the directive, there are no good reasons for such limitations: they might actually pose a barrier to the conservation and sustainable use of such varieties as the quantities allowed might not be large enough to justify investing in the production of such varieties. It is also worth noting that Louwaars et al. consider Article 21 on notification of recognized organizations to be important because of what they see as its potential to enable the participation of farmers and seed networks.

On the whole, Louwaars et al. (2010) conclude that while Directive 2008/62/EC can be seen as providing a framework for the cultivation of conservation varieties in areas where they were not formerly grown and opening up for activities that were previously illegal on paper but tolerated in practice, it also might serve to create barriers to the conservation and sustainable use of plant genetic resources in the EU. In their view, the implementing rules drafted at the national level will be of high importance.

Discussing various concepts and standards used in variety testing and seed controls Louwaars et al. (2010) argue that although varietal uniformity is important in relation to some characteristics for agronomic reasons, such as maturity and plant architecture, varietal uniformity in relation to morphological characteristics is useful only for administrative reasons. As a key characteristic of conservation varieties is their genetic heterogeneity, agronomic and other characteristics where most such varieties display uniformity are the most central tools for distinguishing and describing them. If, in addition, morphological uniformity is demanded, this will in most cases be problematic for such varieties and might therefore work against the objective of the directive.

Although the directive does open up for legal distribution of seeds (within certain limits) of what are defined as conservation varieties, some varieties that Louwaars et al. (2010) regard as central to increased genetic diversity in the field are excluded. This is because the concept of 'conservation varieties' demands a historic connection with a region of origin – as a result, what the authors call New Population Varieties and New Farmers' Varieties are not covered.

Based on interviews and correspondence with anonymous country representatives and other officials, Louwaars et al. (2010) conducted an investigation of country positions during the discussions leading to Directive 2008/62/EC. Their conclusion is that these discussions were dominated by countries with a significant commercial seed sector, and that the main difference between countries concerned whether they emphasized biodiversity issues or coherence with existing EU legislation.

Chable et al. (2010) argue that Directive 2008/62/EC connects two terms with somewhat different meanings when it links 'local adaptation' to 'region of origin'. In their view, the term 'region of origin' emphasizes historical and cultural aspects and assumes that all relevant varieties belong to a specific area, whereas 'local adaptation' has more agronomic and ecological connotations. Further, they hold, this connection ignores the travels all cultivated species have done and the resulting adaptation to various new environments. Landraces may be introduced to new regions and adapt to the local conditions: as these authors see it, discounting this possibility by linking a variety to a specific area is equivalent to classifying such a variety as a thing of the past rather than a still-evolving resource.

Chable et al. (2010) have also gone through the translations for the term 'landraces' used by various countries in their national translations of Directive 2008/62/EC and note the differences in how EU member countries interpret the term; some focus on the cultural aspect of these varieties, whereas others emphasize the physical aspect. Chable et al. (2010) also argue that a considerable number of landraces and peasant varieties will fall outside the scope of Directive 2008/62/EC if the homogeneity rate for conservation varieties is set at 90 % and less – and thus that marketing of seed from such varieties will still not be allowed. As conservation varieties also have to be stable, the authors underline that nearly-stable varieties, varieties with stability connected to certain traits, and unstable varieties will all be excluded from this status.

Frese et al. (2009) also analyse the terms 'landrace', 'genetic erosion' and 'adaptation'. They conclude that the criteria of the directive on conservation varieties of agricultural species are not directly related to hard scientific evidence, and argue that implementation may prove difficult due to lack of clarity as to which actions can be undertaken within its limits.

Particularly the term 'landrace' is found by Frese et al. to be difficult in practice; they feel that 'the dynamic and cyclic nature of plant breeding is seldom taken into consideration' in efforts to define it (Frese et al. 2009: 86). As a solution they suggest distinguishing between landraces, varieties and accessions depending on biological state, legal state, adaptation and seed supply system. A landrace will then be characterized by an active and evolving biological state; its adaptation will be evidenced by practical proof; the seed system within which it exists is informal; and it is not protected by plant breeders' rights. However, the mere existence of a geographical name is not seen as sufficient proof that a historical variety is adapted to a specific area and deserves to be called a 'landrace'.

According to Frese et al. (2009) adaptation is a problematic criterion for conservation varieties because most gene-bank accessions will not be sufficiently adapted to current environmental conditions, compared with other genetic material. Further, if the breeding systems of various crops are taken into consideration when analysing the risk of genetic erosion, then the breeding category most likely to suffer from genetic erosion within populations is population varieties. With regard to genetic erosion between populations or varieties, they argue that for clonal accessions priority should be given to landraces not conserved in gene banks; for line varieties, priority should be go to crops with declining breeding activities; and for outbreeding crops, priority might be accorded to varieties from heterotic groups and varieties with decreasing breeding activities.

Frese et al. (2009) conclude that if all of the four central conditions of the directive must be proven – local adaptation, regional adaptation, risk of genetic erosion, and conservation interest – very few candidate varieties will meet the criteria and be accorded status as conservation varieties.

3.4.2 National Implementation Efforts

In Finland, allowing uncertified seed from landraces to be marketed was incorporated in the Seed Trade Act of 2000 (728/2000), with a Statute on Registration of Conservation Varieties (437/2001) and a Statute on Seed Trade of Landraces of Cereal and Fodder Plants (117/00) specifying the rules, on the basis of Council Directive 98/95/EC, which opened up the possibility of establishing such conditions prior to Directive 2008/62/EC (Paavilainen 2009). The Finnish rules might offer lessons for implementation of the latter directive.

Under these requirements, landraces, old commercial varieties and old modified commercial varieties are considered eligible for registration as conservation varieties, if they are not listed on the EU common catalogue of varieties of agricultural plant species or any national lists or protected by plant breeders' rights (Paavilainen 2009).

As of September 2008, 12 varieties had been list as conservation varieties in Finland; 11 of these were defined as landraces. In Finland, it is also possible to apply for support in the form of subsidies for maintenance of conservation varieties (Paavilainen 2009).

Also legislation in Italy may be of interest for the debate about national implementation of Directive 2008/62/EC and the Plant Treaty. The objective of Italian Law 46/2007 was to implement Articles 5, 6, and 9 of the Plant Treaty; and Decree of 18 April 2008 provided further specifications. In addition, there is a body of regional laws on the conservation of plant genetic resources, most of them passed prior to Law 46/2007 (Lorenzetti et al. 2009). According to Lorenzetti et al. (2009) there is a need to first harmonize Law 46/2007 with Directive 2008/62/EC and then the relevant regional laws.

Fig. 3 Norwegian sour cherry, *Prunus cerasus*. Norway, as a member of the European Economic Area, must implement EU seed legislation (Source: The Norwegian Genetic Resource Centre, Norwegian Forest and Landscape Institute. Photographer: Åsmund Asdal)

Both Law 46/2007 and Directive 2008/62/EC provide limitations on the quantities of seed of conservation varieties that can be sold, and which areas the seed can be sold – but while the Italian law limits the amount of seed each farmer can sell of each variety, the EU directive limits the total amount of seed that can be sold per conservation variety and per species (Lorenzetti et al. 2009). According to Lorenzetti et al. (2009: 202), Directive 2008/62/EC seems to be a compromise between those who regard the varieties in question as particularly adapted varieties that are important for re-creating agriculture, and those who see such varieties as relics from the past that are being 'used to break up the seed market'.

As Lorenzetti et al. (2009) see it, the regional laws on conservation of agricultural biodiversity passed in six of Italy's regions demonstrate the high degree of local interest in the issue and the importance of taking the local level as the point of departure as regards recognition of conservation varieties and their inclusion in catalogues. Italy's various regional laws on the conservation of agricultural biodiversity have many elements in common, including: the creation of regional inventories, identification of key farmers for each species, enabling non-profit diffusion of a limited amount of seed, and promoting equitable benefit-sharing and traditional knowledge.

According to Lorenzetti et al., the restrictions concerning the quantity of seed allowed to be distributed and the areas where the varieties can be grown and seed produced are so strict that the Italian system 'does not interfere with large-scale seed trade' (2009: 204). They also argue that only those conservation varieties that are regarded as of commercial interest should be included in the national catalogue and the common catalogue.

While Lorenzetti et al. (2009) think that the implementation of Directive 2008/62/EC can have positive consequences for the conservation of crop diversity through the commercialization of landraces, they argue that the best way to maintain Italian agricultural biodiversity is through a bottom–up approach that coordinates regional initiatives.

In Germany, an inventory of landraces still being grown and 'other varieties' (from gene banks) will constitute the first step towards implementing Directive 2008/62/EC. Frese et al. (2009) outline three possible approaches to creating such an inventory. A crop-based approach would take as its point of departure the origin of accessions listed in databases, while a regional approach would try to determine the range of crops and accessions originating from a certain area. An explorative approach, on the other hand, would aim to map the landraces still being grown in a particular area.

As a member of the European Economic Area, also Norway must implement EU seed legislation. Discussing Norway's implementation of the directive on conservation varieties of agricultural species Andersen (2012) argues that the country's implementation of the EU seed legislation is still detrimental to Farmers' Rights, as the concept is used in the Plant Treaty, even though some improvements were introduced when Norway passed new legislation in 2010.

The revised national legislation enables Norwegian farmers to exchange and sell seeds on a non-commercial basis and to register as professional seed suppliers of conservation varieties. As of 2012, seven conservation varieties had been added to the official Norwegian list of varieties, and a considerable number of applications were being prepared (Andersen 2012).

3.4.3 Suggested Changes

Bocci (2009) argues that the directive on conservation varieties of agricultural species can be seen as a first step towards opening up the seed market for varieties that fail to fulfil the standard criteria of EU seed legislation. However, he also stresses that only certain types of varieties – those for which a link to a specific territory can be historically proven – will be included in the new category 'conservation varieties'. Other types of varieties – such as those produced by participatory plant breeding and not fulfilling the distinctness, uniformity and stability criteria, old varieties that are no longer listed in the national and common catalogues, varieties without a specific area of origin and varieties adapted to different areas than their region of origin – can still not be legally marketed. Bocci (2009) underlines that the certification system for conservation varieties under the new directive is too similar to the standard EU certification system, and that this is a bigger problem than the limitations with regard to quantity and region.

Concerning the directive on conservation varieties of agricultural species in connection with the Plant Treaty, Bocci (2009) writes that if the directive is implemented in the right way it can contribute to the realization of Article 6 on sustainable use of the Plant Treaty by providing incentives for localized production and by legalizing the marketing of a wider range of varieties. In connection with implementation of the Plant Treaty, he argues that the directive presents new opportunities for civil society to become involved in the identification of conservation varieties.

Goldringer et al. (2010) stress that legislation concerning conservation varieties must become more flexible with regard to descriptive criteria, region of origin and the definition of genetic erosion risk; moreover, an appropriate legislative framework is needed for non-conventional varieties that cannot be classified as conservation varieties. The need to create legal space for this type of varieties, such as populations created within participatory plant breeding or other breeding methods favouring diversity, is underlined by Bocci et al. (2010) as well.

Bocci et al. (2010) emphasize that current EU seed legislation does not offer any solutions for non-conventional varieties that do not fall into the 'conservation variety' category, such as population varieties, farmers' varieties and other non-uniform varieties, and that it is important to create the necessary legal space for their cultivation and commercialization. The need to focus on distinctness (for the purpose of identification) rather than uniformity and stability when it comes to the implementation of the directive on conservation varieties of agricultural species is also underlined, along with the need to make the geographical limitations optional, and to adapt and increase the quantitative limitations.

3.5 In Summary: Seed Legislation in Europe and Crop Diversity in the Literature

As the literature reviewed here shows, landraces and other genetically diverse varieties are still being maintained in Europe despite the dominance of genetically uniform varieties, and the informal seed market is still quite important. However, genetic erosion is believed to be widespread, and landraces appear to be threatened. According to many, today's seed legislation constitutes a further threat to the conservation of genetic diversity and poses a barrier to the expansion of conservation initiatives. One main reason is that the registration requirements are problematic for genetically diverse varieties.

Various suggestions have been offered for changing EU seed legislation: that only commercial seed trade should be regulated; that heterogeneous varieties should be allowed to be registered; that such varieties should be exempt from the registration requirement; and that voluntary registration should be introduced. With regard to the directives aimed at the conservation of genetic resources it has been suggested that distinctness, rather than uniformity and stability, should be in focus; that the

Table 4 The directive on conservation varieties of agricultural species: main relevant points in the literature

Literature reference	Main relevant points
Andersen R (2012) Plant Genetic Diversity in Agriculture and Farmers' Rights in Norway. FNI Report 17/2012. Fridtjof Nansen Institute, Lysaker, Norway	EU seed legislation, and Norway's implementation of it, still detrimental to Farmers' Rights after Directive 2008/62/EC
	Important improvement in Norway; now possible for farmers to exchange and sell seeds on a non-commercial basis
Bocci R (2009) Seed legislation and agrobiodiversity: conservation varieties. Journal of Agriculture and Environment for International Development, 103: 31–49	Opening up of seed market for increased variety, but only some types of varieties
	Too similar to standard EU certification
	Might contribute to implementation of Plant Treaty
Bocci R, Chable V, Kastler G, Louwaars N (2010) Policy Recommendations. Farm Seed Opportunities	Create legal space for other non-uniform varieties
	Distinctness rather than uniformity and stability
	Make geographical limitations optional
	Increase quantitative limitations
Chable V, Thommens A, Goldringer I, Valero Infante T, Levillain T, and Lammerts van Bueren E (2010) Report on the Definitions of Varieties in Europe, of Local Adaptation, and of Varieties Threatened by Genetic Erosion. Farm Seed Opportunities and the French National Institute for Agricultural Research (INRA), Paris	'Landrace' interpreted and translated in different ways across EU
	Many landraces and peasant varieties fall outside scope of directive on conservation varieties
	'Local adaptation' and 'region of origin' not the same
	Landraces travel, adapt and develop
	Linking varieties to specific areas takes away their evolving nature
Frese L, Reinhard U, Bannier HJ, Germeier CU (2009) Landrace inventory in Germany – Preparing the national implementation of the EU Directive 2008/62/EC. In: European Landraces: On-farm Conservation, Management and Use. Bioversity Technical Bulletin No. 15. Bioversity International, Rome, Italy	'Landrace', 'genetic erosion' and 'adaptation' seen as problematic
	Directive's criteria not directly related to hard scientific evidence
	Directive may be difficult to implement due to lack of clarity
	Cyclic nature of plant breeding not considered
	Adaptation as criterion rules out gene bank accessions
	Crop-based approach, regional approach or explorative approach to create national inventory
	Few varieties will meet all four criteria

(continued)

Table 4 (continued)

Literature reference	Main relevant points
Goldringer I, Dawson J, Serpolay E, Schermann N, Giuliano S, Chable V, Lammerts van Bueren E, Osman A, Pino S, Bocci R, Pimbert M, Levillain T (2010) Report on the Analysis of the Bottlenecks and Challenges Identified for On-farm Maintenance and Breeding in European Agricultural Conditions. Farm Seed Opportunities and the French National Institute for Agricultural Research (INRA), Paris	More flexibility needed for descriptive criteria, region of origin and genetic erosion risk
	Framework needed for non-conventional varieties other than conservation varieties
Lorenzetti F, Negri V (2009) The European seed legislation on conservation varieties. In: European Landraces: On-farm Conservation, Management and Use. Bioversity Technical Bulletin No. 15. Bioversity International, Rome, Italy	Central concepts: agricultural landraces and varieties, region of origin and genetic erosion risk
	Likely to cause variation in interpretation and implementation
	Commercialization of gene-bank material not foreseen in directive
	Compilation of national inventories of landraces as starting point for determining genetic erosion
Lorenzetti F, Lorenzetti S, Negri V (2009) The Italian laws on conservation varieties and the national implementation of Commission Directive 2008/62 EC. In: European Landraces: On-farm Conservation, Management and Use. Bioversity Technical Bulletin No. 15. Bioversity International, Rome, Italy	Objective of Italian law and directive different, but both prescribe limitations
	Directive seen as result of compromise
	Regional agrobiodiversity laws in Italy seen as evidence of local interest
	Local level as point of departure seen as important
	Regional inventories, identification of key farmers, non-profit diffusion of limited seed amounts, equitable benefit-sharing and traditional knowledge central in regional laws
	Bottom–up coordination of regional initiatives best way forward
Louwaars N (2007) Seeds of Confusion: The Impact of Policies on Seed Systems. PhD dissertation. Wageningen University, Wageningen, the Netherlands	Directive includes farmers' seed systems in regulatory framework
	Allows 'conservation varieties' to be marketed under different rules
Louwaars N, Kik C, Lammerts van Bueren E (2010) Matches and Mismatches of the 2008/62/EC Directive, Text, Practice, and Positions. Farm Seed Opportunities and the French National Institute for Agricultural Research (INRA), Paris	'An interest for the conservation of plant genetic resources' leaves room for interpretation
	Wide interpretation seen as most beneficial
	Prefers focus on distinctness of landraces, rather than uniformity and stability
	No scientific reason for two-year exclusion
	Necessary with wide interpretation of 'region of origin'

(continued)

Table 4 (continued)

Literature reference	Main relevant points
	No good reasons for quantitative restrictions
	Morphological uniformity requirements will work against goal
	New Population Varieties and New Farmers' Varieties excluded
	Prior discussions dominated by countries with big commercial seed sectors
Paavilainen K (2009) National policies and support systems for landrace cultivation in Finland. In: European Landraces: On-farm Conservation, Management and Use. Bioversity Technical Bulletin No. 15. Bioversity International, Rome, Italy	Landraces, old commercial varieties and old modified varieties can be registered as conservation varieties if not in common catalogue or protected by plant breeders' rights
	11 of 12 registered conservation varieties classified as landraces

Fig. 4 Norwegian oat field (Avena sativa) (Source: The Norwegian Genetic Resource Centre, Norwegian Forest and Landscape Institute. Photographer: Dan Aamlid)

quantitative limitations should be increased; that the geographical limitations should be made optional; that greater flexibility and/or wide interpretation is needed with regard to the key terms; and that a framework is needed for other non-conventional varieties apart from conservation varieties.

As detailed in the next chapter, some of these issues were also debated during the EU seed legislation review process.

4 The Road to Legislative Reform: EU Seed Legislation Review

The review of EU legislation on the marketing of seed and propagating material may result in legislative reform in the form of a regulation covering all plant reproductive material. As part of the review process an external evaluation of the EU legislation on the marketing of seed and propagating material was carried out by a Food Chain Evaluation Consortium (FCEC) team headed by Arcadia International from December 2007 to August 2008, and one of the conclusions of this evaluation was that the legislation ought to be modified (FCEC 2008).

An 'Action Plan for Review of the Community legislation on marketing of seed and plant propagating material and related issues' was then approved in July 2009 within the Directorate General for Health and Consumers (DG SANCO) and on 2 October 2009 presented to the EU member states during a Council Working Group meeting (DG SANCO 2011). This action plan outlined a work programme with a time frame of two and a half years; the stated overall objective being to develop a single horizontal legal framework for the marketing of seed and plant propagating material – an EU Seed Law (Commission of the European Communities 2009).

To give various stakeholders and the general public the opportunity to provide inputs, the Directorate General for Health and Consumers then published the document 'Options and Analysis of Possible Scenarios for the Review of the EU Legislation on the Marketing of Seed and Plant Propagating Material' on its website,[68] along with a questionnaire with a 30 May 2011 response deadline. The 'Options' document outlines and analyses five scenarios for modification of EU legislation on marketing of seed and propagating material, and invites feedback on several issues (DG SANCO 2011). The inputs received through this consultation process were intended to enable the Commission services to develop 'a well-founded proposal for a comprehensive review of the legislation, in view of discussion and adoption by the European Parliament and Council' (DG SANCO 2011: 3).

4.1 The Evaluation

The aim of the evaluation of the Community *acquis* on the marketing of seed and plant propagating material (hereinafter 'the evaluation') was to find out 'how effectively and efficiently the legislation has met its original objectives and to identify its strengths and areas for improvement and its robustness with regard to potential new challenges affecting this field' (FCEC 2008: 2). Because it was conducted within the context of the Better Regulation initiative of the Community, it also sought to identify current and future difficulties and needs and to suggest how the Community could respond. Social, environmental and economic consequences were all taken

[68] See http://ec.europa.eu/food/plant/propagation/evaluation/index_en.htm

into consideration when the various options were evaluated; feasibility, stakeholder support, strengths and weaknesses were also considered (FCEC 2008).

4.1.1 Stakeholder Consultation

Central to the evaluation was a comprehensive stakeholder consultation consisting of a qualitative survey (244 responses were analysed), a cost survey (with 38 return questionnaires) and 55 interviews. This consultation showed that a majority of the stakeholders consulted felt that EU seed legislation has been effective in achieving improved agricultural productivity, increased competitiveness of related sectors and harmonization for the purpose of more open markets. However, stakeholders involved with crops of minor importance, niche and emerging markets underlined that the current costs of registration and certification are disproportionately high when viewed in terms of the market size of landraces, populations or organic varieties.

A majority of the stakeholders interviewed also felt that the system created by the EU seed legislation, where the data to be evaluated are produced by official authorities, is to be preferred because it levels the playing field and promotes equal access to the EU seed market for all players regardless of size. In addition, value for cultivation and use and distinctness, uniformity and stability requirements were generally seen as important and useful tools for conventional agriculture with regard to ensuring agronomic performance and establishing varietal identity. A majority of the stakeholders consulted therefore wanted to maintain these provisions (FCEC 2008).

Nevertheless, quite a few respondents indicated that the distinctness, uniformity and stability requirements had limited the marketing of varieties of interest to users. Among other things it was mentioned that these requirements generally limit the marketing of adaptive populations – like many conservation varieties, amateur varieties and landraces – which build on genetic diversity instead of uniformity and stability. It was also mentioned that the distinctness requirement serves to restrict the marketing of gradual improvements in the agronomical description of the same variety (FCEC 2008).

To address the issue of what were seen as overly strict rules for uniformity, stakeholders active in organic farming recommended that this requirement be made optional and that a system for traceability be developed that could inform the user about the origin of the variety in question, the varieties used to breed it and the specific breeding methods employed. The rationale was that this would allow the greater marketing of conservation varieties, amateur varieties and landraces, thereby widening the users' choice (FCEC 2008).

Also with regard to the value for cultivation and use provision some concerns were raised during the consultation. For organic and other forms of alternative agriculture, the requirement was seen as an obstacle to the release of varieties of interest. The value for cultivation and use trials for example do not allow for the selection of low-input varieties, as the examination conditions and the examined characteristics are poorly suited for such varieties. It was also noted that there has been too much focus on yield: in the future, broader assessments should be done; further, because trials last for two years, they do not evaluate 'yield stability' (FCEC 2008).

While certification standards on the whole were perceived as relevant by a majority, and a substantial majority of the respondents were in favour of maintaining both the certification structure and the certification standards of the EU legislation, stakeholders active in niche and emerging markets saw current costs as disproportionate to the market size of niche varieties like landraces and organic varieties (FCEC 2008).

One suggestion discussed was to remove species of minor economic importance or species for which certification adds no additional value from the legislation. It was also suggested that subspecies with special end-uses for organic farming or adapted to local conditions should be shifted to a list with less stringent rules. And although most respondents opposed the introduction of a voluntary certification scheme, some felt that the flexibility needed for alternative farming practices could be provided by maintaining mandatory certification for non-direct sales and mass seed sales whereas certification could be voluntary for small quantities, niche markets and direct sales (FCEC 2008).

With regard to the revision of the EU seed legislation in general, the aims that received most support from the majority of stakeholders consulted were productivity, plant health and sufficient quality of seed and plant propagating material. However, it was also argued that legislation should be sufficiently flexible and that improving agricultural biodiversity will be important to mitigation efforts related to climate change and to reducing chemical inputs (FCEC 2008).

The evaluation also briefly addressed Commission Directive 2008/62/EC, which had just been approved when the evaluation was conducted. Among some stakeholders, there was concern that the new directive would undermine the main commercial system for introducing new varieties and would offer a quick and cheap way for varieties to be registered. Concerns were also voiced regarding specific provisions: some stakeholders preferred a longer interval before a variety that has been removed from the Common Catalogue can become a conservation variety, and found the quantitative restrictions to have been set too high (FCEC 2008).

On the other hand, stakeholders involved with organic and low-input varieties considered the quantitative restrictions to be too limiting. It was also believed that it would be difficult for member states to define 'regions' in their implementation of the directive. In addition, there was some concern about the influence of the main commercial breeders, and that implementation would prove unnecessarily restrictive (FCEC 2008).

As the results of the stakeholder consultation show, and as the Kokopelli court case highlighted, there is considerable disagreement among the various actors in the seed sector regarding the ideal nature of both variety registration and seed certification.

4.1.2 Problems and Potentials

Although most of the stakeholders consulted felt that the costs associated with implementing EU seed legislation were reasonable, the evaluation points to the high quality of the seed and propagating material currently produced in the EU as a factor that could enable a reduction of the rather high certification costs. For many member states this issue has become central: for instance, France and the United Kingdom have already taken steps to reduce costs and relieve the administrative burdens within the limits set by the EU legislation by changing the certification system to one ‘under official supervision’ (FCEC 2008).

In addition, the evaluation points out that the context within which seed legislation operates in has changed since it was first enacted, and the seed sector is now part of an increasingly international environment that is constantly evolving. New consumer demands, for example related to sustainability, also play a role, as well as developments in biotechnology and plant breeding (FCEC 2008).

Other problems with the EU seed legislation are also identified by the evaluators, such as its complexity and inadaptability to a changing market and what they see as an uneven playing field. According to the evaluation, this last problem stems from inharmonious implementation of provisions, for example those on value for cultivation and use and distinctness, uniformity and stability, some countries’ additional implementation measures, inharmonious national systems for costs and responsibility, and lack of information sharing among member states when it comes to implementation (FCEC 2008).

The evaluation also recognizes the negative impact that the legislation can have on cultivation of agricultural biodiversity. Interestingly, the Food Chain Evaluation Consortium team notes that it ‘believes that the two different systems of the large commercial breeding companies and the smaller market or regional breeders and producers could run side by side because they are targeting completely different markets’ (2008: 172). This can be seen as a counterargument to the fear expressed by some that the legal space provided by the conservation directives would undermine the commercial system.

With regard to Commission Directive 2008/62/EC, the evaluation expresses fears that member states might not understand how to implement it ‘with the flexibility, freedom and adaptability that the Commission intended’ (FCEC 2008: 172), and that, as a result, this directive may prove restrictive. Similar fears regarding the restrictiveness of this directive has, as seen in Sect. 3, also been expressed by researchers and other academics.

4.1.3 Recommendations

The evaluation examined three scenarios with regard to the future of the EU seed legislation: a ‘status quo’ scenario, where the legislation remains unchanged and therefore the current difficulties remain; a ‘suppress’ scenario, where the current EU provisions are suppressed and it becomes up to the member states to retain the national regulations or leave listing and certification up to the market; and a ‘modify’ scenario, where the EU seed legislation is changed (FCEC 2008).

The evaluation recommends modification of the current legislation: a large majority of the stakeholders do not support suppressing the Community provisions, and having different regulatory approaches at the national level might threaten the internal market and decrease transparency; choosing the ‘status quo’ scenario is not in line with the Better Regulation initiative, and most stakeholders prefer to change the current EU legislation.

As possible objectives for a modification scenario the evaluation suggests simplification of the current EU legislation, introducing flexibility within the regulatory framework, reducing implementation differences among member states, promoting cost-reduction approaches, securing long-term consistency with other EU policies, and finalizing the discussion of the possible extension of the role of the Community Plant Variety Office and how to make the seed and propagating material sector benefit from the expertise of the Community Plant Variety Office and improve information to users. In the evaluation's assessment of the various implementing options associated with these objectives, it is only the options for introducing greater flexibility that are associated with increased agricultural biodiversity (FCEC 2008).

The implementing options for this objective are presented as being to make the official rules for uniformity more flexible, for the rules regarding value for cultivation and use to 'evolve to adapt to any type of agriculture and to test varieties created by new technologies' (FCEC 2008: 182) and to 'adapt the requirement for the marketing of seed to defined categories' (FCEC 2008: 182). Further, the first two options will lead to greater diversity in available varieties, and as a result the various agronomic needs of farmers will more easily be met, whereas the third option is believed to offer greater genetic diversity in commercial varieties (FCEC 2008).

As illustrated by the developments since, and in particular the Commission proposal, the 'modify' scenario has been chosen.

4.2 The Action Plan

The Action Plan for Review of the Community legislation on marketing of seed and plant propagating material and related issues states that the Council has acknowledged the findings of the evaluation and welcomed the Commission's intention of undertaking an impact assessment and develop a proposal intended to lead to simpler legislation and reduced administrative burdens for all stakeholders (Commission of the European Communities 2009).

Central to the Action Plan is the goal of creating a modern, harmonized framework for marketing of seed and plant propagating material which should be easier to implement and understand than the current system. To this end, the plan includes a thorough review of this legislation, emphasizing legislative as well as non-legislative measures.

A collection of clear outcomes is outlined: one single horizontal legal framework for the marketing of seed and propagating material (a seed law); harmonized implementation through audits and training; lower administrative burdens and costs through efficient, effective and flexible procedures; consistency with other EU policies such as those for agriculture, environment, genetically modified organisms, plant health and food safety; an enhanced role for the Common Catalogues as a source of information; greater Community influence on international standards; the establishment of a system for stakeholder involvement and a possible extension of the role of the Community Plant Variety Office to the seed and plant propagating material sector (Commission of the European Communities 2009).

The purposes of the overall objective of developing an EU seed law that, in the form of a Regulation, would replace the current 12 Council Directives are stated as being to ensure the availability of good-quality, healthy seed and plant propagating material; to make sure that user expectations regarding seed and plant propagating material are met; to make a contribution to halting the loss of biodiversity; to achieve harmonized implementation; and to boost economic competitiveness (Commission of the European Communities 2009).

The Action Plan also mentions that the Commission should consider whether it is appropriate to keep the current requirement for seed testing for crops of minor importance, and that part of the work to ensure consistency with other EU policies will involve improving coherence with environmental policies such as those on biodiversity (Commission of the European Communities 2009).

The objectives are recognized as challenging in the Action Plan, not just for the EU institutions and the member states, but for breeders, farmers and other seed users as well (Commission of the European Communities 2009).

The original timeframe was two and a half years. Thus, according to the Plan a legislative proposal for an EU Seed Law should have been ready in 2011 (Commission of the European Communities 2009). However, it would take until fall 2012 before a first draft proposal, in the form of a 'non paper', was circulated to stakeholders, and until May 2013 before the Commission adopted a proposal for a Regulation to replace the current directives.

4.3 *'Options and Analysis of Possible Scenarios'*

As mentioned, the document 'Options and Analysis of Possible Scenarios for the Review of the EU Legislation on the Marketing of Seed and Plant Propagating Material' presents and assesses five scenarios for modification of the EU legislation on marketing of seed and propagating material. The analysis presented in the document takes into account the problems identified in the evaluation, supported by the conference and reiterated in the Action Plan, and notes four key reasons why the current system should be reformed: the complexity and fragmentation of the legislation; the high level of administrative burden for public authorities in particular; the distortions in the internal market created by the non-harmonized implementation: and the room for improvement with regard to sustainability. Agreeing with the evaluation, the document concludes, that despite the achievements of the current system, the preferred option should be to modify it, as its identified shortcomings would otherwise persist (DG SANCO 2011).

The paper lists a set of general policy objectives, specific objectives and operational objectives, which build on the objectives in the Action Plan. One of the general policy objectives is listed as being to 'contribute to improve biodiversity, sustainability and favour innovation' (DG SANCO 2011: 7). Among the specific objectives are to improve farmer access to a diversity of varieties, and promote innovative plant breeding that focuses on sustainable cultivation (DG SANCO 2011). While this indicates that issues related to agricultural biodiversity are considered, the document also underlines that not all the objectives can be realized to the same extent, so prioritizing among them will be necessary. As already noted, the paper presents five scenarios for modification of EU seed legislation. One of these, scenario 4, is meant to enable marketing of conservation varieties to a greater extent than today. This scenario, referred to as the 'enhanced flexibility system', introduces basic provisions for registration that are mandatory as well as a voluntary higher level for registration and certification. Thus certification becomes a right that only tested varieties have, instead of being an obligation, and that the national and common catalogues will consist of two sections (DG SANCO 2011).

The variety description criteria are to be in line with Community Plant Variety Office rules and the rules of the International Union for the Protection of New Varieties of Plants for both sections, but whereas Sect. 1 will consist of varieties that have undergone testing for distinctness, uniformity and stability and for value for cultivation and use (for value for cultivation and use the health and adaptation criteria will be mandatory for these varieties, while the yield and value tests will be optional), Sect. 2 will comprise varieties that have not been tested in the same way and that have been registered on the basis of harmonized descriptions, with only denomination, registration and labelling being checked by competent authorities (DG SANCO 2011).

Registration will be compulsory for all breeders and suppliers, and EU-level administrative tasks concerning variety registration will be handled by the Community Plant Variety Office. This scenario would allow conservation varieties and other heterogeneous or 'niche' varieties to be marketed as 'non-tested'. The rationale for proposing a seed and plant propagating material category that can be marketed at a very low cost is to offer new opportunities for the commercialization of varieties with smaller markets (DG SANCO 2011).

The enhanced flexibility system is expected to have positive environmental impacts – a result of the opportunities for marketing of varieties that now fulfil the current criteria, and the introduction of sustainability as part of the screening of tested varieties (DG SANCO 2011). However, one might question why this positive impact has been rated as 'minor'.

For non-tested varieties, there is believed to be a certain risk related to plant health and quality of seed and propagating material in the long run. As certification will no longer be mandatory and the quality of the suppliers' inspection work will be central here as well, the impact is rated as a small negative one. It is also expected that this system would have a medium negative impact on employment and jobs. With respect to administrative burdens and costs, however, the system is expected to have a large positive impact on both the private and the public sectors. The increased flexibility is also expected to have a positive effect on competitiveness, markets, trade and investment flows, as well as on innovation and research (DG SANCO 2011).

Overall, the enhanced flexibility system is the scenario for which most positive effects are expected if the presumed impacts under the various areas (plant health and seed quality, employment and jobs, administrative burden and costs for authorities and private sector, competitiveness, markets, trade and investment flows, innovation and market and environmental impact) are seen together. Moreover, the same result can be found if the expected achievements of the various scenarios with regard to the stated objectives of the review process are seen together.

These conclusions on impact derive from the tables in the paper where the various scenarios were rated and compared. However, the positive and negative ratings were not counted together in the paper, as has been done here.

4.4 The Response to the Option and Analysis Paper

Altogether 257 replies to the online consultation on the review of the EU legislation on the marketing of seed and plant propagating material were received by the Directorate General for Health and Consumers.[69] These are available on the website of the European Commission.[70]

Not surprisingly, a quick review of the replies shows that differences exist with regard to views and stakeholder groups. The European Seed Association for example, offers the opinion that 'the issue of niche markets is overestimated throughout the paper.'[71] As regards the objectives of the review, improving farmers' choice and access to a wide diversity of plant varieties is seen as an inappropriate goal: the focus should be on 'varieties which are beneficial, fit for use and fit for sustainable intensification'[72] rather than on achieving broader diversity. Scenario 4 is the option criticized in most detail by the European Seed Association: this scenario 'seems to focus on turning existing niche markets into large markets'.[73]

By contrast, most stakeholders involved in the conservation of plant genetic diversity seem to prefer scenario 4, or a scenario with new features based on scenario 4. Such stakeholders include seed savers' associations,[74] the European Consortium for

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[69] See the website of the Directorate General for Health and Consumers: http://ec.europa.eu/food/plant/propagation/evaluation/index_en.htm

[70] See http://ec.europa.eu/food/plant/propagation/evaluation/options_review_legislation_replies_en.htm

[71] ESA questionnaire, page 3: http://ec.europa.eu/food/plant/propagation/evaluation/docs/stakeholder_replies_2011/ESA_EuropeanSeedAssociation.pdf

[72] ESA questionnaire, page 4 (see link in footnote 72).

[73] ESA questionnaire, page 5 (see link in footnote 72).

[74] Both the Irish Seed Savers Association (their responses can be found here: http://ec.europa.eu/food/plant/propagation/evaluation/docs/stakeholder_replies_2011/IrishSeedSaversAssociation.pdf) and the Danish Seed Savers Association (their responses can be found here: http://ec.europa.eu/food/plant/propagation/evaluation/docs/stakeholder_replies_2011/DanishSeedSaversAssociationFrosamlerne.pdf) are among the organizations that prefer scenario 4.

Organic Plant Breeding,[75] Association Kokopelli[76] and European Coordination Via Campesina.[77] The European Consortium for Organic Plant Breeding emphasizes that it would be logical to differentiate between the requirements for seed and plant propagating material with relatively high market shares, and seed and plant propagating material for quite small niche markets, with stricter requirements for the former than for the latter.

As opposed to the European Seed Association, Association Kokopelli argues that the issue of biodiversity and the need to strengthen sustainability is underestimated in the options and analysis paper, and that current legislation has led to a dramatic loss in crop diversity. The association prefers scenario 4, but would like to see some changes made to it: there should be no application of the rules of the International Union for the Protection of New Varieties of Plants in connection with the registration of non-tested varieties, and they stress the importance of not confusing intellectual property rules and seed market regulation. In addition, this organization underlines the need for excluding 'non-professional uses of seeds' from the scope of the revised legislation.[78]

It should also be noted that many respondents indicate that they do not know which scenario they prefer or that all scenarios are equally undesirable. Many also respond that they prefer a combination of scenarios, or scenarios with new features.

4.5 The Way Forward for European Seed Legislation and Crop Genetic Diversity

The process towards the adoption of a legislative proposal took longer than originally anticipated. Although it was still believed in July 2012 that a proposal would be submitted by the Commission to the European Parliament and the member states by the end of 2012 (ESA 2012), the Commission did not adopt the proposal on plant reproductive material until May 2013. This proposal was part of the Commission's proposed package of measures to 'modernise, simplify and strengthen the agri-food chain in Europe' (European Commission 2013: 1).

The proposal would, if adopted, replace the 12 basic Directives currently in force, and as this is a proposal for a regulation, it would become binding in all member countries in its entirety. If a new plant reproductive material law based on the Commission proposal is enacted, mandatory registration and certification would

[75] Their responses can be found here: http://ec.europa.eu/food/plant/propagation/evaluation/docs/stakeholder_replies_2011/EuropeanConsortiumforOrganicPlantBreeding.pdf

[76] Their responses can be found here: http://ec.europa.eu/food/plant/propagation/evaluation/docs/stakeholder_replies_2011/KokopelliFrance.pdf

[77] Their responses can be found here: http://ec.europa.eu/food/plant/propagation/evaluation/docs/stakeholder_replies_2011/EuropeanCoordinationViaCampesina.pdf

[78] Association Kokopelli questionnaire, page 4: http://ec.europa.eu/food/plant/propagation/evaluation/docs/stakeholder_replies_2011/KokopelliFrance.pdf

still form the basis of EU seed legislation. However, the proposal provides exclusions that are relevant to the maintenance of crop genetic diversity. Central in this connection is the specification that the proposed regulation 'shall not apply to plant reproductive material exchanged in kind between persons other than professional operators' (Article 2). As can be seen, some new concepts and definitions are also introduced. The proposed exclusion of 'plant reproductive material intended solely for, and maintained by, gene banks, organisations and networks of conservation of genetic resources, or persons belonging to those organisations or networks' (Article 2) is also of interest in this connection.

However, this proposal will be reviewed by the European Parliament and the Council as part of the 'co-decision' procedure of the EU,[79] and that process could lead to some amendments. It can be expected that the central stakeholders will try to influence this process, and it remains to be seen what the results of this will be for the future maintenance of crop genetic diversity in the EU.

According to the Commission, the new regulation might enter into force in 2016 (European Commission 2013). However, progress thus far could indicate that further delays should be expected.

Fig. 5 Sharing seed of fruit varieties, like this traditional Norwegian apple variety, 'Red Torstein', is something many people do without being familiar with seed legislation (Source: The Norwegian Genetic Resource Centre, Norwegian Forest and Landscape Institute. Photographer: Åsmund Asdal)

[79] The EU's ordinary decision-making procedure is known as 'co-decision': both the European Parliament (which is directly elected) and the Council (the governments of the EU member countries) have to approve EU legislation. Commission proposals are reviewed by both institutions and they have to agree on proposed amendments.

5 Conclusion

As this article has shown, current EU seed legislation is quite complicated. Thus, the review process represented a welcome step toward simplification and clarification. Indeed, one of the main conclusions of the external evaluation that was conducted as part of the review of EU seed legislation was that the legislation ought to be modified. The evaluation concluded that efforts must be made to rein in the costs for governments, and that the complexity and lack of ability to adapt to a changing market are main problems of today's EU seed legislation.

In addition to complexity, a frequently cited drawback of the current legislation is its impact on agricultural biodiversity. Experts and practitioners engaged in the maintenance of such biodiversity argue that the EU seed legislation functions as a barrier to this work. Under today's seed legislation, varietal change cannot take place during the commercial life of a variety; moreover, it is difficult to market old, traditional and/or locally adapted varieties legally, as these usually do not fulfil the requirements for distinctness, uniformity and stability. Efforts to develop or maintain such varieties, to create local seed enterprises and to upscale existing initiatives are therefore struggling. Altering the legislation is seen as necessary. Suggested changes include exempting traditional varieties from variety registration and introducing voluntary registration.

In this context it is relevant to compare the control-based EU seed system with the voluntary system in the USA. In the latter, variety registration, performance testing and seed certification are all voluntary, and there is no national variety release authority. By contrast, all of the above are mandatory in the EU. It can be argued that the need of seed users to know what they are buying could be met without all varieties having to adhere to strict distinctness, uniformity and stability requirements, and that a system with more voluntary elements could work also in the EU. As long as the labelling clearly states the extent to which the seed can be expected to be distinct, uniform and stable, surely the interest of users can be regarded as sufficiently protected here.

However, seed legislation is a contested subject and considerable disagreement exists between stakeholders regarding to what extent and how the current EU seed legislation should be changed. These differences were showcased by the Kokopelli court case. When Advocate General Kokott concluded that the prohibition on the marketing of seed of varieties that do not fulfil the distinctness, uniformity and stability criteria, and, where relevant, the value for cultivation and use criteria, as established in Council Directive 2002/55/EC on the marketing of vegetable seed, was invalid because it infringes on the principle of proportionality, the freedom to conduct a business, the free movement of goods and the principle of equal treatment, it was both hoped and feared that the judgment of the Court of Justice of the EU would include a similar conclusion. This was not to be, however.

Now that the Commission has adopted a proposal on plant reproductive material, stakeholder attention will shift to the European Parliament and the Council. It is these two institutions that will determine how much legal space is provided for the maintenance and sustainable use of crop genetic diversity in the EU in the years to come.

References

Literature

Almekinders CJM, Louwaars NP (2002) The importance of the farmers' seed systems in a functional national seed sector. In: Louwaars NP (ed) Seed policy, legislation and law: widening a narrow focus. Food Products Press/The Haworth Press, Haworth

Andersen R (2009) Information paper on Farmers' Rights submitted by the Fridtjof Nansen Institute, Norway, based on the Farmers' Rights Project. Input paper submitted to the Secretariat of the Plant Treaty, 19 May 2009 (IT/GB-3/09/Inf. 6 Add. 3), Lysaker, Norway

Andersen R (2012) Plant Genetic Diversity in Agriculture and Farmers' Rights in Norway. FNI Report 17/2012. Fridtjof Nansen Institute, Lysaker, Norway

Andersen R, Winge T (2011) The 2010 Global Consultations on Farmers' Rights: results from an email-based survey. FNI report 2/2011. Fridtjof Nansen Institute, Lysaker, Norway

Bocci R (2009) Seed legislation and agrobiodiversity: conservation varieties. J Agri Environ Int Develop 103:31–49

Bocci R, Levillain T, Kastler G, Serpolay E, Pino S, Nonne MF, Almekinders C, González JM, Valero T, Casado S (2009) National survey on the role of innovative market mechanisms. Farm Seed Opportunities and the French National Institute for Agricultural Research (INRA), Paris

Bocci R, Chable V, Kastler G, Louwaars N (2010) Policy recommendations. Farm Seed Opportunities and the French National Institute for Agricultural Research (INRA), Paris

Ceddia MG, Cerezo ER (2008) A descriptive analysis of conventional, organic and GM crop and certified seed production in the EU. Office for Official Publications of the European Communities, Luxembourg

Chable V, Goldringer I, Dawson J, Bocci R, Lammerts van Bueren E, Serpolay E, González JM, Valero T, Levillain T, Van der Burg JW, Pimbert M, Pino S, Kik C (2009) Farm seed opportunities: a project to promote landrace use and renew biodiversity. In: European landraces: on-farm conservation, management and use. Bioversity technical bulletin no. 15. Bioversity International, Rome

Chable V, Thommens A, Goldringer I, Valero Infante T, Levillain T, Lammerts van Bueren E (2010) Report on the definitions of varieties in Europe, of local adaptation, and of varieties threatened by genetic erosion. Farm Seed Opportunities and the French National Institute for Agricultural Research (INRA), Paris

Frese L, Reinhard U, Bannier HJ, Germeier CU (2009) Landrace inventory in Germany – preparing the national implementation of the EU Directive 2008/62/EC. In: European landraces: on-farm conservation, management and use. Bioversity technical bulletin no. 15. Bioversity International, Rome

Goldringer I, Dawson J, Serpolay E, Schermann N, Giuliano S, Chable V, Lammerts van Bueren E, Osman A, Pino S, Bocci R, Pimbert M, Levillain T (2010) Report on the analysis of the bottlenecks and challenges identified for on-farm maintenance and breeding in European agricultural conditions. Farm Seed Opportunities and the French National Institute for Agricultural Research (INRA), Paris

Lorenzetti F, Negri V (2009) The European seed legislation on conservation varieties. In: European landraces: on-farm conservation, management and use. Bioversity technical bulletin no. 15. Bioversity International, Rome

Lorenzetti F, Lorenzetti S, Negri V (2009) The Italian laws on conservation varieties and the national implementation of Commission Directive 2008/62 EC. In: European landraces: on-farm conservation, management and use. Bioversity technical bulletin no. 15. Bioversity International, Rome

Louwaars N (2002a) Seed policy, legislation and law. J New Seeds 4(1):1–14

Louwaars N (2002b) Variety controls. J New Seeds 4(1):131–142

Louwaars N (2007) Seeds of confusion: the impact of policies on seed systems. PhD dissertation. Wageningen University, Wageningen

Louwaars N, Kik C, Lammerts van Bueren E (2010) Matches and mismatches of the 2008/62/EC Directive, text, practice, and positions. Farm Seed Opportunities and the French National Institute for Agricultural Research (INRA), Paris

Negri V, Maxted N, Veteläinen M (2009) European landrace conservation: an introduction. In: European landraces: on-farm conservation, management and use. Bioversity technical bulletin no. 15. Bioversity International, Rome

Osman A, Chable V (2007) Breeding initiatives of seeds of landraces, amateur varieties and conservation varieties. Farm Seed Opportunities and the French National Institute for Agricultural Research (INRA), Paris

Osman A, Chable V (2009) Inventory of initiatives on seeds of landraces in Europe. J Agri Environ Int Develop 103:95–130

Paavilainen K (2009) National policies and support systems for landrace cultivation in Finland. In: European landraces: on-farm conservation, management and use. Bioversity technical bulletin no. 15. Bioversity International, Rome

Pimbert M (2011) Participatory research and on-farm management of agricultural biodiversity in Europe. International Institute for Environment and Development, London

Thommen A, Lammerts van Bueren ET, Serpolay E, Levillain T, Valero Infante T, Bocci R (2010) Characterisation of stakeholder expectations – an expert survey. Research Institute of Organic Agriculture (FIBL), Frick

Tripp R (1997) Regulation and regulatory reform. In: Tripp R (ed) New seed and old laws: regulatory reform and the diversification of national seed systems. Intermediate Technology Publications and ODI, London

Tripp R (2002) Seed regulatory reform: an overview. J New Seeds 4(1/2):103–115

Tripp R, Louwaars NP (1997) Seed regulation: choices on the road to reform. Food Policy 22(5):433–446

United Nations (2009) Seed policies and the right to food: enhancing agrobiodiversity and encouraging innovation. Report of the Special Rapporteur on the right to food. Sixty-fourth session of the General Assembly. A/64/170. United Nations, New York

Vellvé R (1992) Saving the seed: genetic diversity and European agriculture. Earthscan and GRAIN, London

Visser B (2002) An agrobiodiversity perspective on seed policies. J New Seeds 4(1):231–245

Treaties, Directives and the Proposal

Convention on Biological Diversity, 5 June 1992

The International Treaty on Plant Genetic Resources for Food and Agriculture (Plant Treaty), 3 November 2001

Council Directive 66/401/EEC on the marketing of fodder plant seed, 14 June 1966

Council Directive 66/402/EEC on the marketing of cereal seed, 14 June 1966

Council Directive 68/193/EEC on the marketing of material for the vegetative propagation of the vine, 9 April 1968

Council Directive 92/33/EEC on the marketing of vegetable propagating and planting material, other than seed, 28 April 1992

Council Directive 92/34/EEC on the marketing of fruit-plant propagating material and fruit plants intended for fruit production, 28 April 1992

Council Directive 98/95/EC amending, in respect of the consolidation of the internal market, genetically modified plant varieties and plant genetic resources, Directives 66/400/EEC, 66/401/EEC, 66/402/EEC, 66/403/EEC, 69/208/EEC, 70/457/EEC and 70/458/EEC on the marketing of beet seed, fodder plant seed, cereal seed, seed potatoes, seed of oil and fibre plants

and vegetable seed and on the common catalogue of varieties of agricultural plant species, 14 December 1998
Council Directive 98/56/EC on the marketing of propagating material of ornamental plants, 20 July 1998
Council Directive 1999/105/EC on the marketing of forest reproductive material, 22 December 1999
Council Directive 2002/11/EC amending Directive 68/193/EEC on the marketing of material for the vegetative propagation of the vine and repealing Directive 74/649/EEC, 14 February 2002
Council Directive 2002/53/EC on the common catalogue of varieties of agricultural plant species, 13 June 2002
Council Directive 2002/54/EC on the marketing of beet seed, 13 June 2002
Council Directive 2002/55/EC on the marketing of vegetable seed, 13 June 2002
Council Directive 2002/56/EC on the marketing of seed potatoes, 13 June 2002
Council Directive 2002/57/EC on the marketing of seed of oil and fibre plants, 13 June 2002
Commission Directive 2008/62/EC providing for certain derogations for acceptance of agricultural landraces and varieties which are naturally adapted to the local and regional conditions and threatened by genetic erosion and for marketing of seed and seed potatoes of those landraces and varieties, 20 June 2008
Council Directive 2008/90/EC on the marketing of fruit-plant propagating material and fruit plants intended for fruit production, 29 September 2008 (Recast version)
Commission Directive 2009/145/EC providing for certain derogations, for acceptance of vegetable landraces and varieties which have been traditionally grown in particular localities and regions and are threatened by genetic erosion and of vegetable varieties with no intrinsic value for commercial crop production but developed for growing under particular conditions and for marketing of seed of those landraces and varieties, 26 November 2009
Commission Directive 2010/60/EU providing for certain derogations for marketing of fodder plant seed mixtures intended for use in the preservation of the natural environment, 30 August 2010
Proposal for a Regulation of the European Parliament and of the Council on the production and making available on the market of plant reproductive material (plant reproductive material law) of 6 May 2013

Other Documents

Advocate General Kokott (2012) Opinion of Advocate General Kokott delivered on 19 January 2012. Case C-59/11, Association Kokopelli v Graines Baumaux SAS. The Court of Justice of the European Union, Luxembourg
Commission of the European Communities (2009) Action plan for review of the community legislation on marketing of seed and plant propagating material and related issues. European Commission, Brussels
Court of Justice of the European Union (2011) Information note on references from national courts for a preliminary ruling (2011/C 160/01). In: Official Journal of the European Union, 28.5.2011, C 160/1, Brussels, Belgium
Court of Justice of the European Union (2012) Judgment of the Court (Third Chamber) 12 July 2012. Case C-59/11, Association Kokopelli v Graines Baumaux SAS. The Court of Justice of the European Union, Luxembourg
DG SANCO (2011) Options and Analysis of Possible Scenarios for the Review of the EU Legislation of the Marketing of Seed and Plant Propagating Material. DG SANCO, Brussels, Belgium
ESA (2012) Kokopelli ruling – CJEU confirms validity of European seed marketing legislation. ESA press release, 12 July 2012. European Seed Association
European Commission (2013) Smarter rules for safer food: commission proposes landmark package to modernise, simplify and strengthen the agri-food chain in Europe. European Commission Press Release, 6 May 2013. Brussels, Belgium

FAO (1998) State of the world's plant genetic resources for food and agriculture. Food and Agriculture Organization of the United Nations, Rome, Italy
FCEC (2008) Evaluation of the community acquis on the marketing of seed and plant propagating material (S&PM). European Commission, Brussels, Belgium
IFOAM (2012) European citizens must stand up for diversity on our plates. The International Federation of Organic Agriculture Movements EU Group Press Release, 12 July 2012. International Federation of Organic Agriculture Movements

Postharvest Management of Fruits and Vegetables Storage

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Be the change you want to see in the world.

Mahatma Gandhi

Abstract Sustainable agriculture is a core part of the concept of sustainable development. Given the forecast in population increase, sustainable agriculture has to achieve food security in combination with economic viability, social responsibility and have as little effect on biodiversity and natural ecosystems as possible. Based on Agenda 21, signed at the world summit in Rio de Janeiro 1992, sustainable agriculture takes a truly global perspective. This concept requires a thorough understanding of agro-ecosystem functions. The protection of soil and water is one necessary prerequisite as well as the efficient use of mineral and organic fertilizers. This might be achieved by means of improved technology and better understanding of the basic processes in soils. Solving the persistent hunger problem is not simply a matter of developing new agricultural technologies and practices. Most poor producers cannot afford expensive technologies. They will have to find new types of solutions based on locally-available and cheap technologies combined with making the best of natural and human resources. Sustainable intensification is the use of the best available technologies and inputs such as best genotypes, best agronomic management practices and best postharvest technologies to maximize yields, while

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at the same time minimizing or eliminating harm to the environment. Clearly, over the next 50 years we will need to learn to do just this. Therefore, this review will be focused on the postharvest physiology and management including harvesting, handling, packing, storage and hygiene of fruits and vegetables to enhance using of new postharvest biotechnology. The postharvest biology including biochemical parameters of horticultural crops quality, postharvest handling under extreme weather conditions, potential impacts of climate changes on vegetable postharvest and postharvest biotechnology will be also highlighted.

Keywords Postharvest • Preharvest • Postharvest management • Postharvest losses • Storage science • Biotechnology

1 Introduction

Postharvest physiology is the scientific study of the physiology of living plant tissues after they have denied further nutrition by picking. It has direct applications to postharvest handling in establishing the storage and transport conditions that best prolong shelf life. An example of the importance of the field to post-harvest handling is the discovery that ripening of fruit can be delayed, and thus their storage prolonged, by preventing fruit tissue respiration. This insight allowed scientists to bring to bear their knowledge of the fundamental principles and mechanisms of respiration, leading to post-harvest storage techniques such as cold storage, gaseous storage, and waxy skin coatings. Another well known example is the finding that ripening may be brought on by treatment with ethylene.

The fruit processing industry is one of the major businesses in the world. While basic principles of fruit processing have shown only minor changes over the last few years, major improvements are now continuously occurring, and more efficient equipment capable of converting huge quantities of fruits into pulp, juice, dehydrated, frozen, refrigerated products, etc. make possible the preservation of products for year-round consumption. The fruit processing and storage, even under the most industrially available “mild conditions,” involves physical and chemical changes that negatively modify the quality. These negative or deteriorative changes include enzymatic and non-enzymatic browning, off-flavor, discoloration, shrinking, case hardening, and some other chemical, thermo-physical, and rheological alterations that modify the nutritive value and original taste, color, and appearance of fruits. The ability of the industry to provide a nutritious and healthy fruit product to the consumer is highly dependent on the knowledge of the quality modifications that occur during the processing.

In postharvest, fresh harvested food crops can be considered isolated small scale systems. Postharvest research aims to understand the quality of these ‘systems’ as influenced by postharvest conditions. The phenotypic quality of horticultural produce is based on genetic traits that are expressed through a cascade of reactions subject to complex regulatory mechanisms and diverse environmental conditions. Ultimately, to fully understand postharvest phenomena, a systemic approach that links genetic and environmental responses and identifies the underlying biological networks is required. Thanks to the development of high throughput omics techniques such system-wide approaches have become a viable option to support traditional postharvest research (Hertog et al. 2011).

The structure of a biological system is defined by its physical parts e.g., tissues, cells, organelles and their composition e.g., DNA, proteins, metabolites, lipids. Their behaviour involves inputs e.g., external stimuli such as light, temperature, atmospheric composition, pH and nutrient levels and internal stimuli such as proteins, metabolites, hormones and various other compounds that might act as signal molecules, processing e.g., via catabolic and anabolic pathways, or processes such as gene expression, differentiation and cell division and outputs of material e.g., proteins, metabolites, information e.g., transcripts or energy e.g., heat, ATP, movement. Finally, a biological system is characterized by a high degree of interconnectivity between its various parts, showing both functional relationships e.g., through metabolic, signaling and gene regulation pathways and structural relationships between each other e.g., through compart-mentalization, receptor molecules, membrane transporters, plasmodesmata, vascular tissue, and the cytoskeleton. Systems biology relies on a multidisciplinary approach to integrate data from various disciplines of biology (Friboulet and Thomas 2005) bringing together molecular disciplines e.g., genetics, biochemistry, molecular biology with those involving more complex systems e.g., cell biology, microbiology, plant or human physiology. The aim of systems biology is to link the quantitative data in a mathematically defined sense across the different scales of biological organization from DNA, RNA, protein to cell, tissue, organs. Mathematical modelling is used to drive integration with an aim of reaching a unified understanding of biological systems (Hertog et al. 2011).

In this review, we have tried to address the following questions. First, what examples do we have of specific postharvest physiological or biochemical traits that have been used to improve crops and more specifically, to improve the environmental sustainability of the agricultural production system? Second, what have been the real effects of these advances on agricultural sustainability? Third, how can researcher's best make postharvest of crops research relevant to the challenge of environmental sustainability? Finally, how can producers use these postharvest technological advances in a viable and sustainable manner to improve their productivity and profitability? These issues will be inspired us to look back at the history of agricultural technologies to determine which ones are more sustainable with a view to providing a discussion of the future focus of researchers, government agencies and the agricultural community.

2 Postharvest Management

Postharvest management is a set of post-production practices that includes: cleaning, washing, selection, grading, disinfection, drying, packing and storage. These eliminate undesirable elements and improve product appearance, as well as ensuring that the product complies with established quality standards for fresh and processed products. Postharvest practices include the management and control of variables such as temperature and relative humidity, the selection and use of packaging, and the application of such supplementary treatments as fungicides (FAO 2009).

After they are harvested, the value of fruits and vegetables is added in successive stages up to the point when someone eats them. The aim of postharvest management is to maximize this added value. This ultimately should benefit the whole community, whether through increased export earnings or extending the availability of fresh produce through the year. Conversely losses hurt everyone. Kader (1992) has estimated that from 5 to 25 % of fruit and vegetables leaving the farm gate is never consumed, but has to be thrown away. Obviously, disease and oversupply contribute to this, but there are many other reasons for the losses. Postharvest management can influence all them, with the two most important areas being temperature management and packaging. Another point to remember is that the loss of value of a down graded product is likely to be substantially greater for highly differentiated branded products which sell at a premium in the market. All the hard work that has gone into promoting and raising the profile of a branded product can be quickly eroded if there are postharvest quality problems with some lines of that product (Jobling 2002).

It could be addressed the postharvest management through the following items:

2.1 The Nature of Postharvest Management

The horticultural produce includes fruits, vegetables, flowers and other ornamental plants, plantation crops, aromatic and medicinal plants and spices. According to Oxford English Dictionary, fruit can be defined as '*the edible product of a plant or tree, consisting of seed and its envelope, especially the latter when it is juicy or pulpy*'. The consumer definition of fruit would be 'plant products with aromatic flavors, which are either naturally sweet or normally sweetened before eating. The classification of fruits and vegetables is arbitrary and according to usage. Botanically many crops, defined as vegetables, are fruits e.g., tomato, capsicum, melons etc. Morphologically and physiologically the fruits and vegetables are highly variable, may come from a root, stem, leaf, immature or fully mature and ripe fruits. They have variable shelf life and require different suitable conditions during marketing. All fresh horticultural crops are high in water content and are subjected to desiccation (wilting, shriveling) and to mechanical injury. Various authorities have estimated that 20–30 % of fresh horticultural produce is lost after harvest and these losses can assume considerable economic and social importance. That is why, these perishable commodities need very careful handling at every stage so that deterioration of produce is restricted as much as possible during the period between harvest and consumption (Dhatt and Mahajan 2007).

Horticultural produce is alive and has to stay alive long after harvest. Like other living material it uses up oxygen and gives out carbon dioxide. It also means that it has to receive intensive care. For a plant, harvesting is a kind of amputation. In the field it is connected to roots that give it water and leaves which provide it with the food energy it needs to live. Once harvested and separated from its sources of water and nourishment it must inevitably die. The role of postharvest handling is to delay that death for as long as possible. Horticultural managers must posses many skills to succeed in this. They need a keen appreciation of horticultural diversity. For example, spinach and apples, bananas and potatoes each have their own requirements. The optimum postharvest management of horticultural products is not the same for all products. Growers, wholesalers, exporters and retailers must all be aware of the specific needs of a product if the postharvest shelf life and quality is to be maximized (Jobling 2002).

It could be concluded that, horticultural produce is alive and has to stay alive long after harvest. Like other living material it uses up oxygen and gives out carbon dioxide. It also means that it has to receive intensive care. The role of postharvest handling is to delay that death for as long as possible. Horticultural managers must posses many skills to succeed in this.

2.2 Understanding Product Maturity

The stage of development at which a product is regarded as mature depends on its final use. Fruit and vegetables are eaten at all stages of development. We eat sprouted seeds, vegetative leaves and flowers, whole fruit as well as seeds and nuts. There are no general rules when it comes to defining horticultural maturity. A lot of research has been done to establish maturity parameters for a whole range of specific horticultural products. Maturity must be defined for each product in some cases for each variety of a particular product. The use of maturity standards provides consumers with a minimum level of quality assurance. Another reason for establishing maturity standards is that most horticultural products are harvested by hand. A simple color guide and size can help pickers harvest produce at the correct stage of development (Fig. 1 and Table 1; Jobling 2002).

Maturity at harvest is the most important factor that determines postharvest-life and final quality such as appearance, texture, flavor, nutritive value of fruit-vegetables. Fruit-vegetables include two groups: (1) immature fruit-vegetables, such as green bell pepper, green chili pepper, cucumber, summer (soft-rind) squash, chayote, lima beans, snap beans, sweet pea, edible-pod pea, okra, eggplant, and sweet corn; and (2) mature fruit-vegetables, such as tomato, red peppers, muskmelons (cantaloupe, casaba, crenshaw, honeydew, persian), watermelon, pumpkin, and winter (hard-rind) squash. For group (1), the optimum eating quality is reached before full maturity and delayed harvesting results in lower quality at harvest and faster deterioration rate after harvest. For group (2) most of the fruits reach peak eating quality when fully ripened on the plant and, with the exception of tomato, all are incapable of continuing their ripening processes once removed from the plant. Fruits picked at less than mature stages are subject to greater shriveling and mechanical damage,

Fig. 1 The stage of development at which a product is regarded as mature depends on its final use. Fruit and vegetables are eaten at all stages of development (Photos of some different fruits at maturity stage were taken in Cesena, Italy by M. Fári)

Table 1 Approximate number of days from planting to market maturity under optimum growing conditions

Crop	Early variety	Common type	Late variety
Beans, bush	46	–	65
Beans, pole	56	–	72
Beans, lima, bush	65	–	78
Beets	50	–	80
Broccoli, sprouting[a]	70	–	150
Brussels sprouts[b]	90	–	100
Cabbage[b]	62	–	110
Carrots	60	–	85
Cauliflower, snowball type[b]	55	–	65
Chinese cabbage	70	–	80
Chives	–	90	–
Corn	70	–	100
Cucumber	60	–	70
Eggplant	70	–	85
Kohlrabi	55	–	65
Lettuce, head	60	–	85
Lettuce, leaf	40	–	50
Melon, Honey Ball	–	105	–
Melon, Honey Dew	–	115	–
Muskmelon	75	83	90
Mustard	40	–	60
Okra	50	–	60
Onions	85	–	120
Parsley	70	–	85
Parsnips	100	—	130
Peas	58	–	77
Pepper, sweet[b]	60	–	80
Potatoes	90	–	120
Pumpkin	110	–	120
Radishes	22	–	40
Radishes, winter type	50	–	60
Rutabagas	–	90	–
Spinach	40	–	50
Squash, winter	50	–	68
Squash, summer	80	–	120
Tomatoes[b]	65	–	100
Turnips	40	–	75
Watermelon	65	75	95

Adapted from Smith (2010)

When these crops are planted under low-temperature conditions, reaching the harvest stage will take longer than indicated above

[a]For a direct-seeded crop. Transplanting may delay maturity by a few weeks, depending on environmental conditions

[b]For a transplanted crop, additional time is needed from seed sowing to transplanting

and are of inferior flavor quality. Overripe fruits are likely to become soft and/or mealy in texture soon after harvest. The necessity of shipping mature fruit-vegetables long distances has often encouraged harvesting them at less than ideal maturity, resulting in suboptimal taste quality to the consumer (Kader 1995).

Several factors in addition to maturity at harvest have major impacts on postharvest behaviour and quality of fruit-vegetables. Fruits of group (1) normally produce only very small quantities of ethylene. However, they are very responsive to ethylene and can be damaged by exposure to 1 ppm or higher concentrations. Ethylene exposure accelerates chlorophyll degradation, induces yellowing of green tissues, encourages calyx abscission (eggplant), and accelerates fruit softening. Most of the fruits in group (2) produce larger quantities of ethylene in association with their ripening, and exposure to ethylene treatment will result in faster and more uniform ripening as indicated by loss of chlorophyll (green color), increase of carotenoids (red, yellow, and orange colors), flesh softening and increased intensity of characteristic aroma volatiles (Fig. 2).

All fruit-vegetables, except peas and sweet corn, are susceptible to chilling injury if exposed to temperatures below 5 °C e.g., cantaloupe, lima bean, snap bean, 7.5 °C e.g., pepper, 10 °C such as cucumber, soft-rind squash, eggplant, okra, chayote, or 12.5 °C e.g., tomato, muskmelons other than cantaloupe, pumpkin, hard-rind squash. A relative humidity range of 90–95 % is optimum for all fruit-vegetables except pumpkin and hard-rind squash where it should be 60–70 %. Atmospheric modification (low oxygen and/or elevated carbon dioxide concentrations) can be

Fig. 2 Photo of some different vegetables at maturity stage in super market was taken in China by M. Fári

a useful supplement to proper temperature and relative humidity in maintaining postharvest quality of some fruit-vegetables, such as tomato and muskmelons (Kader 1995).

Fruits harvested too early may lack flavor and may not ripen properly, while produce harvested too late may be fibrous or have very limited market life. Similarly, vegetables are harvested over a wide range of physiological stages, depending upon which part of the plant is used as food. For example, small or immature vegetables possess better texture and quality than mature or over-mature vegetables. Therefore, harvesting of fruits and vegetables at proper stage of maturity is of paramount importance for attaining desirable quality. The level of maturity actually helps in selection of storage methods, estimation of shelf life, selection of processing operations for value addition etc. The maturity has been divided into two categories i.e. physiological maturity and horticultural maturity:

- **Physiological maturity**: It is the stage when a fruit is capable of further development or ripening when it is harvested i.e. ready for eating or processing.
- ***Horticultural maturity***: It refers to the stage of development when plant and plant part possesses the pre-requisites for use by consumers for a particular purpose i.e. ready for harvest (Dhatt and Mahajan 2007).

Importance of maturity indices:

- Ensure sensory quality (flavor, color, aroma, texture) and nutritional quality.
- Ensure an adequate postharvest shelf life.
- Facilitate scheduling of harvest and packing operations.
- Facilitate marketing over the phone or through internet (Table 2).

Definitions related to maturity and ripening:

(i) **Mature**: It is derived from Latin word '*Maturus*' which means ripen. It is that stage of fruit development, which ensures attainment of maximum edible quality at the completion of ripening process.

(ii) **Maturation**: It is the developmental process by which the fruit attains maturity. It is the transient phase of development from near completion of physical growth to attainment of physiological maturity. There are different stages of maturation e.g. immature, mature, optimally mature, over mature.

(iii) **Ripe**: It is derived from Saxon word '*Ripi*', which means gather or reap. This is the condition of maximum edible quality attained by the fruit following harvest. Only fruit which becomes mature before harvest can become ripe.

(iv) **Ripening**: Ripening involves a series of changes occurring during early stages of senescence of fruits in which structure and composition of unripe fruit is so altered that it becomes acceptable to eat. Ripening is a complex physiological process resulting in softening, coloring, sweetening and increase in aroma compounds so that ripening fruits are ready to eat or process. The associated physiological or biochemical changes are increased rate of respiration and ethylene production, loss of chlorophyll and continued expansion of cells and conversion of complex metabolites into simple molecules.

Table 2 Maturity indices for selected fruits and vegetables (Adapted from Dhatt and Mahajan 2007)

Fruits/vegetables	Maturity indices or characteristics
Almonds	Splitting of hull, separation of hull from shell, development of abscission zone
Apple	
'Golden Delicious'	12 % SSC, 18 lb firmness
'Red Delicious'	11 % SSC, 18 lb firmness
Banana	Disappearance of angularity in a cross section of the finger
Bear	Color break stage (when light yellow color appear)
Cherry	TSS = 14–15 %, light red color
Grapes (table)	Minimum SSC % of 14–17.5, depending on cultivars, SSC/TA of 20 or higher
Guava	Color break stage (when skin color changes from dark green to light green)
Lemon	30 % or more juice by volume
Litchi	TSS: total acid ratio of 30–40, bright red in color
Kiwi fruit	TSS – 6.5 %, Firmness = 14 lbs
Mango	Changes in shape (increase fullness of cheeks or bulge of shoulder), flesh color yellow to yellowish-orange
Papaya	Skin shows yellowing
Peach	Ground color change from green to yellow (varied for different cultivars)
Plums	Skin color changes
Pomegranate	Minimum 1.85 % TA and red juice color
Strawberry	2/3 of berry surface showing pink or red color
Beans	Pods are filled, seeds immature
Broccoli	Adequate diameter, compact, all florets should be closed
Cabbage	Firm head
Cantaloupe	¾ to full slip under slight pressure, abscission from vine
Carrot	Immature, roots reached adequate size
Cucumber	Immature and glossy skin
Garlic	Well filled bulbs, tops dry down
Ginger	8–9 months after planting
Melon	Ground color change to white with greenish tint, slightly waxy peel
Mushroom	Caps well rounded, partial veil completely intact
Okra	Pod 2–4″ long, not fibrous, tips of pods pliable
Onion (dry bulbs)	When 10–20 % of tops fall over
Peas	Pods well filled but not faded in color
Pepper	Fruit size and color (depends on color and intended market)
Potatoes	Harvest before vines die completely, cure to heal surface wounds
Radish (spring)	20–30 days after planting
Radish (winter)	45–70 days after planting
Tomato	Seeds fully developed, gel formation advanced in at least one locule
Watermelon	Flesh color 75 % red, TSS = 10 %

Source: Kitinoja and Gorny (1998)
Soluble solid content (*SSC*) also called total soluble solids (*TSS*), can be determined in a small sample of fruit juice using hand refractometer. Titratable acidity (*TA*) can be determined by titrating a know volume of juice with 0.1 N NaOH to end point of pink color as indicated by phenolphethalin indicator. The milliliters of NaOH needed are used to calculate the TA. The TA expressed as percent malic, citric or tartaric acid can be calculated as follows:

$$TA = \frac{\text{ml NaOH} \times \text{N}(\text{NaOH}) \times \text{acid meq. Factor}^{*} \times 100}{\text{Juice titrated}}$$

*The following acid meq. factor may be used for different fruits (for ex. for citric acid = 0.0064)

(v) **Senescence:** Senescence can be defined as the final phase in the ontogeny of the plant organ during which a series of essentially irreversible events occur which ultimately leads to cellular breakdown and death (Dhatt and Mahajan 2007).

Types of indices and their components:

(i) Visual indices (size, shape and color)
(ii) Physical indices (firmness and specific gravity)
(iii) Chemical measurement: Soluble Solids Content (SSC) or total soluble solids (TSS) and Titratable acidity (TA)
(iv) Calculated indices: calendar date and heat units (Dhatt and Mahajan 2007).

It could be concluded that, the stage of development at which a product is regarded as mature depends on its final use. Fruit and vegetables are eaten at all stages of development. We eat sprouted seeds, vegetative leaves and flowers, whole fruit as well as seeds and nuts. There are no general rules when it comes to defining horticultural maturity. It is worth to mention also that, the most important fruits and vegetables maturity indices are visual, physical, chemical and calculated indices.

2.3 Harvest Handling

Postharvest handling is the stage of crop production immediately following harvest, including cooling, cleaning, sorting and packing. The instant a crop is removed from the ground, or separated from its parent plant, it begins to deteriorate. Postharvest treatment largely determines final quality, whether a crop is sold for fresh consumption, or used as an ingredient in a processed food product. The most important goals of postharvest handling are keeping the product cool, to avoid moisture loss and slow down undesirable chemical changes, and avoiding physical damage such as bruising, to delay spoilage. Sanitation is also an important factor, to reduce the possibility of pathogens that could be carried by fresh produce, for example, as residue from contaminated washing water. After the field, postharvest processing is usually continued in a packing house. This can be a simple shed, providing shade and running water, or a large-scale, sophisticated, mechanized facility, with conveyor belts, automated sorting and packing stations, walk-in coolers and the like. In mechanized harvesting, processing may also begin as part of the actual harvest process, with initial cleaning and sorting performed by the harvesting machinery (Simson and Straus 2010).

Postharvest handling is the final stage in the process of producing high quality fresh produce. Being able to maintain a level of freshness from the field to the dinner table presents many challenges. Production practices have a tremendous effect on the quality of fruits and vegetables at harvest and on postharvest quality and shelf life. It is well known that some cultivars ship better and have a longer shelf life than others. In addition, environmental factors such as soil type, temperature, frost, and rainy weather at harvest can have an adverse effect on storage life and quality. Management practices can also affect postharvest quality. Produce that has been stressed by too much or too little water, high rates of nitrogen, or mechanical injury (scrapes, bruises, abrasions) is particularly susceptible to postharvest diseases. Food safety also begins in the field, and should be of special concern, since a number of outbreaks of food borne illnesses have been traced to contamination of produce in the field. Harvest should be completed during the coolest time of the day, which is usually in the early morning, and produce should be kept shaded in the field. Handle produce gently. Crops destined for storage should be as free as possible from skin breaks, bruises, spots, rots, decay, and other deterioration. Bruises and other mechanical damage not only affect appearance, but provide entrance to decay organisms as well. The care taken during harvesting is repaid later, because fewer bruises and other injuries mean less disease and enhanced value. Good managers train their pickers so that they select the product at the correct stage of maturity with adequate care. It is worthwhile reducing the amount of hard physical work required in picking fruit and vegetables as far as possible. In recent years conveyors have been introduced for vegetable crops such as lettuce or celery and "cherry pickers" for tree crops. Such as increase the comfort and speed of harvesting and help the pickers to devote more energy to the care of the product (Jobling 2002).

It could be summarized the handling of vegetable and fruits postharvest as follows:

(A) Packing house operations

It is important to minimize mechanical damage by avoiding drops, rough handling and bruising during the different steps of pack house operations. Secondly the pack house operations should be carried out in shaded area. Shade can be created using locally available materials like, shade cloth, woven mats, plastic tarps or a canvas sheet hung from temporary poles. Shade alone can reduce air temperatures surrounding the produce by 8–17 °C. The packing house operations include the following steps:

(i) *Dumping*: The first step of handling is known as dumping. It should be done gently either using water or dry dumping. Wet dumping can be done by immersing the produce in water. It reduces mechanical injury, bruising, abrasions on the fruits, since water is more gentle on produce. The dry dumping is done by soft brushes fitted on the sloped ramp or moving conveyor belts. It will help in removing dust and dirt on the fruits.

(ii) *Pre-sorting*: It is done to remove injured, decayed, misshapen fruits. It will save energy and money because culls will not be handled, cooled, packed or transported. Removing decaying fruits are especially important, because these will limit the spread of infection to other healthy fruits during handling.

(iii) *Washing and cleaning:* Washing with chlorine solution (100–150 ppm) can also be used to control inoculums build up during pack house operations. For best results, the pH of wash solution should be between 6.5 and 7.5

(iv) *Sizing/grading*: Grading can be done manually or by automatic grading lines. Size grading can be done subjectively (visually) with the use of standard size gauges. Round produce units can be easily graded by using sizing rings (Dhatt and Mahajan 2007).

(B) Pre-cooling of horticulture produce

Pre-cooling of the produce soon after their harvest is one of the important components of the cool chain, which ultimately affect the shelf life of the produce. The main purpose of precooling is to immediately remove the field heat from the produce. It could be summarized the method of pre-cooling as follows:

I. *Room cooling*: It is low cost and slow method of cooling. In this method, produce is simply loaded into a cool room and cool air is allowed to circulate among the cartons, sacks, bins or bulk load.

II. *Forced air cooling*: Forced air-cooling is mostly used for wide range of horticultural produce. This is the fastest method of pre-cooling. Forced air-cooling pulls or pushes air through the vents/holes in storage containers. In this method uniform cooling of the produce can be achieved if the stacks of pallet bins are properly aligned. Cooling time depends on (i) the airflow, (ii) the temperature difference between the produce and the cold air and (iii) produce diameter.

III. *Hydrocooling*: The use of cold water is an old and effective cooling method used for quickly cooling a wide range of fruits and vegetables before packaging. For the packed commodities it is less used because of difficulty in the movement of water through the containers and because of high cost involved in water tolerant containers. This method of cooling not only avoids water loss but may even add water to the commodity. The hydrocooler normally used are of two types: shower and immersion type.

IV. *Vacuum cooling*: Vacuum cooling takes place by water evaporation from the product at very low air pressure. In this method, air is pumped out from a larger steel chamber in which the produce is loaded for pre-cooling. Removal of air results in the reduction of pressure of the atmosphere around the produce, which further lowers, the boiling temperature of its water. As the pressure falls, the water boils quickly removing the heat from the produce. Vacuum cooling cause about 1 % produce weight loss (mostly water) for each 6 °C of cooling.

V. *Package icing*: In some commodities, crushed or flaked ice is packed along with produce for fast cooling. However, as the ice comes in contact with the produce, it melts, and the cooling rate slows considerably. The ice keeps a high relative humidity around the product. Package ice may be finely crushed ice, flake ice or slurry of ice. Liquid icing distributes the ice throughout the container, achieving better contact with the product. Packaged icing can be used only with water tolerant, non-chilling sensitive products and with water tolerant packages such as waxed fiberboard, plastic or wood (Dhatt and Mahajan 2007).

(C) Postharvest treatments

Fresh fruits are living tissues subject to continuous change after harvest. Some changes are desirable from consumer point of view but most are not. Postharvest changes in fresh fruit cannot be stopped, but these can be slowed down within certain limits to enhance the shelf life of fruits. The post-harvest treatments play an important role in extending the storage and marketable life of horticultural perishables. The most important postharvest treatments include:

(i) ***Washing with chlorine solution***: Chlorine treatment (100–150 ppm available chlorine) can be used in wash water to help control inoculums build up during packing operations. Maintain pH of wash water between 6.5 and 7.5 for best results.

(ii) ***Ethylene inhibitors/Growth regulator/fungicide treatments***: 1-MCP (1-methyl cyclopropene), AVG (Amenoethoxyvinyl gycine), silver nitrate, silver thiosulfate, cycloheximide, benzothiadiazole etc. are some of the chemicals which inhibit ethylene production and/or action during ripening and storage of fruits. The growth regulators or fungicidal application such as GA_3 can be effectively used to extend/enhance the shelf life of fruits.

(iii) ***Calcium application***: The post-harvest application of $CaCl_2$ or Ca $(NO_3)_2$ play an important role in enhancing the storage and marketable life of fruits by maintaining their firmness and quality. Calcium application delays aging or ripening, reduces postharvest decay, controls the development of many physiological disorders and increases the calcium content, thus improving their nutritional value. The post-harvest application of $CaCl_2$ (2–4 %) or Ca $(NO_3)_2$ for 5–10 min dip extend the storage life of pear up to 2 months, plum up to 4 weeks and apple up to 6 months at 0–2 °C with excellent color and quality. Calcium infiltration reduces chilling injury and increase disease resistance in stored fruit.

(iv) ***Thermal treatments***: Thermal treatments included (a) hot water treatment: Fruits may be dipped in hot water before marketing or storage to control various post-harvest diseases and improving peel color of the fruit (Table 3). (b) Vapor heat treatment (VHT): This treatment proved very effective in controlling infection of fruit flies in fruits after harvest. The boxes are stacked in a room, which are heated and humidified by injection of steam.

Table 3 Hot water treatments for different fruits

Commodity	Pathogens	Temperature (°C)	Time (min)
Apple	*Gloeosporium* sp.	45	10
	Penicillium expansum		
Grapefruit	*Phytophthora citrophthora*	48	3
Lemon	*Penicillium digitatum*	52	5–10
	Phytophthora sp.		
Mango	*Collectotrichum gloeosporioides*	52	5
Orange	*Diplodia* sp.	53	5
	Phomopsis sp.		
	Phytophthora sp.		
Papaya	Fungi	48	20
Peach	*Monolinia fructicola*	52	2.5
	Rhizopus stolonifer		

Adapted from Kitinoja and Gorny (1998)

The temperature and exposure time are adjusted to kill all stages of insects (egg, larva, pupa and adult), but fruit should not be damaged. A recommended treatment for citrus, mangoes, papaya and pineapple is 43 °C in saturated air for 8 h and then holding the temperature for further 6 h. VHT is mandatory for export of mangoes.

(v) ***Fumigation***: The fumigation of SO_2 is successfully used for controlling post-harvest diseases of grapes. This is achieved by placing the boxes of fruit in a gas tight room and introducing the gas from a cylinder to the appropriate concentration. However, special sodium metabisulphite pads are also available which can be packed into individual boxes of a fruit to give a slow release of SO_2. The primary function of treatment is to control the *Botrytis Cinerea*. The SO_2 fumigation is also used to prevent discoloration of skin of litchis.

(vi) ***Irradiation***: Ionizing radiation can be applied to fresh fruits and vegetables to control micro-organisms and inhibit or prevent cell reproduction and some chemical changes. It can be applied by exposing the crop to radiations from radioisotopes (normally in the form of gamma-rays measured in Grays (Gy), where 1 Gy = 100 rads.

(vii) ***Waxing***: Waxing of fruits or vegetables is a common post-harvest practice. Food grade waxes are used to replace some of the natural waxes removed during harvesting and sorting operations and can help reduce water loss during handling and marketing. It also helps in sealing tiny injuries and scratches on surface of fruits and vegetables. It improves cosmetic appearance and prolongs the storage life of fruits and vegetables. The wax coating must be allowed to dry thoroughly before packing (Dhatt and Mahajan 2007).

It could be concluded that, postharvest handling is the stage of crop production immediately following harvest, including cooling, cleaning, sorting and packing. The instant a crop is removed from the ground, or separated from its parent plant, it begins to deteriorate. Postharvest treatment largely determines final quality, whether a crop is sold for fresh consumption, or used as an ingredient in a processed food product. The most important goals of postharvest handling are keeping the product cool, to avoid moisture loss and slow down undesirable chemical changes, and avoiding physical damage such as bruising, to delay spoilage.

2.4 Fruits and Vegetables Quality

Quality cannot be improved after harvest, only maintained; therefore it is important to harvest fruits, vegetables, and flowers at the proper stage and size and at peak quality. Quality is a complex perception of many attributes that are simultaneously evaluated by the consumer either objectively or subjectively. The brain processes the information received by sight, smell, and touch and instantly compares or associates it with past experiences or with textures, aromas, and flavors stored in its memory. For example, just by looking at the color, the consumer knows that a fruit is unripe and that it does not have

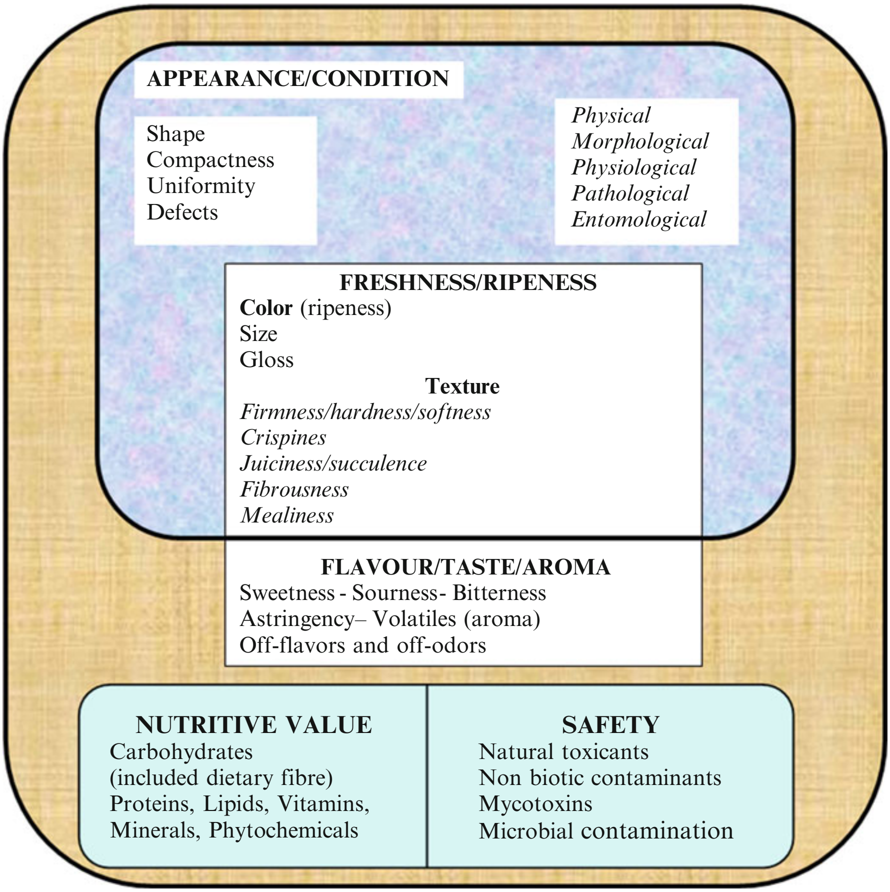

Fig. 3 Consumer perception of fruit and vegetable quality from nutritive value, safety to the physical properties of fruit and vegetable (Adapted from FAO 2004)

good flavor, texture or aroma. If color is not enough to evaluate ripeness, he/she uses the hands to judge firmness or other perceptible characteristics. The aroma is a less used parameter except in those cases where it is directly associated to ripeness like in melon or pineapple. This comparative process does not take place when the consumer sees for the first time an exotic fruit whose characteristics are unknown. Fruits and vegetables are consumed mainly for their nutritive value as well as for the variety of shapes, colors and flavors that make them attractive for food preparation. When they are consumed raw or with very little preparation, the consumer's main concern is that they must be free of biotic or non-biotic contaminants that may affect health (Figs. 3 and 4; FAO 2004).

The following tips can be followed for storage of high quality horticultural produce:

- Store only high quality produce, free of damage, decay and of proper maturity (not over-ripe or under-mature).
- Know the requirements for the commodities you want to put into storage, and follow recommendations for proper temperature, relative humidity and ventilation.

Fig. 4 Quality control of fruits is a constant challenge in food and life science industry with respect to contaminations and frauds like wrong labeling of the product type or the type and origin of ingredients. There are a lot of quality papmeters for fruits such as Brix analysis and juice quality (Photos by M. Fári in Cesena, Italy)

- Avoid lower than recommended temperatures in storage, because many commodities are susceptible to damage from freezing or chilling.
- Do not over load storage rooms or stack containers closely
- Provide adequate ventilation in the storage room.
- Keep storage rooms clean.
- Storage facilities should be protected from rodents by keeping the immediate outdoor area clean, and free from trash and weeds.
- Containers must be well ventilated and strong enough to with stand stacking. Do not stack containers beyond their stacking strength.
- Monitor temperature in the storage room by placing thermometers at different locations.
- Don’t store onion or garlic in high humidity environments.
- Avoid storing ethylene sensitive commodities with those that produce ethylene.
- Avoid storing produce known for emitting strong odors (apples, garlic, onions, turnips, cabbages, and potatoes) with odor-absorbing commodities.
- Inspect stored produce regularly for signs of injury, water loss, damage and disease.
- Remove damaged or diseased produce to prevent the spread of problems (Dhatt and Mahajan 2007).

Fruit and vegetables are important sources of a wide range of vital micronutrients, phytochemicals and fibre, and there is now strong evidence that fruit and vegetable consumption can prevent a number of chronic non-communicable diseases, including cardiovascular diseases (CVD), diabetes, obesity, cancer and respiratory conditions (Robertson et al. 2004). Phytochemicals are bioactive non-nutrient plant compounds found in fruit, vegetables, grains and other plant foods that have been linked to reductions in the risk of major chronic diseases. They are almost ubiquitous in plant-derived foods and inherently have more subtle effects than nutrients. Phytochemicals can accumulate in relatively high amounts in plants and appear to have a myriad of supplemental roles in a plant's life cycle. Although these secondary metabolites account for the bioactive chemicals responsible for medicinal actions in humans, they are actually produced to provide the plant itself with unique survival or adaptive strategies. As sessile organisms, plants rely on the production of secondary compounds for defence, protection, cell-to-cell signaling and as attractants for pollinators. Phytochemicals can act as a 'shield' between plant tissues and the environment, thereby providing protection against abiotic stresses such as UV-B irradiation, temperature extremes, low water potential or mineral deficiency. One of the most versatile groups of phytochemicals in this regard, the anthocyanins, protect chloroplasts from photodegradation by absorbing high-energy quanta, while also scavenging free radicals and reactive oxygen species (Gould 2004). Flavonols, as well as providing protection against the damaging effects of UV-B, are also involved in promoting the growth of pollen tubes in the style to facilitate fertilization of the ovule. In addition, lignans, terpenoids and isoflavonoids play important defence roles against pathogen and insect attack (Table 4; Jaganath and Crozier 2008).

It could be concluded that, fruit and vegetables are important sources of a wide range of vital micronutrients, phytochemicals and fibre, and there is now strong evidence that fruit and vegetable consumption can prevent a number of chronic non-communicable diseases, including cardiovascular diseases, diabetes, obesity, cancer and respiratory conditions.

2.5 Preparation for the Fresh Market

After harvest, fruits and vegetables need to be prepared for sale. This can be undertaken on the farm or at the level of retail, wholesale or supermarket chain. Regardless of the destination, preparation for the fresh market comprises four basic key operations: (i) removal of unmarketable material, (ii) sorting by maturity and/or size, (iii) grading and (iv) packaging. Any working arrangement that reduces handling will lead to lower costs and will assist in reducing quality losses. Market preparation is therefore preferably carried out in the field. However, this is only really possible with tender or perishable products or small volumes for nearby markets. Products need to be transported to a packinghouse or packing shed for large operations, for distant or demanding markets or for special operations like washing, brushing, waxing, controlled ripening, refrigeration, storage or any specific type of treatment or

Table 4 List of fruit and vegetable (FAV) phytochemicals and major plant food sources

Phytochemicals		Major plant food sources
Carotenoids		Yellow, orange, red and green FAV. As a rule of thumb, the greater the intensity of the color of the fruit or vegetable, the more carotenoids it contains
	α-carotene, β-carotene, β-cryptoxanthin	Carrots, sweet potatoes, winter squash, pumpkin, papaya, mango, cantaloupe, oranges, broccoli, spinach, lettuce.
	Zeaxantine, Lutein	Green leafy vegetables such as spinach and kale.
	Lycopene	Tomatoes, watermelons, pink grapefruits, apricot, pink guavas.
Phenolics		
Phenolic acid		Seeds, berries, fruit and leaves of plants.
		Blueberries and other wild berries, dark plum, cherry, green and black teas, broccoli florets, olive oil.
Hydroxybenzoic acid	Gallic	Gallnuts, sumach, witch hazel, tea leaves, oak bark.
	Protocatechuic	Onion, roselle (*Hibiscus sabdariffa*)
	Vanillic	Vanilla.
	Syringic	Grapes.
Hydroxycinnamic acid	*p*-Coumaric	Wide variety of edible plants such as peanuts, tomatoes, carrots, garlic.
	Caffeic	Coffee beans.
	Ferulic	Seeds of plants such as brown rice, whole wheat and oats, coffee, apple, artichoke, peanut, orange, pineapple.
	Sinapic	All plants (lignin precursor).
Flavonoids		All citrus fruits, berries, onions, parsley, legumes, green tea, red grapes, red wine, dark chocolate.
Flavonols	Quercetin	Apples, green and black tea, onions (higher concentrations of quercetin occur in the outermost rings), red wine, red grapes, broccoli, leafy green vegetables, cherries and several wild berries (raspberries, bog whortleberries, cranberries, sweet rowan, rowanberries, sea buckthorn berries), prickly pear.
	Kaempferol	Green and black tea.
	Myricetin, Galangin, Fisetin	Grapes, berries, fruits, onions, broccoli, turnip, watercress.
Flavones	Apigenin, Chrysin, Luteolin	Celery, pepper, rutabagas, spinach.

(continued)

Table 4 (continued)

Phytochemicals		Major plant food sources
Catechins	Catechin, Epicatechin, Epigallocatechin	Apricot, berries, broad beans, peas, white tea, green tea, black tea, grapes and wine, oolong tea, peach, plums, strawberries, cocoa.
Flavanones	Eriodictyol, Hesperetin, Naringenin	Citrus fruits.
Anthocyanidins	Cyanidin, Pelargonidin, Peonidin	Red and blue FAV, blackberries, blueberries, hawthorn, raspberries, cranberries, elderberries, loganberries, strawberries and other berries, apples, cherry, plums (the highest concentrations of cyanidin are found in the skin of the fruit).
	Malvidin	Red and blue FAV (primarily responsible for the color of red wine).
	Delphinidin	Grape, blueberries, cranberries
Isoflavonoids	Genistein, Daidzein, Glycitein, Formonentin	Soybeans and soy products (tofu and textured vegetable protein) and, in small quantities, several other seeds.
Stilbenes	Resveratrol	Red grapes and red wine.
Coumarins		Notably woodruff and at lower levels in licorice.
Tannins		Tea, red grapes, red wine, pomegranates (punicalagins), persimmons, berries (cranberries, strawberries, blueberries).
Lignans		Flaxseeds (linseeds) and pulses, whole grain cereals, carrot, squash, sweet potatoes, green pepper, broccoli, garlic, asparagus, leek.
Alkaloids		Potatoes, tomatoes, cocoa, kola nut, guarana berries, tea plant, mushrooms.
Nitrogen containing compounds	Polyamines (spermidine, spermine, putrescine)	Fruits (with exception of berries) and fruity vegetables (tomatoes, eggplants), potatoes, cereal germ.
Organosulfur compounds	Isothiocyanates, Indoles	Cruciferous vegetables, broccoli, cabbage, cauliflower, kale, turnips, collards, brussels sprouts, radish, turnip, watercress
	Allylic sulfur compounds	Garlic.
Vitamin C		Citrus fruits (orange, lemon, grapefruit, lime), strawberries, cranberries, blackcurrants, papaya, kiwifruit, tomatoes, potatoes, broccoli, brussels sprouts, cauliflower, spinach, cantaloupe, red peppers.

(continued)

Table 4 (continued)

Phytochemicals		Major plant food sources
Vitamins B	Thiamine (B_1)	Green peas, spinach, navy beans, nuts, pinto beans, soybeans, whole-grain cereals, breads, pulses.
	Riboflavin (B_2)	Leafy green vegetables, legumes, almonds.
	Pantothenic acid (B_5)	High amounts in whole-grain cereals and pulses.
	Pyridoxine (B_6)	Lima beans, peanuts, whole-grain cereals, avocado, bananas, dragon fruit.
	Cyanocobalamin ($B1_2$)	Seaweeds (nori), barley grass.
Folic acid		Leafy vegetables such as spinach and turnip greens, dried beans and peas, sunflower seeds and certain other FAV.
Vitamin E (tocopherol)		Vegetable oils such as palm, olive, sunflower, soybean and corn, nuts, sunflower seeds, seabuckthorn berries, kiwi fruit, wheat germ.

Adapted from Jaganath and Crozier (2008)

packaging. These two systems (field vs. packinghouse preparation) are not mutually exclusive. In many cases partial field preparation is completed later in the packing shed. Because it is a waste of time and money to handle unmarketable units, primary selection of fruits and vegetables is always carried out in the field where products with severe defects, injuries or diseases are removed. Field preparation of lettuce is an example where a team of three workers cut, prepare and pack. For distant markets, boxes prepared in the field are delivered to packinghouses for palletizing, pre-cooling, and sometimes cold storage before shipping. Mobile packing sheds provide an alternative for handling large volumes in limited time. Harvest crews feed a mobile grading and packing line. On completion of loading, the consignment is shipped to the destination market. In mechanized harvesting, the product is transported to the packinghouse where it is prepared for the market. In many cases, harvest crews make use of an inspection line for primary selection in the field (Simson and Straus 2010).

Preparation and packing operations should be designed to minimize the time between harvest and delivery of the packaged product. Delays frequently occur in the reception area; therefore the produce should be protected from the sun as much as possible. Produce is normally weighed or counted before entering the plant and, in some cases; samples for quality analysis are taken. Records should be kept, particularly when providing a service to other producers. Preparation for the fresh market starts with dumping onto packinghouse feeding lines. Dumping may be dry or in water. In both cases it is important to have drop decelerators to minimize injury as well as to control the flow of produce. Water dipping produces less bruising and can be used to move free-floating fruits; however, not all products tolerate wetting. Products with a specific density lower than water will float. Salts e.g. sodium sulphate are diluted in the water to improve the flotation of other products. Water dipping through washing helps to remove most dirt from the field. For thorough cleaning, more washing and brushing are required. Water rinsing allows produce to maintain cleanliness and be free of soil, pesticides, plant debris and rotting parts. However, in some cases this is not possible because of insufficient water. If recycled water is used, it needs to be filtered and the settled dirt removed. Chlorination of dumping and washing waters with a concentration of 50–200 ppm active chlorine eliminates fungi spores and bacteria on the surface of diseased fruits, which prevents the contamination of healthy fruit. Bruising should be avoided because it creates the entry for infection by decay organisms. At depths greater than 30 cm and for periods of immersion longer than 3 min, water tends to penetrate inside fruits, particularly those that are hollow, for example, peppers. Water temperature also contributes to infiltration. It is recommended that the temperature of fruit is at least 5 °C lower than that of water (FAO 2004).

It could be concluded that, fruits and vegetables need to be prepared for sale after harvest and this can be undertaken on the farm or at the level of retail, wholesale or supermarket chain. Regardless of the destination, preparation for the fresh market comprises four basic key operations i.e., removal of unmarketable material, sorting by maturity and/or size, grading and packaging. Preparation and packing operations should be designed to minimize the time between harvest and delivery of the packaged product.

2.5.1 Packaging

Packaging is the act of putting the produce inside a container along with packing materials to prevent movement and to cushion the produce such as plastic or moulded pulp trays, inserts, cushioning pads, etc. and to protect it i.e., plastic films, waxed liners, etc. Packaging must satisfy three basic objectives: (i) contain product and facilitate handling and marketing by standardizing the number of units or weight inside the package. (ii) Protect product from injuries (impact, compression, abrasion and wounds) and adverse environmental conditions (temperature, relative humidity) during transport, storage and marketing. (iii) Provide information to buyers, such as variety, weight, number of units, selection or quality grade, producer's name, country and area of origin. Frequently included are recipes, nutritional value, bar codes or any other relevant information on traceability. A well-designed package needs to be adapted to the conditions or specific treatments required for the product (Fig. 5). If hydro-cooling or ice-cooling is required, the package must tolerate wetting without losing strength. For a product with a high respiratory rate, the packaging should have sufficiently large openings to allow good gas exchange. When produce dehydrates easily, the packaging should be designed to provide a good barrier against water loss, etc. Semi-permeable materials make it possible to generate special atmospheres inside packages. This helps in maintaining produce freshness. There are three types of packaging: (1) consumer units or prepackaging, (2) transport packaging and (3) unit load packaging or pallets (FAO 2004).

Fig. 5 Some vegetables in the final package in the super market and ready for consumers (Photos by M. Fári in China)

Fresh fruits and vegetables are generally packed in bamboo baskets, plastic crates, plastic bags, or nylon sacks for transportation, in many developing countries. Often, they are transported in an unpackaged form. After harvest, fresh fruits and vegetables are generally transported from the farm to either a packing house or distribution center. Farmers sell their produce either in fresh markets or in wholesale markets. At the retail level, fresh produce is sold in an unpackaged form, or is tied in bundles. This type of market handling of fresh produce greatly reduces its shelf life if it is not sold quickly. The application of proper postharvest technologies, would, however, extend postharvest shelf life, retain fresh quality and reduce losses. Packaging plays a very important role in protecting fresh produce:

- It provides protection from dust;
- It reduces microbial contamination from the surrounding environment and from consumer contact;
- It helps to maintain the freshness of produce;
- It extends the postharvest shelf life;
- It increases the sale of fresh produce.

The following are among the more important general requirements and functions of food packaging materials/containers: (a) they must be non-toxic and compatible with the specific foods; (b) sanitary protection; (c) moisture and fat protection; (d) gas and odor protection; (e) light protection; (f) resistance to impact; (g) transparency; (h) ease of opening; (i) pouring features; (j) reseal features; (k) ease of disposal; (l) size, shape, weight limitations; (m) appearance, printability; (n) low cost and (o) special features (Simson and Straus 2010).

Films and foils have different values for moisture and gas permeability, strength, elasticity, inflammability and resistance to insect penetration and many of these characteristics depend upon the film's thickness. Important characteristics of the types of films and foils commonly used in food packaging are given in Table 5.

The development of packaging which is suited to the handling of fresh produce necessitates an understanding of the physiological characteristics of the produce. Fruits and vegetables may be characterized as being either climacteric or non-climacteric, depending on their respiratory pattern (Fig. 6):

- *Non-climacteric fruit* ripen only while still attached to the parent plant. Their eating quality suffers if they are harvested before they are fully ripe because their sugar and acid contents do not increase further. Their respiration rate gradually declines during growth and after harvesting. Maturation and ripening are a gradual process. Examples of non-climacteric fruit include: cherries, cucumbers, grapes, lemons and pineapples (Sirivatanapa 2006).
- *Climacteric fruit* can be harvested when mature but before the onset of ripening. These fruits may undergo either natural or artificial ripening. The onset of ripening is accompanied by a rapid rise in respiration rate, generally referred to as the respiratory climacteric. After the climacteric, the respiration rate slows down as the fruit ripens and develops good eating quality. Examples of climacteric fruit include: apples, bananas, melons, papaya and tomatoes (Sirivatanapa 2006).

Table 5 Properties of packaging films

Material	Properties
Paper	Strength; rigidity; opacity; printability.
Aluminum foil	Negligible permeability to water-vapor, gases and odors; grease proof, opacity and brilliant appearance; dimensional stability; dead folding characteristics.
Cellulose film (coated)	Strength; attractive appearance; low permeability to water vapor (depending on the type of coating used), gases, odors and greases; printability.
Polythene	Durability; heat-sealability; low permeability to water-vapor; good chemical resistance; good low-temperature performance.
Rubber hydrochloride	Heat-sealability;. low permeability to water vapor, gases, odors and greases; chemical resistance.
Cellulose acetate	Strength; rigidity; glossy appearance; printability; dimensional stability.
Vinylidene chloride	Low permeability to water vapor, gases, copolymer odors and greases; chemical resistance; heat-sealability.
Polyvinyl chloride	Resistance to chemicals, oils and greases; heat-sealability.
Polyethylene terephtalate	Strength; durability; dimensional stability; low permeability to gases, odors and greases.

Adapted from Simson and Straus (2010)

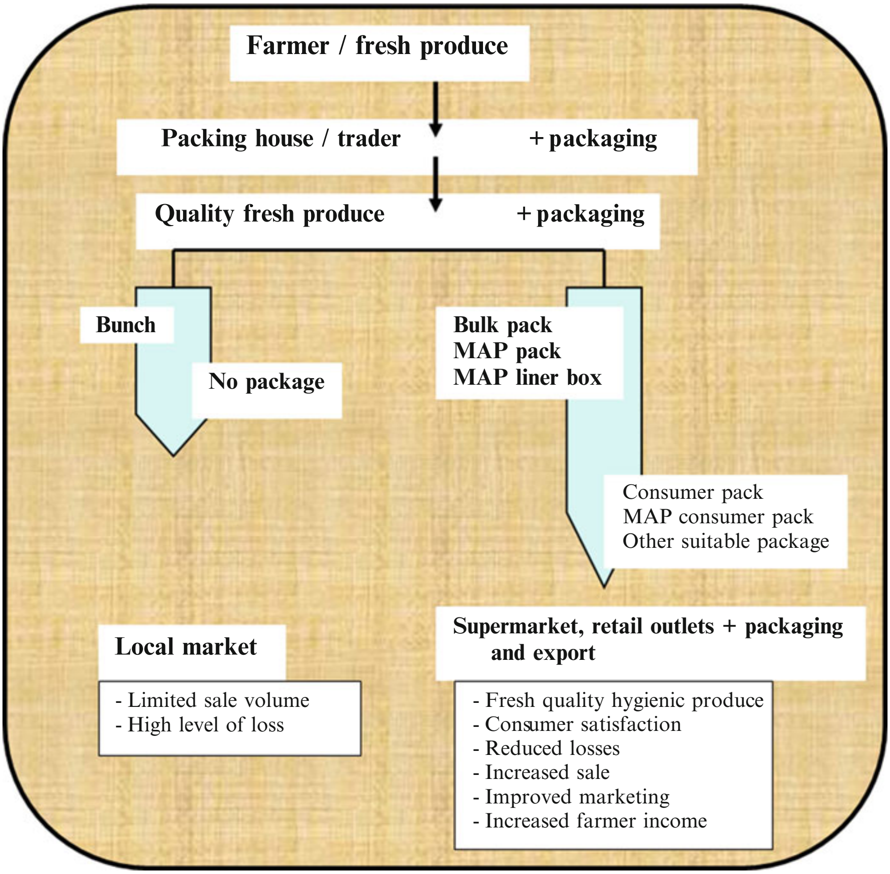

Fig. 6 Packaging and transportation of fruits and vegetables to local or supermarket. MAP is modified atmospheric package (Adapted from Sirivatanapa 2006)

Therefore, it could be concluded that, packaging is the act of putting the produce inside a container along with packing materials to prevent movement. Packaging must satisfy three basic objectives: (i) contain product and facilitate handling and marketing by standardizing the number of units or weight inside the package. (ii) Protect product from injuries and adverse environmental conditions during transport, storage and marketing. (iii) Provide information to buyers, such as variety, weight, number of units, selection or quality grade, producer's name, country and area of origin.

2.6 Storage Process

Without doubt, the ability to store harvested plant organs for extended periods of time has played a critical role in the development of agriculture. Simple baskets were used as early as 7000 BC to gather and store food until consumed. Advances in technology such as fired clay pottery would enable primitive societies to store crops in concealed environments, creating a simple modified atmosphere. Middle Eastern artisans specialized in making pottery of numerous shapes and sizes for varied usage (ca. 4500 BC), while pre-Neolithic, Middle Eastern societies held grain in underground pits 9,000–11,000 years ago. Ancient Egyptians and Samarians are thought to have stored some of their crops in sealed limestone crypts to prolong storage life about 2500 BC. Pits were and are still used by primitive societies for storing various types of fruits and vegetables. Silos for long-term grain storage were used by the Romans and continued to be popular until well into the nineteenth century. Ancient (and modern) people often dried fruits such as apples, apricots, figs, and grapes to prolong the storage longevity of these perishable crops (De Long and Prange 2003).

In temperate areas most fruit and vegetable production is seasonal. In contrast, cultivation and harvest periods are much longer in tropical and subtropical areas. Demand is year round and it is normal practice to use storage in order to ensure continuity of supply. Storage is also used as a strategy for achieving higher returns. Produce can be held temporarily to overcome gluts thus limiting price falls or to address shortage periods when prices are high. Storage time depends on the intrinsic characteristics and perishability of the product. Shelf life ranges from short periods for products such as raspberries and other berries to long periods for products such as onions, potatoes, garlic and pumpkins. Storage conditions also depend on specific product characteristics. For example, leafy vegetables tolerate temperatures close to 0 °C, while most tropical fruits cannot tolerate exposure to temperatures below 10 °C. In order to optimize storage conditions, only one crop should be stored in a room unless it is for a short period of time. Using the same storage area for different products can result in product damage because of incompatibility of temperature and relative humidity conditions, chilling and ethylene sensitivity, odor contamination and other problems affecting shelf life and quality. Generally, storage facilities are linked or integrated to packinghouses or other areas where there is a concentration of produce. However, often produce can be stored on farm, either naturally or in specifically designed facilities. Location and design have an impact on system operations and efficiency even when mechanical refrigeration is used. Climate is an important factor for the location of the storage facility. For example, altitude reduces temperature by 10 °C for every 1,000 m of elevation. It also increases the overall efficiency of refrigeration equipment by facilitating heat exchange with ambient temperature, thereby reducing energy costs. Shading, particularly of loading and unloading areas, reduces thermal differences between field and storage temperatures (Tables 6 and 7; FAO 2004).

Therefore, it could be concluded that, the ability to store harvested plant organs for extended periods of time has played a critical role in the development of agriculture. Ancient Egyptians and Samarians are thought to have stored some of their crops in

Table 6 Maximum storage life (days) in normal atmosphere storage (NA), controlled atmosphere (CA) and low-pressure storage (LP)

Commodity	Maximum storage time (days)		
	Normal atmosphere storage	Controlled atmosphere	Low-pressure storage
Apple (various)	200	300	300
Asparagus	14–21	Slight benefit – off-odors	28–42
Avocado (Lula)	30	42–60	102
Banana	14–21	42–56	150
Carnation (flower)	21–42	No benefit	140
Cherry (sweet)	14–21	28–35	56–70
Cucumber	9–14	14+ (slight benefit)	49
Green pepper	14–21	No benefit	50
Lime (Persian)	14–28	Juice loss, peel thickens	90
Mango (Haden)	14–21	No benefit	42
Mushroom	5	6	21
Papaya (Solo)	12	12+ (slight benefit)	28
Pear (Bartlett)	60	100	200
Protea (flower)	< 7	No benefit	30
Rose (flower)	7–14	No benefit	42
Spinach	10–14	Slight benefit	50
Strawberry	7	7+ (off-flavor)	21
Tomato (mature-green)	7–21	42	84

Adapted from Burg (2004)
To simplify comparisons between results obtained at atmospheric and sub-atmospheric pressures, the O_2, CO_2 and NH_3 concentrations are expressed as percent [O_2], [CO_2] and [NH_3], where 2 % [O_2] refers to an O_2 partial pressure of 0.02 atm. According to international conventions, the pressure-unit conversion constants are: 1 atm (standard) = 101.33 kPa (kilopascals) = 1013.3 mbar (millibar) = 760 mm Hg (mm mercury) = 760 Torr = 14.696 psi (lb in^{-2})

Table 7 The most important different methods of cooling

Method	Details
Precooling	Precooling is the rapid reduction of field temperature prior to processing, storage, or refrigerated transport. Generally it is a separate operation requiring special facilities, but complementary to cold storage. As deterioration is proportional to the time produce is exposed to high temperatures, precooling is beneficial even when produce is later returned to ambient conditions. It is critical in maintaining the quality of fruits and vegetables and forms part of the "cold chain" to maximize postharvest life.
Room cooling	Room cooling is the most widely used system and is based on the product's exposure to cold air inside a refrigerated room. It is simple to operate as the product is cooled and stored in the same room. However, the slow removal of heat makes this system unsuitable for highly perishable commodities because at least 24 h is needed to reach the required storage temperature. Almost all other crops are suitable for this type of cooling; however, it is mainly used for potatoes, onions, garlic, citrus, etc.
Forced-air cooling	Cold air is forced to pass through produce by means of a pressure gradient across packages. Cooling is four to ten times more rapid than room cooling and its rate depends on airflow and the individual volume of produce.
Hydrocooling	The refrigerating medium for hydrocooling is cold water. Because of its higher capacity to absorb heat, it is faster than forced-air cooling. Hydrocooling can be achieved by immersion or through means of a chilled water shower. In the latter case produce must be arranged in thin layers for uniform cooling. This system cannot be used for crops that do not tolerate wetting, chlorine and water infiltration. Tomatoes, asparagus and many other vegetables are hydrocooled commercially. Chlorination of water (150–200 ppm) is important to prevent the accumulation of pathogens.
Ice cooling	Ice cooling is probably one of the oldest methods used to reduce field temperature. It is most commonly used for individual packages – crushed ice is placed on top of the produce before the package is closed. Ice layers may also be interspersed with produce. As it melts, cold water cools the lower layers of produce. Liquid icing is another system where a mix of water and crushed ice (40 % water + 60 % ice + 0.1 % salt) is injected into open containers so that a big ice block is formed. The main disadvantage of ice cooling is that it is limited to ice-tolerant crops. It also increases costs because of the heavier weight for transportation and the need for oversized packages. Another disadvantage is that as water melts, storage areas, containers and shelves become wet.
Evaporative	This is one of the simplest cooling systems. It involves forcing dry air through wet products. Heat is absorbed from the product as water evaporates. This method has a low energy cost but cooling efficiency is limited by the capacity of air to absorb humidity.
Vacuum cooling	Vacuum cooling is one of the more rapid cooling systems; however, cooling is accomplished at very low pressures. At a normal pressure of 760 mmHg, water evaporates at 100 °C, but it evaporates at 1 °C if pressure is reduced to 5 mmHg. Produce is placed in sealed containers where vacuum cooling is performed. This system produces about 1 % product weight loss for each 5 °C of temperature reduction. Modern vacuum coolers add water as a fine spray in the form of pressure drops. Similar to the evaporation method, this system is in general appropriate for leafy vegetables because of their high surface-to-mass ratio.

Adapted from FAO (2004)

sealed limestone crypts to prolong storage life about 2500 BC. In order to optimize storage conditions, only one crop should be stored in a room unless it is for a short period of time. Using the same storage area for different products can result in product damage because of incompatibility of temperature and relative humidity conditions, chilling and ethylene sensitivity, odor contamination and other problems affecting shelf life and quality.

2.7 Storage Systems

The marketable life of most fresh vegetables can be extended by prompt storage in an environment that maintains product quality. The desired environment can be obtained in facilities where temperature, air circulation, relative humidity, and sometimes atmosphere composition can be controlled. Storage rooms can be grouped accordingly as those requiring refrigeration and those that do not. Storage rooms and methods not requiring refrigeration include: *in situ*, sand, coir, pits, clamps, windbreaks, cellars, barns, evaporative cooling, and night ventilation as follows:

1. ***In situ:***
   This method of storing fruits and vegetables involves delaying the harvest until the crop is required. It can be used in some cases with root crops, such as cassava, but means that the land on which the crop was grown will remain occupied and a new crop cannot be planted. In colder climates, the crop may be exposed to freezing and chilling injury.
2. ***Sand or coir:***
   This storage technique is used in countries like India to store potatoes for longer periods of time, which involves covering the commodity under ground with sand.
3. ***Pits or trenches:***
   Pits are dug at the edges of the field where the crop has been grown. Usually pits are placed at the highest point in the field, especially in regions of high rainfall. The pit or trench is lined with straw or other organic material and filled with the crop being stored, then covered with a layer of organic material followed by a layer of soil. Holes are created with straw at the top to allow for air ventilation, as lack of ventilation may cause problems with rotting of the crop.
4. ***Clamps:***
   This has been a traditional method for storing potatoes in some parts of the world, such as Great Britain. A common design uses an area of land at the side of the field. The width of the clamp is about 1–2.5 m. The dimensions are marked out and the potatoes piled on the ground in an elongated conical heap. Sometimes straw is laid on the soil before the potatoes. The central height of the heap depends

on its angle of repose, which is about one third the width of the clump. At the top, straw is bent over the ridge so that rain will tend to run off the structure. Straw thickness should be from 15 to 25 cm when compressed. After 2 weeks, the clamp is covered with soil to a depth of 15–20 cm, but this may vary depending on the climate.

5. ***Windbreaks:***
   Windbreaks are constructed by driving wooden stakes into the ground in two parallel rows about 1 m apart. A wooden platform is built between the stakes about 30 cm from the ground, often made from wooden boxes. Chicken wire is affixed between the stakes and across both ends of the windbreak. This method is used in Britain to store onions.
6. ***Cellars:***
   These underground or partly underground rooms are often beneath a house. This location has good insulation, providing cooling in warm ambient conditions and protection from excessively low temperatures in cold climates. Cellars have traditionally been used at domestic scale in Britain to store apples, cabbages, onions, and potatoes during winter.
7. ***Barns:***
   A bam is a farm building for sheltering, processing, and storing agricultural products, animals, and implements. Although there is no precise scale or measure for the type or size of the building, the term bam is usually reserved for the largest or most important structure on any particular farm. Smaller or minor agricultural buildings are often labeled sheds or outbuildings and are normally used to house smaller implements or activities.
8. ***Evaporative cooling:***
   When water evaporates from the liquid phase into the vapor phase energy is required. This principle can be used to cool stores by first passing the air introduced into the storage room through a pad of water. The degree of cooling depends on the original humidity of the air and the efficiency of the evaporating surface. If the ambient air has low humidity and is humidified to around 100 % RH, then a large reduction in temperature will be achieved. This can provide cool moist conditions during storage.
9. ***Night ventilation:***
   In hot climates, the variation between day and night temperatures can be used to keep stores cool. The storage room should be well insulated when the crop is placed inside. A fan is built into the store room, which is switched on when the outside temperature at night becomes lower than the temperature within. The fan switches off when the temperatures equalize. The fan is controlled by a differential thermostat, which constantly compares the outside air temperature with the internal storage temperature. This method is used to store bulk onions (Simson and Straus 2010).

There are many ways of storing a product. The length of storage time can be longer in specifically designed structures. With refrigeration and controlled atmospheres, storage periods can be even longer. The technology utilized depends on whether the benefits i.e., higher prices outweigh the costs. The most important storage systems are as follows:

(I) Natural or field storage:

This is the most rudimentary system and is still in use for many crops such as roots such as carrots, sweet potatoes, and cassava and tubers (potatoes). Crops are left in the soil until preparation for the market. This is similar to the way citrus and some other fruits are left on the tree.

(II) Natural ventilation:

Amongst the wide range of storage systems, natural ventilation is the simplest. It takes advantage of the natural airflow around the product to remove the heat and humidity generated by respiration. Structures that provide some form of protection from the external environment and gaps for ventilation can be used. Produce is placed in bulk, bags, boxes, bins, pallets, etc. Although natural storage is widely practiced, it leaves products exposed to pests and diseases as well as to adverse weather conditions that can have a detrimental effect on quality. Although simple, some key concepts must be taken into account for the efficient operation of this system:

- Differences in internal temperature and relative humidity conditions compared with external conditions need to be minimal. This system can only be used with crops that store well under natural conditions such as potatoes, onions, sweet potatoes, garlic and pumpkins.
- Openings need to be wide for adequate ventilation and they should be fitted with screens to keep out animals, rodents and pests.
- As with fluids, air follows the path of least resistance. If produce is stored in a compact mass, air will circulate to remove heat and gases that have accumulated as a result of respiration. Efficient ventilation requires adequate space; however, this reduces storage capacity.
- Hot and humid air rises within the storage facility. If no ventilation gaps exist, hot and humid areas build up, which in turn affect the quality of stored goods and create ideal conditions for the development of disease.

(III) Forced-air ventilation:

Heat and gas exchange can be improved provided air is forced to pass through the stored produce. This system allows for more efficient utilization of space for bulk storage. Air conducts run under a perforated floor and air is forced through the produce. As air follows the path of least resistance, loading patterns as well as fan capacity and conduct dimensions should be carefully calculated to ensure that there is uniform distribution of air throughout the stored produce.

(IV) Refrigeration:

Refrigeration is the most widely used method for extending the postharvest life of fruits and vegetables and temperature control is one of the main tools for extending postharvest life. Low temperatures slow product metabolism and the activity of microorganisms responsible for quality deterioration. As a result, reserves are maintained with a lower respiration rate, ripening is retarded and vapor pressure between products and ambient is minimized reducing water loss. These factors contribute towards maintaining freshness by reducing the rate at which quality deteriorates and the nutritional value of the product is preserved. A refrigerated room is a relatively airtight and thermally insulated environment. The refrigeration equipment should have an external escape outlet to release the heat generated by the product. Refrigeration capacity of the equipment should be adequate to extract the heat generated by crops with a high respiration rate. It is also important to control precisely the temperature and relative humidity conditions inside the refrigerated storage environment. It could be concluded the different methods of cooling in Table 8 and the different crop according to cooling method in Table 9.

Therefore, it could be concluded that, the marketable life of most fresh vegetables can be extended by prompt storage in an environment that maintains product quality. The desired environment can be obtained in facilities where temperature, air circulation, relative humidity, and sometimes atmosphere composition can be controlled. Storage rooms can be grouped accordingly as those requiring refrigeration and those that do not. Storage rooms and methods not requiring refrigeration include: *in situ*, sand, coir, pits, clamps, windbreaks, cellars, barns, evaporative cooling, and night ventilation. The length of storage time can be longer in specifically designed structures. With refrigeration and controlled atmospheres, storage periods can be even longer. The technology utilized depends on whether the benefits i.e., higher prices outweigh the costs.

2.8 Hygiene and Sanitation

Sanitation is of great concern to produce handlers, not only to protect produce against postharvest diseases, but also to protect consumers from food borne illnesses. *E. coli* 0157:H7, *Salmonella, Chryptosporidium, Hepatitis,* and *Cyclospera* are among the disease-causing organisms that have been transferred via fresh fruits and vegetables. Use of a disinfectant in wash water can help to prevent both postharvest diseases and food borne illnesses. The different stages of operations that a product goes through after harvest provide many occasions for contamination besides those that naturally occur in the field. Consumers strongly reject foreign materials on products or inside packages, such as dirt, animal feces, grease or lubricating oil, human hairs, insects and plant debris (Fig. 7).

However, because this type of contamination is usually caused by insufficient care in handling, it is relatively easy to detect and to eliminate. A more serious problem is the presence of human pathogens on produce. These may not be visible or detected because of changes in appearance, flavor, color or other external characteristics. It has been shown that specific pathogens are able to survive on produce sufficiently long to constitute a threat. In fact, many cases of illness related to consumption of produce have been reported. Three types of organisms that can be transported on fruits and vegetables may

Table 8 Classification of crops according to cooling method

Room cooling	Forced-air cooling	Hydrocooling	Ice cooling	Vacuum cooling
Artichoke	Avocado	Artichoke	Belgian endive	Belgian endive
Banana	Banana	Asparagus	Broccoli	Brussels sprouts
Beans (dry)	Barbados cherry	Beet	Brussels sprouts	Carrot
Beet	Berries	Belgian endive	Cantaloupe	Cauliflower
Breadfruit	Brussels sprouts	Broccoli	Chinese cabbage	Chinese cabbage
Cabbage	Cactus leaves	Brussels sprouts	Carrot	Celery
Cactus leaves	Cassava	Cantaloupe	Escarole	Escarole
Cassava	Coconut	Cauliflower	Green onions	Leek
Coconut	Cucumber	Carrot	Kohlrabi	Lettuce
Custard apple	Eggplant	Cassava	Leek	Lima bean
Garlic	Fig	Celery	Parsley	Mushrooms
Ginger	Ginger	Chinese cabbage	Pea/snow peas	Snap beans
Grapefruit	Grape	Cucumber	Spinach	Snow peas
Horse radish	Grapefruit	Eggplant	Sweet corn	Spinach
J. artichoke	Guava	Escarole	Swiss chard	Sweet corn
Kohlrabi	Kiwi fruit	Green onions	Watercress	Swiss chard
Kumquat	Kumquat	Horse radish		Watercress
Lime	Lima bean	J. artichoke		
Lemon	Mango	Kiwi fruit		
Melons	Melons	Kohlrabi		
Onion	Mushrooms	Leek		
Orange	Okra	Lima bean		
Cucumber	Orange	Orange		
Pineapple	Papaya	Parsley		
Potato	Passion fruit	Parsnip		
Pumpkin	Pepper (Bell)	Peas		
Radish	Pineapple	Pomegranate		
Summer squash	Pomegranate	Potato (early)		
Sweet potato	Prickly pear	Radish		
Tomato	Pumpkin	Snap beans		
Turnip	Snap beans	Snow peas		
Watermelon	Snow peas	Spinach		
	Strawberry	Summer squash		
	Summer squash	Sweet corn		
	Tangerine	Swiss chard		
	Tomato	Watercress		

Adapted from Sargent et al. (2000)

constitute a risk to human health: virus e.g. hepatitis A, bacteria e.g. *Salmonella* spp., *Escherichia coli*, *Shigella* spp. and parasites e.g. *Giardia* spp. Mycotoxins and fungi do not usually constitute a problem because fungi development is usually detected and eliminated well before the formation of mycotoxins (FAO 2004).

Table 9 Potential risks of microbial contamination and recommended preventive measures

Production step	Risks	Prevention
Production field	Animal fecal contamination	Avoid animal access, either wild, production or even pets.
Fertilizing	Pathogens in organic fertilizers	Use inorganic fertilizers.
		Proper composting
Irrigation	Pathogens in water	Underground drip irrigation
		Check microorganisms in water
Harvest	Fecal contamination	Personal hygiene. Portable bathrooms.
		Risk awareness
	Pathogens in containers and tools	Use plastic bins. Cleaning and disinfecting tools and containers
Packhouse	Fecal contamination	Personal hygiene. Sanitary facilities. Avoid animal entrance. Eliminate places may harbor rodents.
	Contaminated water	Alternative methods for precooling. Use potable water. Filtration and chlorination of recirculated water. Multiple washing
Storage and transportation	Development of microorganisms on produce	Adequate temperature and relative humidity. Watch conditions inside packaging. Cleaning and disinfection of facilities. Avoid repackaging. Personal hygiene. Do not store or transport with other fresh products. Use new packing materials
Sale	Product contamination	Personal hygiene. Avoid animal access.
		Sell whole units. Cleaning and disinfection of facilities. Discard garbage daily.

Adapted from FAO (2004)

In most cases, bacteria are responsible for illnesses related to the consumption of fruits and vegetables. Some human pathogens are naturally present in the environment. However, fecal deposits (human as well as from domestic and wild animals) are the main source of contamination of produce. Entry is mainly through irrigation or washing water. Microorganisms in surface water (rivers, lakes, etc.) may come from the upstream dumping of untreated municipal wastes. Underground water may also be contaminated from septic tanks leaching through soil into aquifers. If only contaminated water is available, underground drip irrigation is the only irrigation system recommended to avoid the contamination of above-ground edible plants. The main causes of contamination are the use of animal manure or sewage waste as organic fertilizer and the presence of animals in production areas. Manure should be composted aerobically to reach between 60 and 80 °C for a minimum of 15 days. Composting of static piles and earthworms do not guarantee that microorganisms have been inactivated. Wastewater and municipal wastes should only be used if effective disinfecting systems are available (FAO 2004).

Therefore, it could be concluded that, sanitation is of great concern to produce handlers, not only to protect produce against postharvest diseases, but also to protect consumers from food borne illnesses. Use of a disinfectant in wash water can help to prevent both postharvest diseases and food borne illnesses.

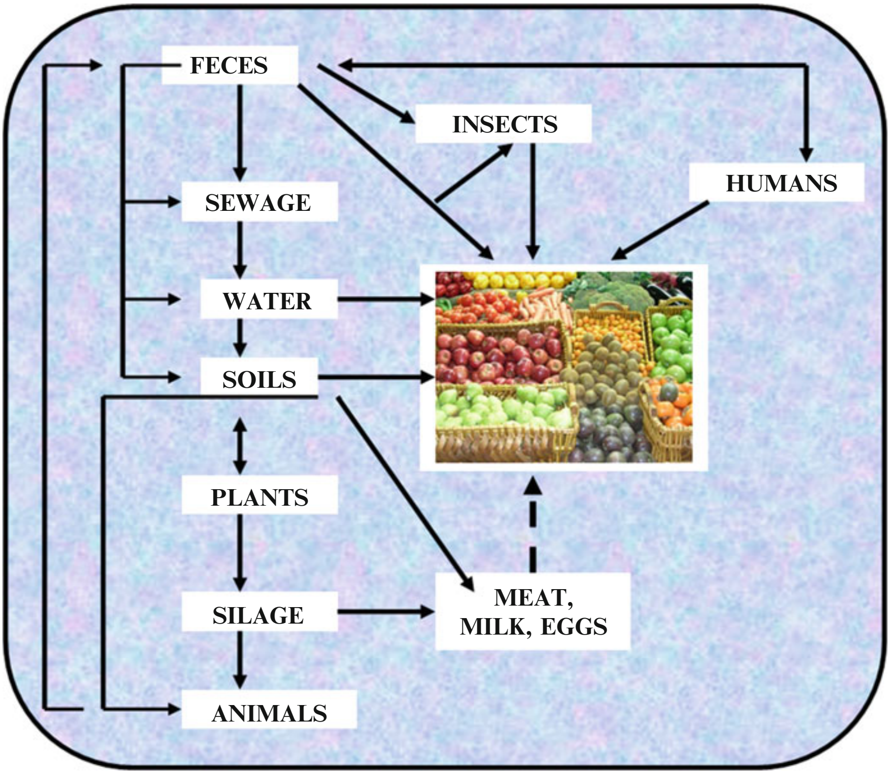

Fig. 7 Mechanisms by which fruit and vegetable can become contaminated with pathogenic microorganisms (Adapted from Harris 1998)

3 Influence of Preharvest Factors on Postharvest Quality

Preharvest factors greatly influence both the condition of the crop at harvest and the crop's storage and nutritive potential. The postharvest quality of fresh horticultural commodities markedly depends upon the quality attained at the time of harvest. Many preharvest factors are known to affect storage quality, including: genotype and cultivar selection; stage of maturity when harvested; climatic conditions such as temperature, light intensity, and rainfall amounts; soil texture and fertility; fertilizer type and application rates; disease and insect pressure; and growth regulator and pesticide application. Evaluating which preharvest conditions exert the most influence on postharvest fruit quality is difficult as they interact during the growing season and can change in degree of influence from year to year. Nonetheless, the goal in managing preharvest factors is ultimately to harvest the crop at the highest degree of quality and to sustain that quality throughout the storage period (De Long and Prange 2003).

The harvest of any crop, whether fruit, vegetable, or flower, is associated with mechanical stresses and because the plant part is separated from the parent, removal from a supply of water, nutrients, hormones, and energy. When harvested, the plant part has an altered ability to respond to stresses in the environment. Stress interrupts, restricts, or accelerates normal metabolic processes in an adverse or negative manner, and therefore is usually considered as potentially injurious to any plant system. However, consideration of postharvest systems is complicated as many storage regimens beneficially utilize stress conditions such as temperature and atmosphere modification to maximize storage potential of fresh crops. As highlighted by Kays, from a postharvest physiologist's position, stress is an external factor that will result in undesirable changes only if the plant or plant part is exposed to it for a sufficient duration or sufficient intensity. The postharvest period, in this context, can be seen as a time of stress management (Watkins 2003).

Therefore, it could be concluded that, preharvest factors greatly influence both the condition of the crop at harvest and the crop's storage and nutritive potential. The postharvest quality of fresh horticultural commodities markedly depends upon the quality attained at the time of harvest. Many preharvest factors are known to affect storage quality, including: genotype and cultivar selection; stage of maturity when harvested; climatic conditions such as temperature, light intensity, and rainfall amounts; soil texture and fertility; fertilizer type and application rates; disease and insect pressure; and growth regulator and pesticide application.

4 Processing of Fruits and Vegetables for Reducing Postharvest Losses

Despite decades of educational efforts, the most common causes of postharvest losses in developing countries continue to be rough handling and inadequate cooling and temperature maintenance. The lack of sorting to eliminate defects before storage and the use of inadequate packaging materials further add to the problem. In general, minimizing rough handling, sorting to remove damaged and diseased produce and effective temperature management will help considerably toward maintaining a quality product and reducing storage losses. Storage life will be enhanced if the temperature during the postharvest period is kept as close to the optimum as feasible for a given commodity (Tables 10 and 11; Kitinoja and Kader 2002).

Time and money are required to cultivate food products, and unless the farmer is providing food only for his own household, he automatically becomes part of the market economy: he must sell his produce, he must recover his costs, and he must make a profit. Estimates of the post-harvest losses of food grains in the developing world from mishandling, spoilage and pest infestation are put at 25 %; this means that one quarter of what is produced never reaches the consumer for whom it was grown, and the effort and money required to produce it are lost-forever. Fruit, vegetables and root crops are much less hardy and are mostly quickly perishable,

Table 10 Classification of some fruits and vegetables according to principal causes of postharvest losses and poor quality and in order of importance

Group	Examples	Principal causes of postharvest losses and poor quality (in order of importance)
Root vegetables	Carrots	Mechanical injuries
	Beets	Improper curing
	Onions	Sprouting and rooting
	Garlic	Water loss (shriveling)
	Potato	Decay
	Sweet Potato	Chilling injury (subtropical and tropical root crops)
Leafy vegetables	Lettuce	Water loss (wilting)
	Chard	Loss of green color (yellowing)
	Spinach	Mechanical injuries
	Cabbage	Relatively high respiration rates
	Green onions	Decay
Flower vegetables	Artichokes	Mechanical injuries
	Broccoli	Yellowing and other discolorations
	Cauliflower	Abscission of florets
		Decay
Immature-fruit vegetables	Cucumbers	Over-maturity at harvest
	Squash	Water loss (shriveling)
	Eggplant	Bruising and other mechanical injuries
	Peppers	Chilling injury
	Okra	Decay
	Snap beans	
Mature-fruit vegetables and fruits	Tomato	Bruising
	Melons	Over-ripeness and excessive softening at harvest
	Citrus	Water loss
	Bananas	Chilling injury (chilling sensitive fruits)
	Mangoes	Compositional changes
	Apples	Decay
	Grapes	
	Stone fruits	

Adapted from Kitinoja and Kader (2002)

and if care is not taken in their harvesting, handling and transport, they will soon decay and become unfit for human consumption. Estimates of production losses in developing countries are hard to judge, but some authorities put losses of sweet potatoes, plantain, tomatoes, bananas and citrus fruit sometimes as high as so percent, or half of what is grown. Reduction in this wastage, particularly if it can economically be avoided, would be of great significance to growers and consumers alike (Simson and Straus 2010).

The fruit and vegetable sector has grown substantially both in volume and in variety of outputs traded globally. Rising incomes, falling transportation costs, improved

Table 11 Classification of fresh horticultural crops according to their relative perishability and potential storage life in air at near optimum temperature and relative humidity

Relative perishability	Potential storage life (weeks)	Commodities
Very high	<2	Apricot, blackberry, blueberry, cherry, fig, raspberry, strawberry; asparagus, bean sprouts, broccoli, cauliflower, green onion, leaf lettuce, mushroom, muskmelon, pea, spinach, sweet corn, tomato (ripe); most cut flowers and foliage; minimally processed fruits and vegetables.
High	2–4	Avocado, banana, grape (without SO2 treatment), guava, loquat, mandarin, mango, melons (honeydew, crenshaw, Persian), nectarine, papaya, peach, plum; artichoke, green beans, Brussels sprouts, cabbage, celery, eggplant, head lettuce, okra, pepper, summer squash, tomato (partially ripe).
Moderate	4–8	Apple and pear (some cultivars), grape (SO2-treated), orange, grapefruit, lime, kiwifruit, persimmon, pomegranate; table beet, carrot, radish, potato (immature).
Low	8–16	Apple and pear (some cultivars), lemon; potato (mature), dry onion, garlic, pumpkin, winter squash, sweet potato, taro, yam; bulbs and other propagules of ornamental plants.
Very low	>16	Tree nuts, dried fruits and vegetables.

Adapted from Kader (1993)

technologies and evolving international agreements, have all contributed to this level of growth. This increased level of fruit and vegetable production has, unfortunately, not been matched by developments in supply chain management, or by vertical integration of production with processing in many developing countries. Processing activities are of critical importance to expansion and diversification within the fruit and vegetable sector in that they increase market opportunities for fresh fruits and vegetables and add value while minimizing postharvest losses. Furthermore, processing improves the viability, profitability and sustainability of fruit and vegetable production systems by increasing farm incomes, and generating rural employment and foreign exchange. Traditional processing technologies such as thermal processing (bottling and canning), freezing, dehydration (salting, brining and candying) drying, and fermentation are widely applied in the processing of fruits and vegetables at various levels (artisanal, intermediate and high) and scales (cottage, small, medium and large). Tropical juices and fruit pulps, canned pineapples, tomato paste and canned and dried mushrooms are examples of fruit and vegetable products produced using traditional processing technologies and which are increasingly entering in international trade (Rolle 2006).

Dried and canned mushrooms produced in China, currently account for 52 % of world trade in processed mushrooms, while canned pineapples produced in Thailand accounts for approximately 45 % of that product in world trade. Minimal processing technologies, specialized packaging and natural preservation systems are increasingly being applied in the preservation of fruits and vegetables for both developed and developing country markets, in response to growing consumer demand for convenience and for "fresh-like" fruits of high quality which are nutritious, flavorful and stable. These processing technologies focus on adding value with comparatively little product transformation while increasing product diversity. While minimal and traditional processing technologies present considerable opportunities for innovation and vertical diversification in the fruit and vegetable sector, relatively few small and medium enterprises (SMEs) are able to tap into and benefit from these opportunities. Many SMEs lack the capacity to operate competitively in the current globalized market environment owing to problems of scale, the poor quality of input supplies, poor access to technology, limited technical expertise and research capacity, low production efficiency, high marketing cost, lack of knowledge and consequently inability to comply with international standards for processed products. Traditional processing technologies, applied in the conservation of horticultural produce employ a gradient of technologies, ranging from artisanal to intermediate to high technologies. Major categories of processed products produced with the use of these technologies include fruit preserves, fruit and vegetable juices, fermented products (wines and vinegars), candied products and frozen and dried products (Rolle 2006).

The keeping and the preparation of fresh produce after harvest affects its nutritional value in several ways, for example:

- Dry-matter content is reduced with time as the continuation of living processes within the produce uses up stored food reserves.
- Vitamin C content decreases with time after harvest, and little may remain after 2 or 3 days (Table 12).
- Cooking partially destroys vitamins C and B_1. Raw fruit and vegetables are particularly valuable provided they are grown and handled hygienically.
- Peeling may cause significant loss of food value, especially in potatoes, where the protein content is just beneath the skin.
- Water used in cooking vegetables or fruit contains the dissolved minerals and trace elements of the food and should not be thrown out but used in soups or in preparing other foods (Simson and Straus 2010).

Table 12 Vitamins supplied by fruit, vegetables and root crops

Vitamin	Name	Source
A	Retinol	From carotene in dark green leaves, tomatoes, carrots, papayas
B_1	Thiamine	Pulses, green vegetables, fruit (cereal grains have B. in germ and outer-seed coat)
B_2	Riboflavin	Green leafy vegetables and pulses
B_6	Pyridoxin	Bananas, peanuts
PP	Niacin (nicotinic acid)	Pulses, peanuts
–	Folic acid	Dark green leaves, broccoli, spinach, beets, cabbage, lettuce, avocados
C	Ascorbic acid	Dark green leaves, spinach, cauliflower, sweet pepper, citrus, guava, mango, papaya

Adapted from Simson and Straus (2010)

Therefore, it could be concluded that, the lack of sorting to eliminate defects before storage and the use of inadequate packaging materials further add to the problem. In general, minimizing rough handling, sorting to remove damaged and diseased produce and effective temperature management will help considerably toward maintaining a quality product and reducing storage losses. Storage life will be enhanced if the temperature during the postharvest period is kept as close to the optimum as feasible for a given commodity. The fruit and vegetable sector has grown substantially both in volume and in variety of outputs traded globally. Processing activities are of critical importance to expansion and diversification within the fruit and vegetable sector in that they increase market opportunities for fresh fruits and vegetables and add value while minimizing postharvest losses.

5 Development of Storage Science

Some of the first experiments in which ripening of fruit was intentionally altered by changing the storage atmosphere were carried out in France by Jacques Berard around 1820. He demonstrated that fruit use O_2 and generate CO_2 while in storage, and if totally deprived of O_2, they did not ripen. Berard found that apples, apricots, peaches, pears, and prunes could be stored in an altered gas environment, and following their removal to ambient air and room temperature, were edible for extended periods. Refrigeration has had a significant effect on the development of fruit and vegetable storage technology. The rationale for quickly lowering the temperature of harvested crops is essentially the same today as it was in antiquity: refrigeration greatly reduces food spoilage and waste. Although ice and snow have been used since the Roman era to preserve perishable foodstuffs, it was not until the 1800s that natural ice from the colder climes of the northern hemisphere was commonly used to refrigerate foods worldwide. It could be summarized development of storage science as follows (Table 13; De Long and Prange 2003; Fig. 8):

Date	Events
In the 1860s	**Benjamin Nyce**, an Ohio commercial storage operator, found that when he limited O_2 surrounding his fruit in an ice-refrigerated store, the produce was greatly improved. Nyce did not license the patent rights for his storage system despite strong commercial interest to implement the method, thus, it was not widely adopted. Ironically, Nyce's work resulted in a de facto prototype for modern controlled atmosphere (CA) storage, which, unfortunately, was not further developed for another 60 years.

(continued)

Date	Events
In the early 1890s	The **San Jose Fruit Packing Company** experimented with augmented CO_2 levels as a preserver of fruit. In a railroad car experiment, a load of grapes, peaches, pears, persimmons, and quinces stored in elevated CO_2 and without refrigeration were in good condition following 11 days of travel, and held up well in the retail chain. In other early experiments with storage gas modification, elevated CO_2 was found to be helpful, but could not completely compensate for lack of refrigeration. Temperature control was/is fundamental for preservation of fruit and vegetable quality, with modification of the O_2 and CO_2 atmosphere being an important but secondary quality preservation method.
In the 1920s	**Kidd and West**, working at the Low Temperature Research Station in Cambridge, UK, conducted a number of experiments to determine the optimal atmospheric gas concentrations, i.e., lower O_2 and higher CO_2, for storing apples. It was known at that time that respiration fueled the generation of metabolic heat, which was deleterious to fruit quality in long-term storage. Therefore, one of the goals of their research was to reduce the rate of storage respiration. Their studies led to assigning the term "climacteric" to indicate the burst of postharvest respiration in apples, which is now known to occur in many important fruit crops. In subsequent years, Kidd and West also became increasingly aware of the role of ethylene in the climacteric rise in apples.
In the 1930s	Following the seminal work of Kidd and West, CA storage became more common globally, although the UK was the first country to initially adopt commercial CA storage practices. This was soon followed by South Africa, the United States, Canada, Australia, New Zealand, Denmark, and the Netherlands. Today, CA storage is standard practice worldwide and is largely responsible for the high marketplace quality attained for many CA responsive fruits and vegetables. Interestingly, the challenge facing each major geographical region that adopted CA technology in the 1920s and 1930s is the same today: finding the optimal combination of temperature, O_2, and CO_2 for each species, cultivar, and even strain that leads to the highest quality retention for the longest duration. This goal is always tempered by the technology available and the economic viability of sustaining the storage environment for the desired length of time.

Therefore, it could be concluded that, some of the first experiments in which ripening of fruit was intentionally altered by changing the storage atmosphere were carried out in France by Jacques Berard around 1820. He demonstrated that fruit use O_2 and generate CO_2 while in storage, and if totally deprived of O_2, they did not ripen. Refrigeration has had a significant effect on the development of fruit and vegetable storage technology. The rationale for quickly lowering the temperature of harvested crops is essentially the same today as it was in antiquity: refrigeration greatly reduces food spoilage and waste.

Table 13 Recommended temperature and relative humidity for fruits and vegetables and the approximate storage life under these conditions

Crop	Temperature (°C)	Relative humidity (%)	Storage life (days)
Apple	−1 to 4	90–95	30–180
Apricot	–0.5 to 0	90–95	7–21
Artichoke	0	95–100	14–21
Asparagus	0–2	95–100	14–21
Avocado	3–13	85–90	14–56
Banana – Plantain	13–15	90–95	7–28
Barbados cherry	0	85–90	49–56
Basil	7–10	85–90	7
Bean (dry)	4–10	40–50	180–300
Beet (bunched)	0	98–100	10–14
Beet (topped)	0	98–100	120–180
Black berry	–0.5 to 0	90–95	2–3
Blue berries	–0.5 to 0	90–95	14
Broad beans	0–2	90–98	7–14
Broccoli	0	95–100	14–21
Cabbage	0	98–100	150–180
Cactus leaves	2–4	90–95	14–21
Cantaloupe (half slip)	2–5	95	15
Cantaloupe (full slip)	0–2	95	5–14
Carrot (bunched)	0	95–100	14
Carrot (topped)	0	98–100	210–270
Cassava	0–5	85–96	30–60
Cauliflower	0	95–98	21–28
Celery	0	98–100	30–90
Cherries	−1 to 0.5	90–95	14–21
Chicory	0	95–100	14–21
Chinese cabbage	0	95–100	60–90
Chives	0	95–100	14–21
Coconut	0–1.5	80–85	30–60
Cucumber	10–13	95	10–14
Dates	−18 to 0	75	180–360
Eggplant	8–12	90–95	7
Fig	–0.5 to 0	85–90	7–10
Garlic	0	65–70	180–210
Ginger	13	65	180
Grape	–0.5 to 0	90–95	14–56
Grapefruit	10–15	85–90	42–56
Green onions	0	95–100	21–28
Guava	5–10	90	14–21
Horse radish	−1 to 0	98–100	300–360
Jerusalem artichoke	–0.5 to 0	90–95	120–150
Kiwi fruit	–0.5 to 0	90–95	90–150
Kohlrabi	0	98–100	60–90
Kumquat	4	90–95	14–28

(continued)

Table 13 (continued)

Crop	Temperature (°C)	Relative humidity (%)	Storage life (days)
Leek	0	95–100	60–90
Lemon	10–13	85–90	30–180
Lettuce	0–2	98–100	14–21
Lima bean	3–5	95	5–7
Lime	9–10	85–90	42–56
Mandarin	4–7	90–95	14–28
Mango	13	90–95	14–21
Melon (Others)	7–10	90–95	12–21
Mushrooms	0–1.5	95	5–7
Nectarine	–0.5 to 0	90–95	14–28
Okra	7–10	90–95	7–10
Onions (dry)	0	65–70	30–240
Olives, fresh	5–10	85–90	28–42
Orange	0–9	85–90	56–84
Papaya	7–13	85–90	7–21
Parsley	0	95–100	30–60
Parsnip	0	95–100	120–180
Peach	–0.5 to 0	90–95	14–28
Pear	−1.5 to 0.5	90–95	60–210
Peas	0	95–98	7–14
Cucumber	5–10	95	28
Pepper (bell)	7–13	90–95	14–21
Persimmon	–1	90	90–120
Pineapple	7–13	85–90	14–28
Plum	–0.5 to 0	90–95	14–35
Pomegranate	5	90–95	60–90
Potato (early)	7–16	90–95	10–14
Potato (late)	4.5–13	90–95	150–300
Pumpkins	10–15	50–70	60–160
Quince	–0.5 to 0	90	60–90
Radish	0	95	21
Raspberries	–0.5 to 0	90	2
Snap beans	4–7	95	7–10
Snow peas	0–1	90–95	7–14
Spinach	0	95–100	10–14
Sprouts	0	95–100	7
Strawberry	0–0.5	90–95	5–7
Sweet corn	0–1.5	95–98	5–8
Sweet potato	13–15	85–90	120–210
Summer squash	5–10	95	7–14
Taro	7–10	85–90	120–150
Tomato (red)	8–10	90–95	8–10
Turnip	0	90–95	120
Water melon	10–15	90	14–21
Yam	16	70–80	60–210

Adapted from FAO (2004)

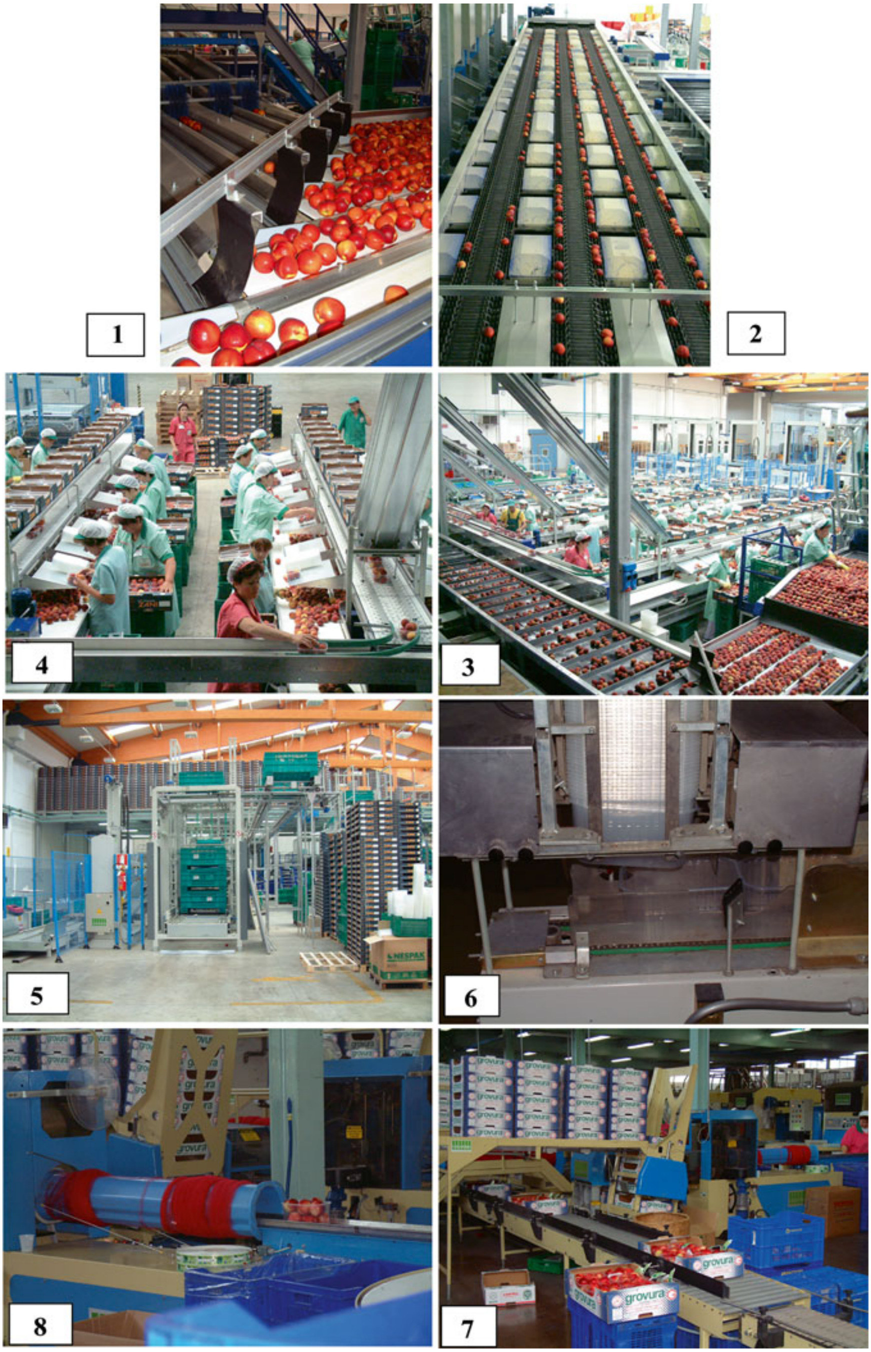

Fig. 8 Processing of fruits for reducing postharvest losses in Cesena in Italy. Sorting to eliminate defects before storage (Photos 1, 2, 3 and 4) and the use of inadequate packaging materials further add to the problem. Storage life will be enhanced if the temperature during the postharvest period is kept as close to the optimum as feasible for a given commodity (Photos 5, 6, 7 and 8) (Photo by M. Fári)

6 Postharvest Biology and Technology: A Perspective

Fruits and vegetables as well as their processed products have become mainstream human dietary choices in recent days, primarily because of several epidemiological studies showing various health benefits associated with the consumption of fruits, vegetables, and their processed products. Fruits and vegetables share several common structural and nutritional properties and also characteristic differences due to differences in their biochemical composition. Fruits, in general, are attractive organs for vectors involved in seed dispersal, and thus have evolved features such as enhanced color, attractive flavor, and taste. Consequently, the developmental and biochemical processes within a fruit are programmed to achieve this goal. The term *vegetables* is more or less arbitrary, comprising products such as leaves, petioles, stems, roots, tubers, and fruits of cucurbits e.g., gourds, melons, squash, and pumpkin and solanaceae members e.g., tomato and eggplant. Morphologically, fruits develop from the ovary, the seed-bearing structure in plants. The developmental processes in fruits are influenced by fertilization, and the hormonal changes induced in the ovary leads to gene expression and biochemical changes resulting in the characteristic fruit that may vary in ontogeny, form, structure, and quality. Fruits originate from different parts of the ovary. Pome fruits such as apple and pear develop from the thalamus of the flower. In drupe fruits such as cherries, peaches, plums, and apricots, the ovary wall (mesocarp) develops into the fruit enclosing a single seed. Berry fruits, such as tomato and grape, possess the seeds embedded in a jellylike pectinaceous matrix, with the ovary wall developing into the flesh of the fruit. Citrus fruits belong to the class known as hesperidium, where the ovary wall develops as a protective structure surrounding the juice-filled locules that are the edible part of the fruit. In strawberry, the seeds are located outside the fruit, and it is the receptacle of the ovary (central portion) that develops into the edible part. Most vegetables are leaves, petioles, or stems containing chlorophyll, or roots, tubers, or fruits that predominantly contain storage components such as starch. Examples include potato and eggplant (Solanaceae), gourds (Cucurbitaceae), several types of yams (Dioscoreaceae and Araceae), vegetables of leaf and flower origin (cabbage, broccoli, cauliflower—Cruciferae), and unripe fruits of leguminous plants such as peas and beans (Leguminosae). The nutritional and food qualities of fruits and vegetables arise as a result of the accumulation of components derived from the intricate biochemical pathways (Paliyath and Murr 2008a).

Postharvest practices for the preservation of fruits, vegetables, and flowers are perhaps one of the oldest in human history. With the understanding of the molecular processes that occur during plant senescence, this discipline has developed unique features of its own. Traditionally, postharvest science is considered as an applied science focusing on the physiological aspects of enhancement of shelf life and preservation of quality of horticultural produce. However, in the past two decades, biochemical and molecular biological aspects have been extensively used for analyzing postharvest issues. Postharvest issues are common around the world. The extent of the loss of horticultural produce after harvest can vary in different countries. In those parts of the world where the methods of agricultural production and storage employ advanced technology, postharvest losses may be minimal, and most of it occurs during the transit of produce from the production site to the destination along the consumer chain. The losses can range from 10 to 20 % by volume. In tropics where the production practices are basic and based on day-to-day demand, the postharvest losses can be as high as 50 % or over. It is surprising and a bit disturbing to see that fruits are considered as luxury items in some parts of the world. In an era where we consider the consumption of fruits and vegetable as a means of health promotion, postharvest science gets a new meaning (Paliyath et al. 2008).

Fruit ripening is characterized by several marked physiological and biochemical changes resulting in the coordinated development of complex characteristics. Following pollination and fertilization, the fruit develops in size leading to the ripening process, which results in the development of ideal organoleptic characters such as taste, color, and aroma that are important quality-determining features. Fruits that are used as vegetables are harvested early prior to their ripening. The physiological process of ripening occurs rapidly when the fruit is mature, and beyond a certain stage, harvested fruits undergo rapid deterioration in quality. Ideally, fruits are harvested at an optimal physiological stage or maturity characteristic to the type of fruit, after which appropriate storage procedures can be adopted for preserving the shelf life and quality of the fruits. Fruits do not ripen fully showing the appropriate quality characteristics if picked at a young stage before the attainment of physiological maturity. Citrus fruits are allowed to fully ripen before they are harvested. Avocado fruits do not ripen if left on the tree and start ripening only after harvest. Irrespective of the nature of the produce whether it is fruits, vegetables, or flowers, various technologies such as cold storage, controlled atmosphere storage, and inhibition of hormone and enzyme action are adopted to slow down the metabolic processes to provide an optimal quality produce for marketing and consumption.

Advances in the biochemistry and molecular biology of the fruit ripening process have enabled the development of biotechnological strategies for the preservation of postharvest shelf life and quality of fruits, vegetables, and flowers. Several metabolic changes are initiated after the harvest of fruits and vegetables. In the case of vegetables, harvesting induces stress responses through reduced availability of water and nutrients, wounding, and exposure to shelf life enhancing storage methods such as cooling. In most cases, these changes help the produce to enhance the shelf life. In the case of fruits, an increase in the biosynthesis of the gaseous hormone ethylene serves as the physiological signal for the initiation of the ripening process. In general, all plant tissues produce a low, basal, level of ethylene. During the ripening process, some fruits evolve large amounts of ethylene, sometimes referred to as an autocatalytic increase in ethylene production, which occurs in conjunction with an increase in respiration referred to as the respiratory climacteric. Fruits are generally classified into climacteric or nonclimacteric types on the basis of the pattern of ethylene production and responsiveness to externally added ethylene. The climacteric fruits characteristically show a marked enhancement in ethylene production and respiration, as noticeable by the evolution of carbon dioxide. By contrast, the nonclimacteric fruits emit a considerably reduced level of ethylene. In climacteric fruits such as apple, pear, banana, tomato, and avocado, ethylene evolution can reach 30–500 ppm/(kg h) (parts per million, μL L^{-1}), whereas in nonclimacteric fruits such as orange, lemon, strawberry, and pineapple, ethylene levels usually range from 0.1 to 0.5 ppm/(kg h) during ripening. Climacteric fruits respond to external ethylene treatment by an early induction of the respiratory climacteric and accelerated ripening in a concentration-dependent manner. Nonclimacteric fruits, on the other hand, show increased respiration in response to increased levels of ethylene concentration without showing acceleration in the time required for ripening. Vegetables produce very low amounts of ethylene most of them with less than 0.1 μL/(kg h), with slightly higher levels as in cassava (1.7 μL/(kg h)), breadfruit (1.2 μL/(kg h)), and cucumber (0.6 μL/(kg h)) when measured at 20–25 °C. After the initiation of ripening or harvest, several biochemical changes occur in fruits and vegetables. As some of these changes such as the development of color, flavor, and sweet taste are desirable for fruits, any sort of quality changes are ideally not desired in vegetables. Thus, strategies for the preservation of shelf life and quality in fruits and vegetables could be entirely different. It is important to know the biochemical differences between fruits and vegetables and several biochemical pathways that operate in these tissues to develop ideal conditions of storage for the preservation of shelf life and quality (Paliyath and Murr 2008a).

Therefore, it could be concluded that, fruits and vegetables share several common structural and nutritional properties and also characteristic differences due to differences in their biochemical composition. Postharvest practices for the preservation of fruits, vegetables, and flowers are perhaps one of the oldest in human history. With the understanding of the molecular processes that occur during plant senescence, this discipline has developed unique features of its own.

7 Biochemical Parameters of Horticultural Crops Quality

There are two major aspects that define the quality of a produce: the first being the inherent biochemical characteristics that provide the color, flavor, texture, and taste to the produce and the second being the consumer perception. The application of postharvest technologies tends to maximize these quality characteristics, though application of some technologies may not provide the optimal quality produce for the consumer. During ripening, activation of several metabolic pathways occurs, often leading to ideal changes in the biochemical composition of fruits. The stage of development in a fruit determines its biochemical composition and the quality-defining parameters. Color is perhaps the first parameter that attracts a consumer to a produce. Hence, fruits that show enhanced yellow-orange-red hue are preferred by the consumer. The composition of anthocyanins and carotenoids in a fruit will determine its color quality characteristics. Consumers also associate the depth of color with the taste, though this is influenced by practical experiences. In general, fruits that are bright red are also sweet. Some of the exceptions include sour cherries and red currants. Brightly colored fruits also tend to possess the ideal texture. Flavor is also an important component to the quality perception, and the degree of ripeness determines the level and types of flavor components such as esters and terpenoids emitted from the fruit. Aroma is derived from several types of compounds that include monoterpenes (as in lime, orange), ester volatiles (ethyl, methyl butyrate in apple, isoamyl acetate in banana), simple organic acids such as citric and malic acids (citrus fruits, apple), and small-chain aldehydes such as hexenal and hexanal (cucumber) (Paliyath and Murr 2008a).

In fruits such as mango, pineapple, strawberry, and grape, the ripening process is associated with the conversion of stored organic acids and starch into sugars, and enhanced evolution of flavor components. The presence of off-flavors resulting from the presence of certain aldehydes e.g., acetaldehyde may negatively impact quality perception, whereas other aldehydes such as hexanal tend to enhance the green flavor and consumer preference of vegetables. The evolution of sulfur volatiles in crucifer vegetables e.g., broccoli and cabbage and *Allium* vegetables (onion, garlic) is characteristic to their quality. In a similar way, the evolution of essential oils in Lamiaceae members (mint, oregano, rosemary, etc.) also attracts consumers. Fruits and vegetables contain a large percentage of water, which can often exceed 95 % by fresh weight. Texture and the degree of softness are determined by the amount of water contained in the produce and the ability to retain that water during postharvest storage. The degradation of cell wall components and the cell membrane negatively affects the rigidity of the tissue in fruits providing the softness that consumers prefer (degradation of stored starch in banana), though excessive degradation of these components reduces the shelf life of the fruits drastically (Paliyath and Murr 2008a).

Most vegetables are preserved to maximize the high textural integrity, and loss of water from vegetables negatively affects their quality. The consumers are increasingly becoming aware of the disease-preventive and health-restoring roles of fruits and vegetables, because of which they are classified as functional foods. Many quality-determining components are also regarded as important functional food ingredients (nutraceuticals) that include soluble and insoluble fibers, color components such as anthocyanins and carotenoids, several polyphenolic components, and sulfur-containing components in crucifer and *Allium* vegetables. Fruits in general contain large amounts of fibrous materials such as cellulose and pectin. The breakdown of these large polymers into smaller water-soluble components during ripening leads to fruit softening as observed during the breakdown of pectin in tomato and cellulose in avocado. Secondary plant metabolites are major ingredients of fruits. Anthocyanins are the major color components in grapes, blueberries, apples, and plums; carotenoids, specifically lycopene and carotene, are the major color components in tomatoes, and these components provide the health benefits to consumers through their antioxidant property and ability to influence metabolic processes within the human body. Fruits are also rich in vitamin C, which is a strong antioxidant. Vegetables such as asparagus are rich in glutathione, another component in the antioxidant defense system (Paliyath and Murr 2008a).

Lipid content is quite low in fruits; however, fruits such as avocado and olives store large amounts of triacylglycerols (oils). The amounts of proteins are usually low in most fruits. It is interesting to note that a majority of edible fruits and vegetables tend to group in certain families. For instance, some of the edible fruit-dominated families include Annonaceae, Rosaceace, Myrtaceae, Rutaceae, Oxalidaceae, and Anacardiaceae among the dicots. Bananas and plantains are the major monocot fruits (Musaceae). The major dicot vegetable families include Fabaceae, Solanaceae, Cruciferae, Cucurbitaceae, Compositae, Umbelliferae, Lamiaceae, and Dioscoreaceae. Monocot families such as the Liliaceae and Araceae are rich in vegetables. The following are some specific characteristics of fruits and vegetables (Paliyath and Murr 2008a).

Several metabolic changes are initiated after the harvest of fruits and vegetables. In the case of vegetables, harvesting induces stress responses through reduced availability of water and nutrients, wounding and exposure to shelf life, enhancing storage methods such as cooling. In most cases, these changes help the produce to enhance the shelf life. In the case of fruits, an increase in the biosynthesis of the gaseous hormone ethylene serves as the physiological signal for the initiation of the ripening process. In general, all plant tissues produce a low, basal, level of ethylene. During the ripening process, some fruits evolve large amounts of ethylene, sometimes referred to as an autocatalytic increase in ethylene production, which occurs in conjunction with an increase in respiration referred to as the respiratory climacteric. Fruits are generally classified into climacteric or nonclimacteric types on the basis of the pattern of ethylene production and responsiveness to externally added ethylene (Paliyath and Murr 2008b).

The climacteric fruits characteristically show a marked enhancement in ethylene production and respiration, as noticeable by the evolution of carbon dioxide. By contrast, the nonclimacteric fruits emit a considerably reduced level of ethylene. In climacteric fruits such as apple, pear, banana, tomato, and avocado, ethylene evolution can reach 30–500 ppm/(kg h) (parts per million, microliter/L), whereas in nonclimacteric fruits such as orange, lemon, strawberry, and pineapple, ethylene levels usually range from 0.1 to 0.5 ppm/(kg h) during ripening. Climacteric fruits respond to external ethylene treatment by an early induction of the respiratory climacteric and accelerated ripening in a concentration-dependent manner. Nonclimacteric fruits, on the other hand, show increased respiration in response to increased levels of ethylene concentration without showing acceleration in the time required for ripening. Vegetables produce very low amounts of ethylene most of them with less than 0.1 μL/(kg h), with slightly higher levels as in cassava (1.7 μL/kg h), breadfruit (1.2 μL/kg h), and cucumber (0.6 μL/kg h) when measured at 20–25 °C. After the initiation of ripening or harvest, several biochemical changes occur in fruits and vegetables. As some of these changes such as the development of color, flavor, and sweet taste are desirable for fruits—any sort of quality changes are ideally not desired in vegetables. Thus, strategies for the preservation of shelf life and quality in fruits and vegetables could be entirely different. It is important to know the biochemical differences between fruits and vegetables and several biochemical pathways that operate in these tissues to develop ideal conditions of storage for the preservation of shelf life and quality (Paliyath and Murr 2008b).

Fruits contain a large percentage of water that can often exceed 95 % by fresh weight. During ripening, activation of several metabolic pathways often leads to drastic changes in the biochemical composition of fruits. Fruits such as banana store starch during development and hydrolyze the starch to sugars during ripening that also results in fruit softening. Most fruits are capable of photosynthesis, store starch, and convert them to sugars during ripening. Fruits such as apple, tomato, and grape have a high percentage of organic acids, which decreases during ripening. Fruits contain large amounts of fibrous materials such as cellulose and pectin. The degradation of these polymers into smaller water-soluble units during ripening leads to fruit softening as exemplified by the breakdown of pectin in tomato and cellulose in avocado. Secondary plant products are major compositional ingredients in fruits. Anthocyanins are the major color components in grapes, blueberries, apples, and plums; carotenoids, specifically lycopene and carotene, are the major components that impart color in tomatoes. Aroma is derived from several types of compounds that include monoterpenes (as in lime, orange), ester volatiles (ethyl, methyl butyrate in apple, isoamyl acetate in banana), simple organic acids such as citric and malic acids (citrus fruits, apple), and small chain aldehydes such as hexenal and hexanal (cucumber). Fruits are also rich in vitamin C. Lipid content is quite low in fruits, the exceptions being avocado and olives, in which triacylglycerols (oils) form the major storage components. The amounts of proteins are usually low in most fruits (Paliyath and Murr 2008b).

Fruit ripening is a dynamic transitional period during which many easily perceived changes, such as alterations in pigmentation, firmness, sweetness, and acidity take place. These changes make fruit desirable for human consumption and capable of seed dispersal by birds, animals, and environments. Fruit firmness is associated with several attributes including crispness, mealiness, grittiness, chewiness, succulence and juiciness, fibrousness, toughness, and oiliness. Additionally, development of various organoleptic components such as sweetness, sourness, astringency, bitterness, and production of volatile compounds leading to characteristic aroma is connected with fruit textural changes. Although most of these changes impart desirable traits to various fruits and vegetables, some of the fruit softening associated changes make them unacceptable for marketing. These include development of off-flavors and off-odors with excessive softening of tissues. Textural softening can also increase susceptibility to phytopathogens due to their proneness to solute leakage that provide rich media for their growth, and resulting in severe losses during postharvest storage and marketing (Prasanna et al. 2007).

Large economic losses results from inability to retard ripening-associated excessive softening of fruits between harvest and marketing. These losses occur due to culling of perishable commodities at field and packinghouses, grading, storage, transit, retail, and consumer. In developing countries, theses losses can range between 10 and 100 %, especially due to phytopathogen-related tissue rotting of certain commodities. The economic consequences of postharvest fruit softening have led to considerable interests of geneticists, physiologists, biochemists, and in recent year's molecular biologists to understand the molecular basis of fruit softening. The last 40 years have seen a significant increase in our understanding of biochemical changes associated to fruit textural modifications. Emerging recombinant DNA technologies including reverse genetics have begun to provide some answers. In this section, it could be described the relationship of cell wall chemistry and various families of cell wall–modifying enzymes to the developmentally regulated softening of fruit during ripening. Also discussed are various postharvest factors affecting structural deterioration of fruit crops and potential of chemical or genetic means to reduce crop losses (Negi and Handa 2008).

Therefore, it could be concluded that, most vegetables are preserved to maximize the high textural integrity, and loss of water from vegetables negatively affects their quality. There are two major aspects that define the quality of a produce: the first being the inherent biochemical characteristics that provide the color, flavor, texture, and taste to the produce and the second being the consumer perception.

8 Postharvest Factors Affecting Structural Deterioration

The major postharvest problem with storage of fruits and vegetables is the excessive softening. Ripening of many fruits is mainly orchestrated by biosynthesis of ethylene that triggers a serial biochemical and physiological process inducing the softening in texture. The most important factors affecting structural deterioration of fruits and vegetables can be summarized as follows:

I. **Processing**
At low extraction percentages (up to 33 %), pectic polysaccharides and hemicellulosic xyloglucans were the main type of polymers affected, suggesting the modification of the cell wall matrix, although without breakage of the walls. At higher extraction rates (up to 64 %), a major disruption of the cell wall occurred as indicated by the losses of all major types of cell wall polysaccharides, including cellulose. At higher extraction rates, fatty acid chains are able to exit the cells either through unbroken walls, or the modification of the pectin-hemicellulose network might have increased the porosity of the wall. Due to high pressure, a progressive breakage of the cell walls was observed, which allows the free transfer of the fatty acid chains from inside the cells (Negi and Handa 2008).

II. **Heat**
Postharvest heat treatments lead to an alteration of gene expression, and fruit ripening can sometimes be either delayed or disrupted. Cell wall–degrading enzymes and ethylene production are frequently the most disrupted, and their appearance is delayed following heating. Fruit sensitivity to heat treatments is modified by preharvest weather conditions, cultivar, rate of heating, and subsequent storage conditions. Prestorage heat treatment appears to be a promising method of postharvest control of decay. Heat treatments against pathogens may be applied to fresh harvested commodities by hot water dips, by vapor heat, by hot dry air, or by a very short hot water rinse and brushing. Prestorage heat treatment could delay the ripening of “Gala” and “Golden Delicious” apples and

maintain storage quality (Shao et al. 2007). Heating "Golden Delicious" apples for 4 days at 38 °C reduced decay and maintained fruit firmness during 6 months of storage at 0 °C. Cooking resulted in an increase in the water-soluble pectins and a decrease in the pectins associated with cellulose. The total cell wall polysaccharide and galactose content of the squash cultivars remained unchanged for up to 2 months of storage and decreased later (Negi and Handa 2008).

III. Physiological disorders

Water soaking developed during the late stages of fruit ripening. The major changes were observed in a protein implicated in calcium signaling processes. While the amount of total calmodulin, the ubiquitous calcium-binding protein, was not modified, a particular calmodulin-binding protein (CaM-BP) was absent in water-soaked but not in sound mature tissues. This CaM-BP may be a marker or a determinant of this physiological disorder. Gel breakdown in inner mesocarp tissue of plums was associated with high viscosities of water-soluble pectin with low levels of extractable juice. In outer mesocarp tissue where extractable juice levels were higher, over-ripeness developed. Cell walls of inner tissue were thicker and had a better developed middle lamella than outer tissue. Inner mesocarp tissue was composed of larger cells than outer tissue (Negi and Handa 2008).

IV. Chilling and freezing injury

Insoluble pectin levels declined during ripening and cold storage of plum fruit with a concomitant increase in soluble pectin levels. Neither harvest maturity nor storage time had a significant effect on the concentration of calcium pectate, and this pectic fraction did not appear to influence development of gel breakdown (GB). Water-soluble pectin and availability of cell fluids indicated a high gel potential in plums. Significant levels of GB developed only in plums harvested at postoptimum maturity. In GB fruit, higher sugar levels and loss of cell membrane integrity probably enhanced formation of pectin sugar gels as cell fluids bind with pectins in cell walls. The initial response to low temperature is considered to involve physical factors such as membrane alteration and protein/enzyme diffusion, but physiological changes that lead to losses of structural integrity and overall fruit quality also occur. During softening, dissolution of the ordered arrangement of cell wall and middle lamella polysaccharides occur. As the fruit ripens, a substantial portion of its cell wall pectins are converted to a water-soluble form affecting the texture. The major changes involved in softening and chilling injury in peaches are the catabolism of cell walls and the development of an intercellular matrix containing pectins. Gel-like structure formation in the cell wall due to the deesterification of pectins without depolymerization leads to the development of woolliness in peach (Lurie et al. 2003). Ruoyi et al. (2005) showed that combination of chitosan coating, calcium chloride, and intermittent warming partially inhibited PG activity, slowed down the increase in soluble pectinefic substances. Addition of calcium chloride and intermittent warming could keep the intactness of cell wall and reduce fruit sensitivity to injury in peach. Endo-PG, PE, and endoglucanase (EGase) activities of delayed-storage nectarines fruit were same as the control fruit at the beginning of storage, although exo-PG was higher. Endo-PG activity was lower in control than delayed-storage fruit at the end of storage, while PE activity was higher, and exo-PG and EGase activities were similar. Prevention of chilling injury by delayed storage (DS) appears to be due to the ability of the fruit to continue progressive and slow cell wall degradation in storage, which allows normal ripening to proceed when the fruits are rewarmed (Negi and Handa 2008).

V. Modified atmosphere

Fruit softening is associated with the disassembly of primary cell wall and middle lamella structure. The changes in cell wall structure and composition result from the composite action of hydrolytic enzymes produced by fruits, which include PG, PE, PL, β-GAL, and cellulases. High-oxygen atmosphere retards the decrease in firmness in grapes (Deng et al. 2005), sweet cherries (Tian et al. 2004), fresh-cut carrots (Amanatidou et al. 2000), and strawberries (Wszelaki and Mitcham 2000). Deng et al. (2005) observed that decrease in firmness of grapes under different oxygen storage was accompanied by a dramatic decrease in hemicellulose and moderate decrease in cellulose and total pectins, which indicates that the softening in grapes is due to increased depolymerization and degradation of cell wall polysaccharides. At higher oxygen storage, grapes maintained firmness, which coincided with higher retention of cell wall polysaccharides. The lower levels of water-soluble pectins in high-oxygen atmosphere were correlated with delayed softening, and the activities of PG, PE, β-GAL increased to lower extent than air storage, which indicates that higher oxygen might have inhibited relative enzyme activities, reducing the degradation and depolymerization of pectin substances. Cellulase activity in grapes increased slightly over time, and its activity was slightly lower in high oxygen compare to air (Deng et al. 2005). In controlled atmosphere (CA)-stored apples, ripening-related softening was inhibited after an initial loss of firmness. However, softening resumed after transfer of apples to normal atmosphere storage at 8 °C (Negi and Handa 2008).

VI. Pathogen attack

In apple and tomato fruits, *Penicillium expansum* infection caused reduction in the molecular mass of hemicelluloses, particularly in the xyloglucan. Xyloglucan endotransglucosylase/hydrolase (XTH)-specific activity decreased drastically during the infection process in both fruits. XTH reduction during the infection might be related with the fungus attack mechanism. Decrease in activity and the consequent lower xyloglucan endotransglucosylation, together with the increase in endoglucanases, would permit fungal access to the cellulose-xyloglucan network, increase the efficiency of cellulose hydrolysis, and thus facilitate the progress of the fungal infection. Hemicellulose degradation is important in the breakdown of plant cell walls, causing cell wall loosening, increasing the porosity of the wall, and allowing the colonization of plant tissue (Miedes and Lorences 2004).

VII. Irradiation

The biological effect of gamma rays is based on the interaction with atoms or molecules in the cell, particularly water, to produce free radicals, which can damage different important compounds of plant cell. The UV-B/C photons have enough energy to destroy chemical bounds, causing a photochemical reaction. Gamma rays accelerate the softening of fruits, causing the breakdown of middle lamella in cell wall. They also influence the plastid development and function, such as starch–sugar interconversion. The penetration of UVB light into the cell is limited, while gamma rays penetrate through the cells. For this reason, UV-B light has a strong effect on surface or near-to-surface area in plant cells. Plant pigments, such as carotenoids and flavonoids, save plant cells against UV-B and gamma irradiation (Kovacs and Keresztes 2002). UV light has been used as a postharvest treatment to enhance shelf life of various fruits and vegetables. The beneficial doses of UV-C are reported to induce the accumulation of phytoalexins and activate genes, encoding pathogenesis-related proteins. Prestorage exposure of peaches with UV-C irradiation significantly reduced chilling injury. A higher accumulation of spermidine and spermine was found in peaches after UV exposure, and it is postulated that these higher levels of polyamines apparently are a response to the UV-C irradiation and might be beneficial in increasing the resistance of fruit tissue to deterioration and chilling injury (Negi and Handa 2008).

Therefore, it could be concluded that, the major postharvest problem with storage of fruits and vegetables is the excessive softening. Ripening of many fruits is mainly orchestrated by biosynthesis of ethylene that triggers a serial biochemical and physiological process inducing the softening in texture. The most important factors affecting structural deterioration of fruits and vegetables are processing, heating, physiological disorders, chilling and freezing injury, pathogen attack, modified atmosphere and irradiation.

9 Challenges in Handling Fresh Fruits and Vegetables

Postharvest losses of fruits and vegetables can be very large depending upon the product and the specific conditions. However, the same factors causing losses will also affect the health-related characteristics of the fruits and vegetables that are eaten. Sometimes there is a direct relation between the appearance of a product and its health-related properties. However, it is probable that in most cases it is impossible to judge the nutritional quality and phytochemical contents by means of our senses. Fresh fruits and vegetables are detached, but still living, plant parts that continue to respire and have metabolic activities. They also to a large extent maintain protective systems such as physical barriers and physiological defence mechanisms. This is in contrast to minimally processed fruits and vegetables that have a larger surface area with tissues that are cut through and open to microbial attack, drying and dust. If not eaten, the products have a limited lifetime before they die and decay. Eating quality is at its highest level at harvest, or it will reach a peak during the postharvest period if the particular product has a clear maturation process and has been picked unripe. Both the keeping quality, or keep ability (the ability to maintain an acceptable quality over time) and the eating quality (the quality at a certain time), as well as the rate of quality change, are determined by a series of biotic and abiotic factors pre- and postharvest. The postharvest factors include species, variety, microbial load, presence of pests, temperature, radiation exposure, atmosphere composition including relative humidity and possible mechanical stress or damage. These factors in turn affect the rate of the deterioration processes, which are essentially physiological (senescence) and microbial (Bengtsson and Matforsk 2008).

The postharvest lifetime is not just the time spent in various stores, but also includes time for washing, sorting, packaging, transport and distribution. In fact, many exported perishable plant products spend most of their time in transport, wholesale and retail. Therefore, different fresh fruits and vegetables can have postharvest lifetimes ranging from less than a week to up to a year. The shelf-life is determined as the time between a starting point (for instance harvest or beginning of distribution) and an end point when the product has reached a minimum acceptable quality for human consumption under defined storage and distribution conditions. Intake of fresh fruits and vegetables of sufficient quantity and quality is not only necessary for maintaining good human health, but is also very important for sensory satisfaction. On the other hand, sufficient sensory quality is a prerequisite for achieving a high enough intake. The present review will focus on the quality of whole fruits and vegetables related to human health as affected by storage and treatments that are common in the commercial distribution of fruit and vegetables, but will also include, when available, data on sensory quality. Health-related quality is defined as the quality related to both nutrients and other constituents that have an effect on human health, preferentially those with a health-promoting effect. The literature on storage effects on fruits and vegetables is very large and the amount of research focusing on health-related quality has increased dramatically since the 1980s (Bengtsson and Matforsk 2008).

In general, at optimum cold storage conditions vitamin C content is decreasing, whereas most phenolics, carotenoids, glucosinolates and dietary fibres are relatively stable (Table 14). Deviations from optimum conditions may indeed affect the contents of health-related constituents. Suboptimum temperature and humidity usually give rise to enhanced rates of breakdown due to increased metabolism leading to faster maturation and senescence. Some constituents, such as phenolics, can increase their content under dehydration (without change in total amount) or when exposed to visible or UV radiation (with increased total amount). Except for vitamin C and phenolics, storage effects on nutrients and health-promoting phytochemicals have not been investigated to any great extent.

Most of the postharvest research on health-related quality has focused on the effects of various factors on constituent levels. In the future, emphasis will be more on the mechanisms behind the observed changes in order to get a deeper understanding of the phenomena. This means that genomics, proteomics and metabolomics

Table 14 General effects of storage on contents of health-related constituents of fruits and vegetables

	Optimal temp.	Suboptimal temp.	Incident light	Reduced O_2	Elevated CO_2	Elevated O_2	Dehydration
Vitamin C	Decrease	Decrease	Decrease	Slower decrease			Decrease
Phenolics, fruits and vegetables	Stable	Decrease	Increase	Stable or increase	Stable or increase		Variable
Phenolics, berries	Increase	Increase	Variable	Stable or decrease	Stable or decrease	Increase	Increase
Carotenoids, fruits	Variable	Variable					
Carotenoids, carrots	Stable		Stable				Decrease
Carotenoids, green vegetables	Variable	Variable	Variable				
Glucosinolates	Stable or decrease	Decrease		Variable	Increase		Decrease
Dietary fibre	Stable	Variable					

Adapted from Bengtsson and Matforsk (2008) should be used in studies of the regulation of the metabolic pathways of constituents. This does not necessarily indicate that the goal is to create a basis for production of transgenic fruits and vegetables, which can be useful in research, but which give rise to a great deal of resistance from large consumer groups. The knowledge can be used in conventional breeding programs to select lines for optimum sensory and health-related quality and for achieving optimized pre- and postharvest treatment for each individual plant product (Bengtsson and Matforsk 2008).

Therefore, it could be concluded that, postharvest losses of fruits and vegetables can be very large depending upon the product and the specific conditions. However, the same factors causing losses will also affect the health-related characteristics of the fruits and vegetables that are eaten. The postharvest lifetime is not just the time spent in various stores, but also includes time for washing, sorting, packaging, transport and distribution. Many exported perishable plant products spend most of their time in transport, wholesale and retail. Therefore, different fresh fruits and vegetables can have postharvest lifetimes ranging from less than a week to up to a year.

10 Postharvest Handling Under Extreme Weather Conditions

The modern era of informatics and high-technology communications has allowed many growers, shippers and retailers to learn and implement practices and technologies that have had success elsewhere in the world. There have been many examples where technological protocols were "*copied on the local level*," and the results were similar to those previously experienced. However, we are also continually reminded that what is done at one site cannot be transmitted directly to another, even when similar cultivars are grown. The interesting point, and the good news for those working to improve postharvest quality of produce, is that a successful plan of production and handling to maintain quality may only need simple adjustments to produce good standards in other operations. It is paramount to stress the importance of environmental factors occurring during the hours before and immediately after harvest, particularly when those factors involve extreme conditions prevalent in places such as low deserts and rainy tropics. Research has shown the direct effects of environmental conditions during the hours before harvest on postharvest quality (Fonseca 2006).

A single stress event can produce major physical changes in the physiology and anatomy of the plant upon recovery. For example, trichomes were formed in feverfew plants after recovering from a wilting event (Fonseca et al. 2005), and grafted watermelon produced adventitious roots and aerenchyma to adapt to a single flooding (Yetisir et al. 2006). Environmental factors encountered during handling of produce that can affect final quality include extremely low or high temperatures, rain and extremely low relative humidity (RH). It is possible that in the future, with more urban areas coexisting with production fields and orchards, other factors may become more important, such as environments with high emissions of CO_2, as well as atmosphere with low pressure and high ethylene content (Fonseca 2009). It could be followed the postharvest handling under extreme weather conditions as follow:

I. ***Postharvest handling in the tropics***

Postharvest losses encountered in tropical areas with a high incidence of rainfall are likely the highest in the world. Losses in developing countries along the tropical belt are estimated to be about 50 % (FAO 1989). Interestingly, there was no significant relationship between solar radiation and mean temperature. It could be concluded that fruit that were exposed on the day of harvest, or during the 3 days before harvest, to minimum temperatures higher than 22.4 °C showed improved tolerance to quarantine heat treatments. This induced-tolerance to postharvest heat treatments has been associated with heat shock proteins. High RH and temperatures around 30°C can enhance wound healing of citrus fruits (Kinay et al. 2005). This is a major reason for the recommendation that degreening treatments occur in high RH and controlled temperatures. This beneficial effect is associated with a fast wound healing rate, triggered by the formation of lignin and the induction of antifungal compounds, such as scoparone and scopoletin. The proliferation and/or penetration in the fruit by pathogens, such as *Penicillium digitatum*, *P. italicum* and *Geotrichum candidum* is reduced when curing is induced under high RH and temperatures around 30 °C (Plaza et al. 2003). High temperatures during postharvest storage are commonly associated with high transpiration rates and subsequent degradation of quality traits. However, in the case of herbs such as marjoram even temperatures as high as 30 °C do not affect oil accumulation, but rather can stimulate synthesis. Temperatures and light intensity in tropical areas fluctuate less than in hot, dry or temperate climates. Although it is not completely clear how the low daily temperature fluctuation impacts the postharvest quality of fruit, some studies suggest that shelf life of vegetative tissue may be significantly impacted by the time of day of harvest (Fonseca 2009).

In tropical countries, the production of shoot tip cutting for export to temperate countries is a major industry. It has been found that late harvests e.g. after 4 pm improve storage quality and subsequent rooting response, an effect that was attributed to higher endogenous carbohydrate status (Rapaka et al. 2007). On the other hand, vegetative tissue is particularly sensitive to storage temperature during the hours after harvest, which may be dependent on the time of the day or harvest if cooling facilities are not available. Chlorophyll degradation significantly increases in leafy vegetables, such as Valeriana lettuce (Ferrante and Maggiore 2007) and fresh-cut rocket and spinach (Ferrante et al. 2004), when storage temperature increases by only 6°C. Diurnal changes in metabolism may explain the prolonged shelf life obtained with harvest during the late hours of the day. Xyloglucan endotransglusylase-hydrolase, a cell wall modifying protein, is more active during the late hours of the day, suggesting a role in preventing cell degradation during postharvest. Moreover, leaf–water relations are also known to fluctuate during the day. For example, stomata opening occurs in response to light and temperature which affects CO_2 uptake productivity and assimilation of carbon productions. The "turgor" hypothesis is another factor that can partially explain the importance of weather conditions at harvest. It is agreed by many that prolonged shelf life may augment with increased turgor, which may be also improved with postharvest calcium treatments (Rico et al. 2007).

Rapid cooling at sunset inhibits the export of sugars from the leaves to the fruit, which results in increased osmotic and turgor pressure. Extreme rainy conditions can have direct implications on plant disease and food safety. Clinical pathogens that invade fruit and vegetables generally benefit from high RH encountered in the field and during postharvest storage, with some exceptions, such as the case of *E. coli* O157:H7 (Stine et al. 2005). Another exception is the Hepatitis A virus, which is quite stable in the environment, with the longest survival having been reported at low RH. In contrast, high RH has been found to afford Salmonella longer survival on the surface of tomato plants (Rathinasabapathi 2004). Because of the potential risk with added moisture in tissue at harvest (Fonseca 2006), it is probably wise to schedule harvest for periods when the rain stops and there has been enough time to dry water from the surface of the tissue to be harvested. Additional concerns exist in the tropics when extreme weather conditions prevail, because efficiency of postharvest operations may decline faster as field workers in charge of harvest, selection or classification may be more at risk of dehydration (Nag et al. 2007).

II. ***Postharvest handling in the desert***

The deserts, which may be present from sea level to 3,500 m, are places where large quantities of produce are grown (Houston 2006). In all deserts, to different degrees, cold nights and/or high temperatures during the day prevail during certain times of the year. Such conditions may be even more extreme than usual during times which may coincide with the harvest of edible tissue. Production of plant crops in the desert often includes high sunlight intensity, low relative humidity (RH) and high temperatures, depending on the season in which the crop is planted, which in some cases is inadequate to support even microbial life (McKay et al. 2003). Low RH is of particular concern, since fresh-harvested commodities tend to lose weight rapidly at low RH. Changes in water status alter the general conditions of the product with economic losses being due to both decreased quality and product weight. Most fruits and vegetables become unmarketable with a loss of 5–10 % of their initial weight. The rate of water loss and subsequent weight are clearly dependent on factors such as temperature and RH. The water status of the product at harvest, in addition to extreme environmental conditions during postharvest, may affect quality (Fonseca 2009).

Water activity, the differential between vapor pressure of water in food and vapor pressure in pure water, affects the water loss rate of a fresh commodity. Higher water activity (or less negative water potential) implies higher amount of water that could be easily released to the environment in the form of water vapor pressure. Lettuce with extremely high water activity showed lower shelf life, particularly if accompanied by extremely low RH during postharvest handling (Fonseca 2006). The loss of membrane integrity associated with desiccations, leads to loss of cellular compartmentation, which consequently allows polyphenol oxidase and polyphenol substrates to mix in the damaged cells, resulting in tissue browning. Color retention and quality of beans was found to be higher when the product had lower moisture content. While initial moisture content of near 14 % yielded shelf life of lower than 10 days, initial moisture content of near 11 % produced shelf life of above 100 days (Karathanos et al. 2006).

Herbs in particular suffer great weight loss when conditions at harvest include high temperatures. The rate of transpiration in herbs is commonly 100 % higher than that of vegetable species showing intensive respiration. Freshly harvested Saint-John's Wort shows a Q10 of 2.60 over the range 10–20 °C and 1.93 over the range 20–30 °C (Bottcher et al. 2003). However, for herbs that are dried, the rapid loss of water did not commonly affect the key active compounds (Fonseca et al. 2006). The quality of asparagus spears clearly varies depending on the weather conditions prevalent during the growth cycle (Bhowmik et al. 2002), but postharvest conditions may be more critical in influencing final quality. In a greenhouse study, the variability in the monthly temperature during the growth of asparagus did not consistently show an affect on breaking force, regardless of the subsequent storage conditions. However, a clear relationship was found between the temperature during postharvest storage and the breaking force of shear asparagus spears (Bhowmik et al. 2003).

High temperatures at harvest time can affect level of decay during postharvest as observed in France with Star Ruby grapefruit (Pailly et al. 2004). Clearly, crops such as broccoli, beans, peas and asparagus harvested at an over-mature stage will be prone to excessive accumulation of fiber content, a factor that will become more pronounced if weather conditions include high temperatures. Extremely high temperatures at harvest impact hormonal levels in the marketed, edible portion of agricultural perishables. Abscisic acid (ABA) is known to fluctuate during the day. ABA seems to peak before the maximum light intensity or prior to the onset of the maximum daily temperatures (Fonseca et al. 2005). This could be explained by the high rate of photo-oxidation during late morning and afternoon hours, which exceeds that rate of synthesis of ABA (Simkin et al. 2003). It has been shown that higher ABA levels at harvest result in a shorter vase-life of cut roses. The accumulation of ABA is tissue specific, as light sources have caused changes in ABA levels in rose petals, but not in their leaves (Fonseca 2009).

Desert crops are particularly exposed to conditions that pose at least a mild stress. Water stress is one of the most common stresses that plants encounter due to excess of transpiration during certain periods of plant growth. Changes in water availability during late phases of growth are so dramatic that a single stress event can trigger alterations to the anatomy of a leaf. Formation of trichomes was observed in feverfew upon recovery from a single wilting event (Fonseca et al. 2005). If plants are under severe stress at the moment of harvest, due to extreme weather conditions or abuse through agricultural practices, the result on the postharvest quality is commonly negative. For example, French fries made from potatoes grown under stress have shown undesirable texture. However, in some cases, mild water stress at harvest under desert conditions has resulted in clear benefits for the quality of intact and fresh-cut produce such as lettuce (Fonseca 2006).

When a mist was induced during the hours prior to harvest, the low temperature breakdown of kiwifruit occurring during postharvest storage was reduced or eliminated, depending on the maturity of the fruit at harvest. This was associated with higher accumulation of chilling time before harvest (Sfakiotakis et al. 2005). Some studies suggest that the time of the year at which harvest takes place may be critical in the shelf life of crops. Increase of dry and fatty matter, polyphenol and antioxidant composition in cauliflower was obtained in a year when plants were subjected to stress conditions (Lo Scalzo et al. 2007). In the same study it was suggested that early harvested product was more suitable for storage due to the higher ration of unsaturated as compared to saturated fatty acids. Regardless of the location of apple fruits in the canopy of the tree, time of the year affected the concentration of ripening associated pigments. For example, in a November harvest content of lutein in apples was lower and violaxanthin higher than in a June harvest (Solovchenko et al. 2006).

Another factor to consider when handling fruits and vegetables in desert conditions is light. Light intensity during postharvest storage is an issue that has been overlooked for a long time; however, it could have a significant effect on final quality, particularly when excess intensity reaches the product. It is now known that harvested product may respond to light in ways that can dramatically affect quality. Solanine content in potatoes exposed to sunlight was increased as high as eight times that found in potatoes stored in the dark. The concentration of solanine obtained with varieties grown in the desert and exposed to sunlight during harvest reached levels of 150 and 90 mg/100 g, far above what has been found in other markets. Levels of solanine above 20 mg/100 g of fresh tuber are considered toxic. Using materials to prevent light from reaching the product has been suggested. The use of reflective tarpaulins for covering bins containing recently-harvested cherries reduced the incidence of pitting in cherries by nearly 20 %. In the case of cherries, water loss is faster in the stems, and the use of reflective tarpaulins for only four hours also increased moisture levels by over 15 % (Schick and Toivonen 2002).

III. Effect of drastic changes occurring during postharvest handling

Adaptation of fruit and vegetables to extreme postharvest handling conditions may be affected by previous conditions occurring during preharvest or during early stages of handling and storage. The effect can be beneficial or detrimental. Cucumbers developed no symptoms of chilling injury during postharvest handling when preharvest temperatures in the greenhouse were 5 °C higher, i.e. fruits grown at 27 °C developed symptoms, cucumber grown at 32 °C did not show any symptoms. Temperature sensitivity and ethylene production was reduced in the fruits from plants grown at the higher temperature. However, weight loss was significantly higher in cucumber grown at the higher temperature. In another study with cucumber, fruit stored at 36–40 °C for 24 h before refrigeration storage significantly reduced subsequent respiration and the appearance of pitting (Fonseca 2009).

Factors occurring before harvest affect the quality of produce. In particular, postharvest responses of fruit and vegetables to chilling stress are often greatly influenced by preharvest field temperature. These factors may be associated with biological agents, physiological mechanisms, mechanical damage, cultural practices, genetic variation or environmental factors. In fact, there are few postharvest disorders of fruits and vegetables that are not affected by preharvest factors. Treatment with air temperature at 36–40 °C reduced chilling injury of Hass avocado fruit. For high quality avocado the temperature and exposure to sunlight during preharvest is critical. For example, skin tissue on the side of the fruit exposed to the sun exhibited less chill injury and heat damage. Tolerance of postharvest heat treatment is affected by air temperatures experienced in the field 3 days before harvest. Retention of nutrients may also be improved with higher preharvest temperature.

Fruit grown in the shady inner areas of the canopy have a greater incidence of chilling injury than fruit grown in the outer areas of the canopy. This finding indicates more efficient preharvest practices to allow higher light penetration would be beneficial. Practices which could be used may include pruning and leaf removal (Forlani et al. 2002). Extremely high temperatures in destination markets during summer months may require adjustments in earlier steps in the postharvest chain to reduce deterioration of quality. Produce in transoceanic shipments often suffers from abrupt changes in temperature when arriving at the destination port. Cassava root for export markets stored at 15 °C kept for a longer time than that stored at 10 °C when the product was shipped during the summer season to the end market (Fonseca and Saborío 2001). This was likely due to a higher transpiration rate and subsequent condensation of water between the skin and paraffin wax in the product stored at lower temperatures, due to a drastic change with the extremely high ambient temperature on the patios of the brokers. The opposite was observed during the winter season. That is, low storage temperatures during transportation extended the shelf life of cassava root (Fonseca 2009).

The activities of antioxidant enzymes, such as catalase and ascorbate peroxidase, decreased under high RH (85–90 %), whereas superoxide reductase increased and glutathione reductase decreased at low RH (55–60 %), an indication of their possible role in lowering rind staining in Navelina oranges (Sala and Lafuente 2004). These observations have important implications for the desert citrus industry, as harvesting may be done during extremely low RH, and the fruit may dehydrate before being stored in coolers, which are expected to be at high RH. Extreme weather conditions trigger responses in plants that involve activity of enzymes and growth regulators. The protective function of enzymes against different stress conditions in plants has been reported (Rubio et al.

Table 15 Fruits and vegetables rich in the different groups of antioxidants

Ascorbic acid	Vitamin E	Carotenoids	Phenolics
Strawberry	Almond	Pineapple	Blueberry
Pepper	Corn	Plum	Plum
Kiwifruit	Broccoli	Peach	Raspberry
Orange	Spinach	Pepper	Strawberry
Pepper	Peanut	Mango	Apple
Broccoli	Avocado	Melon	Blackberry
Guava		Tomato	
Persimmon		Carrot	

Adapted from Vicente et al. (2009)

2002). The involvement of plant hormones in the stress-induced antioxidant system has also been reported (Table 15; Arora et al. 2002).

The time of the day of harvest may critically affect the shelf life of horticultural crops. The shelf life of sweet basil increased by almost 100 % when harvest was done late in the day compared to harvest done in the morning, a reaction that could be due partially to changes in carbohydrate composition. Accumulation of carbohydrates increases during the day as a product of photosynthesis. Increased accumulation of carbohydrates reduces CO_2 sensitivity in lettuce and chilling sensitivity in tomato seedlings. Improved shelf life due to increased extensible cell walls has been correlated to harvesting product during the late hours of the day. The same was found with leafy greens subjected to mechanical stress (Clarkson et al. 2003). Time of day of harvest has also been shown to affect the shelf life of commodities, particularly leafy greens. Shelf life was extended by as much as 6 days in arugula and 2 days in lollo rosso and red chard, when the harvest was done at the end of the day (Clarkson et al. 2005). The time of the month or the time of the day of harvest has been associated with produce quality (Fonseca 2009).

Even when following strict quality control programs, harvested products could still be subjected to stress-producing conditions during handling and storage. The stress can be a result of a single environmental factor, or the outcome of a combination of factors encountered throughout different stages of production and handling. Extreme weather conditions may cause severe losses to growers, especially when operations fail to manage quality in other steps, or when conditions are part of multiple failures. If environmental conditions at harvest and during postharvest handling differ, the result may range from severely-affected products to maximum-quality products. Although it is not possible to eliminate losses due to environmental conditions, the extent of these losses can be reduced through a better understanding of the nature of the problem and by being proactive with implementing potential solutions. Extreme weather conditions differ in nature and affect different commodities in various ways. However, based on studies described in this chapter, some general conclusions can be drawn (Table 16). Research has shown that single

Table 16 Extreme environmental conditions encountered during different postharvest steps and factors that influence the impact of those conditions on quality of the final product

Extreme environmental conditions	Potential negative impact	Factor that may enhance or reduce the impact
Extremely low relative humidity at harvest	Rapid water loss and early development of dehydration symptoms.	Water activity/potential at harvest affects the weight loss. Product with extremely high water activity shows symptoms of dehydration more quickly.
Extremely high relative humidity at harvest	Excess moisture on surface of product can result eventually in large loss of water and oxidation of tissue. It can also produce condensation of water vapor in packages, affording ideal conditions for proliferation of pathogens.	Depending on the product, a time dedicated to release excess water may aid in prolonging shelf life.
Extremely low temperature at harvest/early day hours	Freezing damage. Early day hours produce higher incidence of leaf abscission of vegetative cuttings.	Materials used to cover crops or equipment to elevate the air temperature are currently used commercially.
Extremely high temperature/sunlight intensity at harvest/late day hours	Accumulation of undesired compounds in certain crops such as potatoes. Reduced production of ethylene and subsequent loss of leaves during transition.	Use of transpirants and rapid movement of product from field to refrigeration conditions is used to ameliorate potential negative effects.
Extremely low temperature during postharvest handling	Chilling injury	Temperature during the growth of the plant can affect sensitivity. Plants grown in higher temperatures better tolerate the chilling temperatures. Postharvest heat treatments may aid in reducing sensitivity as well.
Extremely high temperature during postharvest handling	Occurrence of proliferation of plant pathogens. Microorganisms in general will be afforded better conditions to grow.	Risk is reduced if a good preharvest program to control diseases is in place, and relative humidity previous to harvest was not high.
Extremely high temperature at destination port	Abrupt raise in respiration and transpiration of the product. Condensation of water in product packages and spaces between wax and cuticles. Potential for microorganisms' growth.	Higher temperature and transpiration during transition may reduce problems by reducing condensation. Packages, films and coatings with sufficient permeability for gas diffusion is an option.

Adapted from Fonseca (2009)

extreme weather factors can be highly detrimental to the final quality of the marketed commodity. It is also clear that the potential negative impact may be ameliorated when the product is previously exposed to determined conditions (Fonseca 2009).

Therefore, it could be concluded that, environmental factors encountered during handling of fruits and vegetables produce that can affect final quality include extremely low or high temperatures, rain and extremely low relative humidity. High temperatures during postharvest storage are commonly associated with high transpiration rates and subsequent degradation of quality traits. However, in the case of herbs such as marjoram even temperatures as high as 30 °C do not affect oil accumulation, but rather can stimulate synthesis.

11 Postharvest Stress Treatments in Fruits and Vegetables

Fruits and vegetables are an important source of carbohydrates, proteins, organic acids, vitamins, and minerals for human nutrition. When humans use plants or plant parts, whether for food or for aesthetic purposes, there is always a postharvest component that leads to loss (Fallik 2004). Their losses in quantity and quality affect horticultural crops between harvest and consumption. Thus, to reduce the losses, producers and handlers must understand the biological and environmental factors involved in deterioration (Kader 1992). Fresh fruits and vegetables are living tissues subject to continuous changes after harvest. While some changes are desirable, most are not (Kader 1992). Their commodities are perishable products with active metabolism and subject to extensive postharvest losses through microbial decay, physical injury, and senescence during the postharvest period. However, postharvest changes in horticultural crops cannot be stopped, but they can be slowed within certain limits (Kader 1992). The maintenance or improvement of the postharvest life of fresh fruits and vegetables is becoming increasingly important (Imahori 2012).

Fresh fruits and vegetables are living tissues subject to continuous changes after harvest. While some changes are desirable, most are not. Their commodities are perishable products with active metabolism during the postharvest period. Proper postharvest handling plays an important role in increasing food availability. Postharvest stress treatments have been shown to be generally effective in controlling both insect and fungal pests, reducing physiological disorder or decay, delaying ripening and senescence, and maintaining storage quality in fruits and vegetables. In addition, a moderate stress not only induces the resistance to this kind of severe stress, but also can improve tolerance to other stresses. Postharvest stress treatments can, therefore, be very important to improving shelf life and quality retention during postharvest handling of fruits and vegetables.

Harvested fruits and vegetables can be potentially exposed to numerous abiotic stresses during production, handling, storage and distribution (Hodges 2003). Some of these stresses can be minor in nature, resulting in no quality loss or, in some cases, in quality improvement (Hodges et al. 2005) during distribution. However, when the abiotic stress is moderate or severe, quality losses almost always are incurred at market. As a consequence it is important to understand the nature and sources for abiotic stresses that affect fruits and vegetables. In addition, with improved understanding, options for better management or resistance become available (Toivonen 2005). One of the challenges facing fruit and vegetable production globally is that regional climate regimes are becoming more unpredictable from year to year. Hence understanding of effects of field abiotic stresses e.g. drought, extreme temperatures, light and salinity on postharvest stress susceptibility will become more important since postharvest stresses limit the storage and shelf life potential of fruits and vegetables (Toivonen 2005). It is the intent of this section to first describe the nature of pre- and post-harvest abiotic stress events, delve into their importance for product quality and marketing and then explore the technologies available to begin managing the sensitivity of fruits and vegetables to stresses they encounter in the handling and distribution chain.

I. Effect of preharvest stresses on fruits and vegetables

Abiotic stresses occurring during production can either be the primary cause (direct) for disorders that exhibit themselves during postharvest handling and storage practices or they can influence the susceptibility of a fruit or vegetable to postharvest conditions that cause abiotic stresses resulting in disorders (indirect). It is important to characterize the relationship between preharvest abiotic stresses occurring during production and postharvest abiotic stresses that the fruit or vegetable is exposed after harvest and during storage and distribution, since the solution to these different problems will be best resolved by focusing on preharvest or postharvest abiotic stress amelioration, respectively. Moderate levels of preharvest stress can potentially work towards enhancing stress resistance of a fruit or vegetable through up-regulating genes and pathways which renders the tissues cross-tolerant to many stresses which may occur subsequently in postharvest handling, storage and distribution (Toivonen and Hodges 2011).

1. Drought

The occurrence of drought conditions during production of fruit and vegetable crops is becoming more frequent with climate change patterns. While much work has been devoted to understanding of drought effects on production and productivity of these crops (Whitmore 2000), there is limited published literature on the effects of preharvest water stress on responses to postharvest stresses and hence on subsequent quality and shelf life. However, the existing literature provides some insight which may lead to better understanding and perhaps also encourage future research. Water stress during the production phase of some fruits and vegetables may affect their physiology and morphology in such a manner as to influence susceptibility to weight loss in storage. There have been both positive effects reported for field water deficits (stress) in tree fruits and root vegetables. In the case of peaches, it has been shown that lower levels of irrigation results in higher density of fruit surface trichomes and consequent lower weight losses in storage. In addition, some studies have shown that deficit irrigation of apples and pears could reduce water loss of these fruit in subsequent storage (Lopez et al. 2011) and this was attributed to reduction in skin permeance of the deficit irrigated fruit. Presumably, fruit grown under moderate water stresses imposed by deficit irrigation practices adapt by developing a less water permeable cuticle. In terms of understanding that water deficits can have negative effects on postharvest stress susceptibility, irrigation of apples has been shown to enhance apple size which was associated with lower to water losses during storage. This observation highlights a main concern about using deficit irrigation, which is the reduced size of fruit from such treatments (Lopez et al. 2011). Size of fruit is important, since larger fruit have lower surface area to volume ratios, which confers lower relative water loss. Another negative affect associated with water deficits is the case of root vegetables, such as carrot, where preharvest water stress (watering to 25–75 % of soil water field capacity) can weaken the cells, resulting in higher membrane leakage (i.e. cell damage) and consequently greater weight loss in storage (Toivonen and Hodges 2011).

2. **Plant nutrition**

There is limited literature regarding the effects of crop nutrition on the susceptibility of fruits and vegetables to postharvest abiotic stress. There is one review dealing with the effect of preharvest nutrition on postharvest physiology and disorders of fruits and vegetables (Sams and Conway 2003), however most of the reviewed literature touches on nutrition effects on postharvest biotic stress effects (i.e. disease resistance). Calcium nutrition during production has been well documented in regard to postharvest disorders of many fruits and some vegetables (Sams and Conway 2003). Calcium is also been suggested as a putative signaling molecule involved in the development of cross tolerance to abiotic stresses. Therefore, the role of preharvest calcium nutrition is postharvest stress resistance may be complex, and dependent on whether the fruit or vegetable is also exposed to environmental abiotic stresses. Potassium nutrition has been shown to have a few important effects on postharvest abiotic stress susceptibility of vegetables. In carrots, deficiency in potassium is associated with greater weight loss (desiccation stress) in storage. At levels below 1 mM potassium in the soil medium, weight loss was directly associated with increased membrane leakage (i.e. damaged cells) in the carrot tissues. Above 1 mM potassium, there were no significant differences in weight loss under standardized storage conditions. Improved potassium nutrition has also been shown to reduce susceptibility of potatoes to internal bruising in response to mechanical stresses imposed during postharvest handling. Relatively high preharvest nitrogen is often associated with poor postharvest quality of many fruits and vegetables (Sams and Conway 2003). In regards to affecting susceptibility to postharvest stress, applying higher than recommended levels of preharvest nitrogen for a specific crop have been linked to storage discoloration susceptibility in both cabbage and potato. In the case of cabbage, it appears that excessive nitrogen fertilization leads to high accumulations of zinc and aluminum and nitrate induced manganese deficiency. High nitrogen applications in the field resulted in increased incidence and severity of black midrib in cold storage, particularly for the susceptible cultivar, 'Safe keeper'. In the case of potatoes, black spot susceptibility (a consequence of bruising) is influenced by nitrogen fertilization, particularly the balance of nitrogen applied in relation to levels of potassium applied. In contrast nitrogen deficiency or lower than recommended nitrogen application rates will most often results in increased vitamin C content in many fruits and vegetables. Vitamin C content has been tightly linked with storage life potential (Hodges et al. 2001), which is likely a consequence of the importance of this antioxidant nutrient in forestalling oxidative injury that leads to quality losses in storage (Toivonen and Hodges 2011).

3. **Temperature extremes**
   Susceptibility to high heat injury inducing or low chill injury inducing, temperatures is known to be reduced by prior exposure of the sensitive fruit or vegetable to low ambient temperatures. However, if the preharvest temperature leads to chilling induced injury in the field, then susceptibility to postharvest chilling injury can be increased. Therefore, the level of the preharvest temperature extreme will be a determinant as to if the exposure will have positive or negative effects on postharvest stress sensitivity. Extreme high temperatures can occur in the field and apple fruit exposed to direct sunlight can reach in excess of 40 °C. High temperatures during the late season, leading up to harvest, can enhance susceptibility of apples to superficial scald which develops in storage. In contrast, the authors found that low temperatures in the preharvest interval could reduce susceptibility (Toivonen and Hodges 2011).
4. **Salinity**
   Tomatoes grown under high salinity will produce smaller fruit with higher soluble solids. Smaller fruit will have higher surface area to volume ratios, hence greater susceptibility to postharvest water loss i.e. desiccation stress. While there is no direct information in the literature to confirm that smaller tomato fruit from saline growing conditions would be subject to greater desiccation stress postharvest, firmness declines for tomatoes grown under 3 and 6 dS m^{-1} salinity levels were increased by 50–130 %, respectively, at 2 weeks holding at 20 °C compared with control fruit (Toivonen and Hodges 2011).
5. **Light**
   It would be considered logical to assume that effects of exposure to high light are difficult to dissociate from effects exposure to high temperatures. However, research has shown that low light (bagging of apples) in the preharvest interval reduced susceptibility of apples to developing superficial scald in cold storage and, in contrast, high ambient temperatures resulted in increased susceptibility. Generally, only sun-exposed surfaces of susceptible cultivars of apples develop scald in storage. In the case of ambient low light, when lettuce is grown under low light which is sub-optimal for photosynthetic activity, shelf life of fresh cut lettuce i.e. lettuce subjected to mechanical stress is much shorter than lettuce produced under optimal light conditions (Witkowska and Woltering 2010). Tomato size is smaller when the crop is grown under ambient low light levels, such as in the early spring season in northern latitudes (Gruda 2005) and since

surface area to volume ratio is greater in smaller fruits, susceptibility to postharvest desiccation stress would increase. Low light also results in lower levels of ascorbate in many greenhouse-grown fruits and vegetables (Gruda 2005), which would render them less fit to deal with postharvest stresses since ascorbate contents are general directly proportional to relative levels or stress tolerance (Toivonen and Hodges 2011).

II. Postharvest stresses during handling and storage

1. Temperature extremes

Postharvest temperature abuse during distribution is an ongoing challenge for many products, particularly those being shipped by air or ocean container. Breaks in cool chain temperatures can result in acceleration of climacteric ripening and softening i.e. reduction in shelf potential for apples harvested and stored at the preclimacteric stage of maturity (East et al. 2008). However, those authors also found that temperature breaks had minimal effects on apples harvested and stored at post-climacteric stage of maturity. Fruit harvested at post-climacteric stages of maturity are not generally of concern to industry since those fruit have shorter storage and shelf life potential. Chilling injury susceptibility is a significant issue for many crops derived from subtropical and tropical growing regions. Generally fruits, fruit vegetables (fruits which are consumed as vegetables) and root and tuber crops are chilling sensitive (Toivonen 2011).

There is a significant literature pertaining to chilling sensitivity of crops and much effort has been devoted to better understanding of chilling injury mechanisms and approaches to ameliorating the disorder. While there are visible (surface pitting, internal browning) and textural (accelerated softening and development of mealiness) changes often associated with chilling injury, flavor generation capacity has been shown to be a sensitive early indicator of chilling stress effects. Use of heat treatments has become popular for disinfestation and disinfection of fruits and vegetables. Appropriately applied levels of heat treatment can also induce temperature tolerance to both high and low temperatures. However, when excessively harsh heat treatments are applied, heat-induced damage can occur (Toivonen and Hodges 2011).

2. Low O_2 and high CO_2

Managing postharvest handling and storage atmospheres to avoid low O_2 or high CO_2 stress is a constant concern and the problem is more severe when handling products in modified atmosphere (MA) packages as opposed to controlled atmosphere (CA) systems since temperature is often not as easily controlled in the MA packages as the produce moves through a distribution chain (Toivonen 2009). As such, the importance atmospheric stress in postharvest systems deserves significant discussion. While low O_2 levels are well-known to induce stress-induced changes in metabolism and resultant metabolite accumulations, acute low oxygen injury is not expressed until the tissue is re-aerated and a consequent uncontrolled oxygen burst (consisting of hydrogen peroxide and other radicals) occurs, resulting in lipid peroxidation, protein denaturation and membrane injury. Different fruits and vegetables have varying thresholds for low O_2 stress, dependent on anatomy, temperature, physiological age, presence of supplemental gases e.g. CO_2, CO, SO_2, C_2H_4 and duration of exposure (Kanellis et al. 2009).

When a fruit or vegetable is converted to fresh-cut format, it generally becomes tolerant to lower levels of oxygen. One of the most notable effects of high CO_2 levels in postharvest handling is to competitively inhibit ethylene binding and action hence delaying ripening in climacteric fruits. High CO_2 will directly inhibit succinate dehydrogenase, thus impairing the functioning of the tricarboxylic acid cycle and aerobic respiration. There are numerous physiological disorders that can be attributed to high CO_2 stress, including black heart of potatoes, brown heart or core in apples and pears, surface bronzing in apples and brown stain of lettuce. High CO_2 can also modulate chilling stress, ethylene induced disorders and susceptibility to pathogenic attack (Kader and Saltveit 2003).

3. **Mechanical injury**

There are two types of mechanical injury that can be incurred during harvest and handling of fruits and vegetables; (1) cuts or punctures, and (2) impacts leading to bruises. Cuts can lead to transitory increases in respiration, ethylene production, phenolics production and cell deterioration near the site of the injury (Toivonen 2005). Several factors influence the severity and size of bruising sustained, including maturity, water potential, tissue or cellular orientation at the site of the injury, shape of the object imparting the bruising force, energy and angle of the impact, and temperature of the product (Miller 2003). Cut type injuries are most prevalent in fresh-cut fruit and vegetable products. Severity of response to cutting is very much dependant on the tissue characteristics, maturity of the fruit or vegetable of interest, the coarseness or sharpness of the cutting implement used and the temperature at which the cutting is done. Cut injuries occur during the harvest process of many fruits and vegetables and is more severe in machine-harvested as compared with hand-harvested product. Impact caused injuries are associated with loading of product for transport, events during transport (particularly when uneven or rough roads or lanes are encountered), during unloading and throughout packaging and processing lines (Miller 2003).

4. **Desiccation**

Loss of water leading to deterioration in fruit and vegetable tissues is a common issue for postharvest handling and distribution. In addition to wilting, water stress can lead to accelerated senescence or ripening which are expressed as softening of tissues, membrane deterioration and yellowing. As mentioned previously, one of the main characteristics of a fruit or vegetable that defines susceptibility to water loss is surface area to volume ratio. In beans, the density of hairs on the cuticle can modulate rates of water loss to some extent and damage to these hairs will lead to increased losses. The driving force for water loss is the vapor pressure deficit (vpd), which is the relationship that describes the difference in water activity of the fruit or vegetable and the water activity of the atmosphere surrounding it (Ben-Yehoshua and Rodov 2003). The greater the vapor pressure deficit, the greater the water loss. Three postharvest handling principles are important in minimizing water loss of any fruit or vegetable; (1) warm product loses water faster than cool product when placed into a cool room, hence the importance of rapid precooling before storage, (2) delays in cooling will lead to longer exposures to higher vapor pressure deficit conditions, hence timely cooling after harvest is of utmost importance, and (3) storing product at the coldest storage temperature and highest relative humidity possible will minimize water loss (Toivonen 2011).

Abiotic stresses are significant determinants of quality and nutritional value of fruits and vegetables during harvest, handling, storage and distribution to consumer. Crop management can have a significant influence on susceptibility to stress. In addition, climate change has created additional environmental variables which may influence postharvest stress susceptibility of fruits and vegetables. While breeding is underway for many crops to develop stress resistance that will allow them to adapt to climate change, it is not clear that breeding for stress resistance in the field will also extend stress resistance characteristics to the harvested portion. It is important understand the basis of molecular and biochemical response networks to various stresses encountered in the field and in the postharvest continuum to better evaluate the benefits that abiotic stress during production may yield for postharvest abiotic stresses. The researcher must determine whether the adaptive stress response is best tested in the field, greenhouse or test tube. In the context of postharvest handling treatments, there is some indication that such approaches may benefit in enhancing tolerance and thereby extending shelf and nutritional life of fruits and vegetables. However, again there is a limited amount of basic understanding to help guide the development of approaches to reliably confer useful levels of stress tolerance to stresses in general, and, more importantly, to specific stresses that a product is known to be subjected to during its normal distribution (Toivonen and Hodges 2011).

Therefore, it could be concluded that, abiotic stresses are significant determinants of quality and nutritional value of fruits and vegetables during harvest, handling, storage and distribution to consumer. Abiotic stresses occurring during production can either be the primary cause (direct) for disorders that exhibit themselves during postharvest handling and storage practices or they can influence the susceptibility of a fruit or vegetable to postharvest conditions that cause abiotic stresses resulting in disorders (indirect).

12 Implications of Physiology on Postharvest Handling

It is well known that, vegetables are cited as being an important part of a healthy diet. Managing retention of the quality and healthful constituents in vegetables requires a significant depth of knowledge of the postharvest physiology of these products in order to control the target processes that are responsible for constituent and quality change (Bartz and Brecht 2003). Quality is of underlying importance since poor-quality product will not be acceptable for consumption, no matter what the nutritional value of the product. There are at least three recently published entire volumes devoted to in-depth discussion of the physiology of vegetables and which provide a very detailed description of the postharvest physiology of vegetables i.e., Bartz and Brecht 2003; Lamikanra et al. 2005 and Sinha (2011).

The understanding of the physiological bases of quality retention in vegetables provides good guidance to the issues which limit the storage or shelf life of a vegetable and also good guidance to the limitations that may exist for application of certain approaches. Examples of the limitations which may exist are that sensitivity to chilling injury or high CO_2 would preclude the use of low storage temperatures or high CO_2 controlled atmospheres for such a vegetable. Therefore, it is important to define the characteristics of the vegetable in question using existing information and then develop possible strategies to enhance storage life potential as define by whatever criterion is important (sensory quality, nutritional quality, or functional quality). Low temperature storage-induced chilling injury may be more subtle in nature than development of visual defects in vegetable (Toivonen 2011). In many cases these subtle effects can be noted as changes in processing quality induced by low storage temperatures. A good example is potato which is known to sweeten in response to low temperature storage. Consequently, the storage temperature recommendations for potatoes vary significant according to the end-use cooking method. At low temperatures the storage starch is converted to sugars and therefore the potato will tend to be susceptible to unacceptable levels of browning under normal chipping conditions. Another consequence of this sweetening is on the pasting quality attributes when the potatoes are being used for potato flour starch manufacture. Low temperature storage starch loss significant reduces the pasting quality of flour made from those potatoes (Kaur et al. 2007). It is therefore, important to understand the physiological responses of vegetables to the storage conditions in direct relation to the end use for which they are intended.

Water loss is one of the most visible changes in vegetables, often being the factor that limits marketing life (Shamaila 2005). Notional estimates of threshold water loss that renders a particular vegetable unacceptable have been listed in older publications and some of these values are presented in Table 17. The table illustrates several general principles about the morphology of a vegetable and the threshold for water loss before the quality is visually unacceptable. First, bulky organs such as tubers, bulbs, head-forming leafy vegetables, and topped root vegetables have a relatively high threshold of water loss, whereas loose leafy vegetables and bunched root vegetables (i.e., vegetables having leafy tops attached) have relatively

Table 17 Classification of vegetable types by anatomical description, and maximum permissible water loss threshold for saleability of selected vegetables at commercial maturity

Vegetable type	Maximum percent water loss	Common name listing of examples
Root	4	Carrot (bunched), beet (bunched)
	7–8	Carrot (topped), beet (topped), parsnip
Bulb	10	Onion
Tuber	7	Potato (cured and immature)
Stem	8	Asparagus
Leafy	3–5	Lettuce, spinach, rhubarb
	7–10	Celery, cabbage, leek, watercress, Brussels sprout
Floral	4	Broccoli
	7	Cauliflower
Immature fruit	5	Peas, cucumber, snap beans
	7	Peppers, sweet corn
Mature fruit	7	Tomato

Adapted from Toivonen (2011)

lower threshold values for unacceptable water loss. A large part of this difference is that loose leafy structures are more prone to show wilting than bulky organs (Ben-Yehoshua and Rodov 2003).

If water loss is identified as a quality issue for vegetables, it can easily be resolved by "recharging" the vegetable with water, a practice that is prevalent via overhead misting systems in North American retail produce displays. The practice of misting is supported by research literature. It has demonstrated that water content can be increased and quality maintained by "recharging" carrots with cold water under simulated retail display conditions. Barth et al. (1990) found that misting broccoli under simulated retail conditions not only maintained visual quality, but also enhanced ascorbic acid retention. From these examples it can be seen that water loss is potentially a simple problem to resolve by "recharging;" however, if level of loss exceeds a cell injury threshold, rehydration may not be possible (Shibairo et al. 2002). Another aspect of water loss is that the size of the vegetable has a significant influence (Toivonen 2011).

Generally, smaller vegetables have a higher surface area to volume ratio (Ben-Yehoshua and Rodov 2003). Consequently, vegetables such as carrot and pepper will have greater weight loss when they are less mature and smaller in size (Dìaz-Pèrez et al. 2007). Therefore, size grades of vegetables will have differential shelf life potentials based on water loss rates. The response of an individual vegetable to controlled or modified atmosphere may determine what the optimal atmosphere is and also whether it should be co-packaged with another vegetable, even if they are closely related botanically. Selection of cultivars for their suitability for either storage or processing normally requires a complete analysis of all the physiological characteristics that define the vegetables suitability for the desired use. For example, in selecting butter head lettuce for fresh-cut use, it was established that cultivars having both lower respiration rates and lower sensitivity to high carbon dioxide injury were the most suitable. Most often there is more than one physiological characteristic that determines the overall acceptability of a vegetable cultivar for the intended use. If all the characteristics required are identified when selecting new cultivars, then there is a greater chance that that cultivar will have a consistent acceptability for that intended use over the long term. Genetic transformation platforms may provide avenues to accelerate vegetable quality improvement for fresh storage and processing uses in the future, once the molecular mechanisms for quality are better understood (Pech et al. 2005).

Therefore, it could be concluded that, vegetable quality and nutritional value are determined by the physiological characteristics of the vegetable. Suitability for end use, including storage capability, shelf life potential, and acceptability for processing either minimal or secondary processing are also very much determined by the physiological characteristics. Therefore, knowledge of the physiological profile of the selected vegetables can be a powerful tool to assist in optimizing commercial utilization.

13 Climate Changes and Potential Impacts on Vegetable Postharvest

Due to its importance around the globe, agriculture was one of the first sectors to be studied in terms of potential impacts of climate change. Many alternatives have been proposed to growers aimed at minimizing losses in yield. However, few studies have addressed changes in postharvest quality of fruits and vegetable crops associated with these alterations. Nowadays, climate changes, their causes and consequences, gained importance in many other areas of interest for sustainable life on Earth. The subject is, however, controversial.

According to studies carried out by the Intergovernmental Panel on Climate Change (IPCC), average air temperatures will increase between 1.4 and 5.8 °C by the end of this century, based upon modeling techniques that incorporated data from ocean and atmospheric behavior (IPCC 2001). The possible impacts of this study, however, are uncertain since processes such as heat, carbon, and radiation exchange among different ecosystems are still under investigation. Less drastic estimates predict temperature increase rates of 0.088 °C per decade for this century (Kalnay and Cai 2003). Other investigators forecast for the near future that rising air temperature could induce more frequent occurrence of extreme drought, flooding or heat waves than in the past (Assad et al. 2004).

In most tropical regions temperature effects were so far lower, but important changes in rain distribution has occurred in Australia, Asia and Africa. In such events monsoon weakening was observed across Asia and Africa, playing an important role by making rain more scarce and irregular, at a point that climate changes are pointed as one of the most important causes of recent famines in areas such as the Sahel region of Africa. Higher temperatures can increase the capacity of air to absorb water vapor and, consequently, generate a higher demand for water. Higher evapotranspiration indices could lower or deplete the water reservoir in soils, creating water stress in plants during dry seasons. For example, water stress is of great concern in fruit production, because trees are not irrigated in many production areas around the world. It is well documented that water stress not only reduces crop productivity but also tends to accelerate fruit ripening (Henson 2008).

Exposure to elevated temperatures can cause morphological, anatomical, physiological, and, ultimately, biochemical changes in plant tissues and, as a consequence, can affect growth and development of different plant organs. These events can cause drastic reductions in commercial yield. However, by understanding plant tissues physiological responses to high temperatures, mechanisms of heat tolerances and possible strategies to improve yield, it is possible to predict reactions that will take place in the different steps of fruit and vegetable crops production, harvest and postharvest. Besides increase in temperature and its associated effects, climate changes are also a consequence of alterations in the composition of gaseous constituents in the atmosphere. Carbon dioxide (CO_2), also known as the most important greenhouse gas, and ozone (O_3) concentrations in the atmosphere are changing during the last decade and are affecting many aspects of fruit and vegetable crops production around the globe (Lloyd and Farquhar 2008).

Carbon dioxide concentrations are increasing in the atmosphere during the last decades. The current atmospheric CO_2 concentration is higher than at any time in the past 420,000 years. Further increases due to anthropogenic activities have been predicted. Carbon dioxide concentrations are expected to be 100 % higher in 2100 than the one observed at the pre-industrial era (IPCC 2007). Ozone concentration in the atmosphere is also increasing. Even low-levels of ozone in the vicinities of big cities can cause visible injuries to plant tissues as well as physiological alterations (Felzer et al. 2007). The above mentioned climate changes can potentially cause postharvest quality alterations in fruit and vegetable crops. Although many researchers have addressed climate changes in the past and, in some cases, focused postharvest alterations, the information is not organized and available for postharvest physiologists and food scientists that are interested in better understanding how these changes will affect their area of expertise (Moretti et al. 2010).

In this section, it could be reviewed how changes in ambient temperature and levels of carbon dioxide and ozone can potentially impact the postharvest quality of fruit and vegetable crops.

I. Effects of temperature on postharvest quality of fruit and vegetable

Fruit and vegetable growth and development are influenced by different environmental factors. During their development, high temperatures can affect photosynthesis, respiration, aqueous relations and membrane stability as well as levels of plant hormones, primary and secondary metabolites. Seed germination can be reduced or even inhibited by high temperatures, depending on the species and stress level. Most of the temperature effects on plants are mediated by their effects on plant biochemistry. For plants that are subjected to water deficit, temperature is a physical facilitator for balancing sensible and latent heat exchange at the shoot, which is modulated by relative humidity and by wind. Most of the physiological processes go on normally in temperatures ranging from 0 to 40 °C. However, cardinal temperatures for the development of fruit and vegetable crops are much narrower and, depending on the species and ecological origin, it can be pushed towards 0 °C for temperate species from cold regions, such as carrots and lettuce. On the other hand, they can reach 40 °C in species from tropical regions, such as many cucurbits and cactus species (Moretti et al. 2010).

Higher than normal temperatures affect the photosynthetic process through the modulation of enzyme activity as well as the electron transport chain (Sage and Kubien 2007). Additionally, in an indirect manner, higher temperatures can affect the photosynthetic process increasing leaf temperatures and, thus, defining the magnitude of the leaf-to-air vapor pressure difference (D), a key factor influencing stomatal conductance (Lloyd and Farquhar 2008). Photosynthetic activity is proportional to temperature variations. High temperatures can increase the rate of biochemical reactions catalyzed by different enzymes. However, above a certain temperature threshold, many enzymes lose their function, potentially changing plant tissue tolerance to heat stresses. Temperature is of paramount importance in the establishment of a harvest index. The higher the temperature during the growing season, the sooner the crop will mature (Moretti et al. 2010).

Exposure of fruit and vegetable crops to high temperatures can result in physiological disorders and other associated internal and external symptoms (Table 18).

It is also well known that exposure of fruit to temperature extremes approaching 40 °C can induce metabolic disorders and facilitate fungal and bacterial invasion. Although symptoms of heat injury and disease incidence are easily observed at the end of storage, the incipient incidence of these disorders is often

Table 18 Symptoms of heat and solar injury of fruit and vegetable crops

Crop	Symptoms
Snap bean	Brown and reddish spots on the pod; spots can coalesce to form a water-soaked area
Cabbage	Outer leaves showing a bleached, papery appearance; damaged leaves are more susceptible to decay
Lettuce	Damaged leaves assume papery aspect; affected areas are more susceptible to decay; tipburn is a disorder normally associated with high temperatures in the field; it can cause soft rot development during postharvest
Muskmelon	Characteristic sunburn symptoms: dry and sunken areas; green color and brown spots are also observed on rind
Bell pepper	Sunburn: yellowing and, in some cases, a slight wilting
Potato	Black heart: occur during excessively hot weather in saturated soil; symptoms usually occur in the center of the tuber as dark-gray to black discoloration
Tomato	Sunburn: disruption of lycopene synthesis; appearance of yellow areas in the affected tissues
Apple	Skin discoloration, pigment breakdown and water-soaked areas
Avocado	Skin and flesh browning; increased decay susceptibility
Lime	Juice vesicle rupture; formation of brown spots on fruit surface
Pineapple	Flesh with scattered water-soaked areas; translucent fruit flesh

Adapted from Moretti et al. (2010)

not recognized in time to effect corrective treatment. In general, visible evidence of heat injury on tomatoes appears as yellowish-white patches on the side of fruits (Table 18).

II. Effects of CO_2 exposure on fruit and vegetable postharvest quality

The Earth's atmosphere consists basically of nitrogen (78.1 %) and oxygen (20.9 %), with argon (0.93 %) and carbon dioxide (0.031 %) comprising next most abundant gases. Nitrogen and oxygen are not considered to play a significant role in global warming because both gases are virtually transparent to terrestrial radiation. The greenhouse effect is primarily a combination of the effects of water vapor, CO_2 and minute amounts of other gases (methane, nitrous oxide, and ozone) that absorb the radiation leaving the Earth's surface (IPCC 2001). The warming effect is explained by the fact that CO_2 and other gases absorb the Earth's infrared radiation, trapping heat. Since a significant part of all the energy emanated from Earth occurs in the form of infrared radiation, increased CO_2 concentrations mean that more energy will be retained in the atmosphere, contributing to global warming (Lloyd and Farquhar 2008). Carbon dioxide concentrations in the atmosphere have increased approximately 35 % from pre-industrial times to 2005 (IPCC 2007).

Many papers published during the last decade have clearly associated global warming with the increase in carbon dioxide concentration in the atmosphere. Changes in CO_2 concentration in the atmosphere can alter plant tissues in terms of growth and physiological behavior. Many of these effects have been studied in detail for some vegetable crops. These studies concluded, in summary, that increased atmospheric CO_2 alters net photosynthesis, biomass production, sugars and organic acids contents, stomatal conductance, firmness, seed yield, light, water, and nutrient use efficiency and plant water potential (Table 19). Högy and Fangmeier (2009) studied the effects of high CO_2 concentrations on the physical and chemical quality of potato tubers. They observed that increases in atmospheric CO_2 (50 % higher) increased tuber malformation in approximately 63 %, resulting in poor processing quality, and a trend towards lower tuber greening (around 12 %). Higher CO_2 levels (550 μmol CO_2/mol) increased the occurrence of common scab by 134 % but no significant changes in dry matter content, specific gravity and underwater weight were observed. Higher (550 μmol CO_2/mol) concentrations of CO_2 increased glucose (22 %), fructose (21 %) and reducing sugars (23 %) concentrations, reducing tubers quality due to increased browning and acryl amide formation in French fries. They also observed that proteins, potassium and calcium levels were reduced in tubers exposed to high CO_2 concentrations, indicating loss of nutritional and sensory quality (Table 19).

III. Effects of O_3 exposure on fruit and vegetable postharvest quality

Ozone in the troposphere is the result of a series of photochemical reactions involving carbon monoxide (CO), methane (CH_4) and other hydrocarbons in the presence of nitrogen species ($NO+NO_2$). It forms during periods of high temperature and solar irradiation, normally during summer seasons (Mauzerall and Wang 2001). It is also formed, naturally during other seasons, reaching the peak

Table 19 Physiological and quality parameters of fruit and vegetable crops affected by exposure to increased CO_2 and the effect of O_3

Physiological or quality parameter	Effect of high CO_2	Product	Effect of O_3	Product
Photosynthesis	↑	Potato; spinach;	↓	Strawberry; conifers; hardwoods
Respiration	↓	Asparagus; broccoli; mung bean sprouts; blueberries; tomato; pears	↑	Blueberry; broccoli; carrot
	↑	Potato; lettuce, eggplant; lemons; cucumber; mango		
	=	Apple		
Firmness	=	Tomato	↑	Cucumber
	↑	Strawberry; raspberry		
Starch	↑	Potato	=	Potato
Citric Acid	↓	Potato; tomato	↑	Potato
Malic Acid	↓	Potato; tomato	↓	Potato
Ascorbic acid	↑	Potato; strawberry; orange; tomato	↓	Potato
Reducing sugars	↑	Potato; tomato	↓	Potato
Nitrate	↓	Potato; celery; leaf lettuce; Chinese cabbage	↑	Potato
Ripening	↓	Tomato		
Stomatal conductance	↓	Spinach		
Color intensity	↑	Grape		
Dry matter	↑	Potato		
Alcohol	↑	Grape; mango; pear		
Titratable Acidity	=	Grape; mango; pear		
Total phenolics	↑	Grape; strawberry		
Anthocyanins	↑	Grape; strawberry		
Glycoalkaloids	↓	Potato		
pH	=	Grape		
Volatile compounds	↓	Mango		
Antioxidant capacity	↓	Scallion; strawberry		
Visible injury			↑	Black cherry
Viscosity			↑	Potato
Electrolyte leakage			↑	Persimmon
Sucrose			=	Potato; carrot
N, P			=	Potato
K, Mg			↑	Potato

Different effects on these crops including increasing (↑) or decreasing (↓) or neutral effect (=) (Adapted from Moretti et al. 2010) of natural production in the spring. However, higher concentrations of atmospheric ozone are found during summer due to increase in nitrogen species and emission of volatile organic compounds. Concentrations are at maximum values in the late afternoon and at minimum values in the early morning hours, notably in industrialized cities and vicinities. The opposite phenomenon occurs at high latitude sites. Another potential source for increased levels of ozone in a certain region is via the movement by local winds or downdrafts from the stratosphere (Moretti et al. 2010).

The effects of ozone on vegetation have been studied both under laboratory and field experiments. Stomatal conductance and ambient concentrations are the most important factors associated with ozone uptake by plants. Ozone enters plant tissues through the stomates, causing direct cellular damage, especially in the palisade cells (Mauzerall and Wang 2001). The damage is probably due to changes in membrane permeability and may or may not result in visible injury, reduced growth and, ultimately, reduced yield. Visible injury symptoms of exposure to low ozone concentrations include changes in pigmentation, also known as bronzing, leaf chlorosis, and premature senescence (Felzer et al. 2007).

Since leafy vegetable crops are often grown in the vicinity of large metropolitan areas, it can be expected that increasing concentrations of ozone will result in increased yellowing of leaves (Table 19). Leaf tissue stressed in this manner could affect the photosynthetic rate, production of biomass and, ultimately, postharvest quality in terms of overall appearance, color and flavor compounds. Greatest impacts in fruit and vegetable crops may occur from changes in carbon transport. Underground storage organs e.g., roots, tubers, bulbs normally accumulate carbon in the form of starch and sugars, both of which are important quality parameters for both fresh and processed crops. If carbon transport to these structures is restricted, there is great potential to lower quality in such important crops as potatoes, sweet potatoes, carrots, onions and garlic. Quality attributes and sensory characteristics were evaluated on tomato fruits cv. Carousel after ozone exposure (concentration ranging from 0.005 to 1.0 μmol/mol) at 13 °C and 95 % RH. Soluble sugars (glucose, fructose), fruit firmness, weight loss, antioxidant status, CO_2/H_2O exchange, ethylene production, citric acid, vitamin C (pulp and seed) and total phenolic content were not significantly affected by ozone treatment when compared to fruits kept under ozone-free air. A transient increase in b-carotene, lutein and lycopene content was observed in ozone-treated fruit, though the effect was not consistent. Sensory evaluation revealed a significant preference for fruits subjected to low-level ozone-enrichment (0.15 μmol/mol) (Tzortzakisa et al. 2007).

Finally, it could be concluded that rising levels of CO_2 also contribute to global warming, by entrapping heat in the atmosphere. Prolonged exposure to CO_2 concentrations could induce higher incidences of tuber malformation and increased levels of sugars in potato and diminished protein and mineral contents, leading to loss of nutritional and sensory quality. Increased levels of ozone in the atmosphere can lead to detrimental effects on postharvest quality of fruit and vegetable crops. Elevated levels of O_3 can induce visual injury and physiological disorders in different species, as well as significant changes in dry matter, reducing sugars, citric and malic acid, among other important quality parameters (Moretti et al. 2010).

Therefore, it could be concluded that, exposure of fruit and vegetable crops to high temperatures can result in physiological disorders and other associated internal and external symptoms. Changes in ambient temperature and levels of carbon dioxide and ozone can potentially impact the postharvest quality of fruit and vegetable crops.

14 Postharvest Biotechnology

As an emerging technology, the plant tissue culture has a great impact on both agriculture and industry, through providing plants needed to meet the ever increasing world demand. It has made significant contributions to the advancement of agricultural sciences in recent times and today they constitute an indispensable tool in modern agriculture. Biotechnology has been introduced into agricultural practice at a rate without precedent. Tissue culture allows the production and propagation of genetically homogeneous, disease-free plant material. Cell and tissue *in vitro* culture is a useful tool for the induction of somaclonal variation. Genetic variability induced by tissue culture could be used as a source of variability to obtain new stable genotypes. Interventions of biotechnological approaches for *in vitro* regeneration, mass micropropagation techniques and gene transfer studies in tree species have been encouraging. *In vitro* cultures of mature and/or immature zygotic embryos are applied to recover plants obtained from inter-generic crosses that do not produce fertile seeds. Genetic engineering can make possible a number of improved crop varieties with high yield potential and resistance against pests. Genetic transformation technology relies on the technical aspects of plant tissue culture and molecular biology for:

- Production of improved crop varieties.
- Production of disease-free plants (virus).
- Genetic transformation.
- Production of secondary metabolites.
- Production of varieties tolerant to salinity, drought and heat stresses.

The past decades of plant cell biotechnology has evolved as a new era in the field of biotechnology, focusing on the production of a large number of secondary plant products. During the second half of the last century the development of genetic engineering and molecular biology techniques allowed the appearance of improved and new agricultural products which have occupied an increasing demand in the productive systems of several countries worldwide (James 2008). Nevertheless, these would have been impossible without the development of tissue culture techniques, which provided the tools for the introduction of genetic information into plant cells. Nowadays, one of the most promising methods of producing proteins and other medicinal substances, such as antibodies and vaccines, is the use of transgenic plants. Transgenic plants represent an economical alternative to fermentation-based production systems. Plant-made vaccines or antibodies (plantibodies) are especially striking, as plants are free of human diseases, thus reducing screening costs for viruses and bacterial toxins. The number of farmers who have incorporated transgenic plants into their production systems in 2008 was 13.3 million, in comparison to 11 million in 2007.

Plant cell culture has made great advances. Perhaps the most significant role that plant cell culture has to play in the future will be in its association with transgenic plants. The ability to accelerate the conventional multiplication rate can be of great benefit to many countries where a disease or some climatic disaster wipes out crops. The loss of genetic resources is a common story when germplasm is held in field gene banks. Slow growth *in vitro* storage and cryopreservation are being proposed as solutions to the problems inherent in field genebanks. If possible, they can be used with field gene banks, thus providing a secure duplicate collection. They are the means by which future generations will be able to have access to genetic resources for simple conventional breeding programs, or for the more complex genetic transformation work. As such, it has a great role to play in agricultural development and productivity (Hussain et al. 2012).

Fruits, being highly nutritive, are important component of human diet but they possess very short post-harvest shelf life. As ripen, they become very soft and more prone to injuries, which make them highly perishable. Over 30 % of the annual produce in India is wasted due to spoilage. Hence, there is an urgent need to develop technologies to overcome post-harvest losses of fruits. Physiologists and biochemists attempted to extend the shelf life of fruits by different means though the results were not very satisfying. It was demonstrated recently that a judicious dose of γ-irradiation (0.1–0.5 kGy) could enhance the shelf life to fruits by about a week to a fortnight, which could help in minimizing the spoilage during storage and transportation. However, stringent quality controls have to be strictly followed to get the best results. Studies revealed that γ-irradiation brings alterations/changes in metabolic pathways, which delay the production of essential precursors and energy required for ripening of fruits. Another strategy, to enhance the shelf life of fruits, could be adopted through regulation of endogenous ethylene production. Most recent studies have shown that it could be achieved by such genetically modified (GM) crops where gene expression of key enzymes responsible for ripening, like PG-ase, EFE and ACC-synthase, by means of antisense RNAs. However, adoption of this technology has so far been deterred due to apprehensions of safety issues associated with GM crops. An alternate method for prevention of spoilage of fruits as well as sustaining the interests in farmers could be the value addition of fruit commodities. This could be achieved by improving the conventional methods as well as development of non-conventional products of commercial interest (Surendranathan 2005).

Fruits become susceptible to damage on ripening for they soften drastically. A ripened fruit, depending on the variety, has a limited shelf life of a few days to

1 or 2 weeks. Besides, the soft and fleshy nature makes a ripened fruit more susceptible to injuries, increasing the losses further due to spoilage. Hence, it is necessary to develop appropriate technology and infrastructure for proper storage and transportation of the fruits. One of the reasons for the low commercial activity in the fruit and vegetable sector is the lack of organized cultivation. As a result, the total domestic production of fruits and vegetables reaches only domestic market. Many a times, fruits like Indian local varieties of mangoes, bananas, etc., do not attract commercial interests due to lack of proper postharvest management. Some fruits like jackfruits and cashew apple are under-utilized to the extent of 80 % and spoiled as they fall around the tree causing environmental problems. Thus, there is an urgent need to develop technologies to overcome postharvest losses of fruits. One way of achieving this could be by developing feasible technology to extend the postharvest shelf life. Another alternative is the value addition of the produce by developing innovative products of consumer interest. For either of these, it is important to have a fair assessment of the available technologies as well as an understanding of the physiology and biochemistry of fruits ripening (Surendranathan 2005).

Therefore, it could be concluded that, there is an urgent need to develop technologies to overcome post-harvest losses of fruits. Physiologists and biochemists attempted to extend the shelf life of fruits by different means though the results were not very satisfying. It was demonstrated recently that a judicious dose of γ-irradiation (0.1–0.5 kGy) could enhance the shelf life to fruits by about a week to a fortnight, which could help in minimizing the spoilage during storage and transportation.

15 Conclusion

The fruit processing industry is one of the major businesses in the world. While basic principles of fruit processing have shown only minor changes over the last few years, major improvements are now continuously occurring, and more efficient equipment capable of converting huge quantities of fruits into pulp, juice, dehydrated, frozen, refrigerated products, etc. make possible the preservation of products for year-round consumption. The fruit processing and storage, even under the most industrially available "mild conditions," involves physical and chemical changes that negatively modify the quality. Postharvest management is a set of post-production practices that includes: cleaning, washing, selection, grading, disinfection, drying, packing and storage. These eliminate undesirable elements and improve product appearance, as well as ensuring that the product complies with established quality standards for fresh and processed products. There is an urgent need to develop technologies to overcome postharvest losses of fruits. One way of achieving this could be by developing feasible technology to extend the postharvest shelf life.

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References

Amanatidou A, Slump RA, Gorris LGM, Smid EJ (2000) High oxygen and high carbon dioxide modified atmospheres for shelf-life extension of minimally processed carrots. J Food Sci 65:61–66

Arora A, Sairam RK, Srivastava GC (2002) Oxidative stress and antioxidative system in plants. Curr Sci 82:1227–1238

Assad ED, Pinto HS, Zullo J Jr, Ávila AMH (2004) Impacto das mudanças climáticas no zoneamento agroclimático do café no Brasil. Pesquisa Agropecuária Brasileira 39:1057–1064

Barth MM, Perry AK, Schmidr SJ, Klein BP (1990) Misting effects on ascorbic acid retention in broccoli during cabinet display. J Food Sci 55:1187–1191

Bartz JA, Brecht JK (2003) Postharvest physiology and pathology of vegetables. Marcel Dekker, Inc, New York, p 733

Bengtsson GB, Matforsk AS (2008) Storage and handling of fruit and vegetables for optimum health-related quality. In: Tomás-Barberán FA, Gil MI (eds) Improving the health-promoting properties of fruit and vegetable products. Woodhead Publishing Limited/CRC Press LLC, Cambridge, England, pp 412–430

Ben-Yehoshua S, Rodov V (2003) Transpiration and water stress. In: Bartz JA, Brecht JK (eds) Postharvest physiology and pathology of vegetables, 2nd edn. Marcel Dekker, Inc, New York, pp 111–159. ISBN 0-8247-0687-0

Bhowmik PK, Matsui T, Kawada K, Suzuki H (2002) Changes in storage quality and shelf life of green asparagus over an extended harvest season. Postharvest Biol Technol 26:323–326

Bhowmik PK, Matsui T, Suzuki H, Kosugi Y, Alam AK, Enriquez FG (2003) Pre- and postharvest fiber development in green asparagus as related to temperature during growth, storage and handing. Food Agric Environ 1:50–53

Bottcher H, Gunther I, Kabelitz L (2003) Physiological postharvest responses of common Saint-John's wort herbs (*Hypericum perforatum* L.). Postharvest Biol Technol 29:342–350

Burg SP (2004) Postharvest physiology and hypobaric storage of fresh produce. CABI Publishing/CAB International, Wallingford

Clarkson GJJ, O'Byrne EE, Rothwell SD, Taylor G (2003) Identifying traits to improve postharvest processability in baby leaf salad. Postharvest Biol Technol 30:287–298

Clarkson GJJ, Rothwell SD, Taylor G (2005) End of day harvest extends shelf life. Hortscience 40:1431–1435

De Long JM, Prange RK (2003) Storage. In: Thomas B, Murphy DJ, Murray BG (eds) Encyclopedia of applied plant sciences. Elsevier Ltd, Oxford/San Diego. doi:10.1016/B0-12-227050-9/00346-X

Deng Y, Wu Y, Li Y (2005) Changes in firmness, cell wall composition and cell wall hydrolases of grapes stored in high oxygen atmospheres. Food Res Int 38:769–776

Dhatt AS, Mahajan BVC (2007) Horticulture post harvest technology harvesting, handling and storage of horticultural crops. Punjab Horticultural Postharvest Technology Centre, Punjab Agricultural University Campus, Ludhiana. http://nsdl.niscair.res.in/bitstream/123456789/314/4/Revised+Harvesting,+Handling+and+Storage.pdf

Dìaz-Pèrez JC, Muy-Rangel MD, Mascorro AG (2007) Fruit size and stage of ripeness affect postharvest water loss in bell pepper fruit (*Capsicum annuum* L.). J Sci Food Agric 87:68–73

East AR, Tanner DJ, Maguire KM, Mawson AJ (2008) The influence of breaks in storage temperature on 'Cripps Pink' (Pink Lady™) apple physiology and quality. HortScience 43(3):818–824, ISSN 0018–5345

Fallik E (2004) Prestorage hot water treatments immersion, rinsing and brushing. Postharvest Biol Technol 32:125–134

FAO (1989) Prevention of postharvest food losses fruits, vegetables and root crops, a training manual, FAO training series no. 17/2. FAO, Rome
FAO (2004) Manual for the preparation and sale of fruits and vegetables: from field to market, FAO agricultural services bulletin no. 151. Food and Agriculture Organization of the United Nations, Rome
FAO (2009) Course on agribusiness management for producers' associations. Module 4 – Post-harvest and marketing. Santacoloma P, Roettger A, Tartanac F (eds) Training materials for agricultural management, marketing and finance, vol 8. Food and Agriculture Organization of the United Nations, Rome
Felzer BS, Cronin T, Reilly JM, Melillo JM, Wang X (2007) Impacts of ozone on trees and crops. C R Geosci 339:784–798
Ferrante A, Maggiore T (2007) Chlorophyll *a* fluorescence measurements to evaluate storage time and temperature of *Valeriana* leafy vegetables. Postharvest Biol Technol 45:73–80
Ferrante A, Incrocci L, Maggini R, Serra G, Tognoni F (2004) Colour changes of fresh-cut leafy vegetables during storage. J Food Agric Environ 2:40–44
Fonseca JM (2006) Postharvest quality and microbial population of head lettuce as affected by moisture at harvest. J Food Sci 71:M45–M49
Fonseca JM (2009) Postharvest handling under extreme weather conditions. In: Florkowski WJ, Prussia SE, Shewfelt RL, Brueckner B (eds) Postharvest handling: a systems approach, Food science and technology series. Academic Press, Elsevier Inc., New York, USA, pp 539–559
Fonseca JM, Saborío D (2001) Postharvest technology of waxed cassava (*Manihot esculenta* L.) for export markets (Spanish). National Press of Costa Rica, Costa Rica
Fonseca JM, Rushing JW, Rajapakse NC, Thomas RL, Riley MB (2005) Parthenolide and abscisic acid synthesis in feverfew are associated but environmental factors affect them dissimilarly. J Plant Physiol 162:485–494
Fonseca JM, Rushing JW, Thomas RL, Riley MB, Rajapakse NC (2006) Postproduction stability of parthenolide in feverfew (*Tanacetum parthenium*). J Herbs Spices Med Plants 12:139–152
Forlani M, Basile B, Cirillo C, Iannini C (2002) Effects of harvest date and fruit position along the tree canopy on peach fruit quality. Acta Hortic 592:459–466
Friboulet A, Thomas D (2005) Systems biology – an interdisciplinary approach. Biosens Bioelectron 20:2404–2407
Gould K (2004) Nature's Swiss army knife: the diverse protective roles of anthocyanins in leaves. J Biomed Biotechnol 5:314–320
Gruda N (2005) Impact of environmental factors on product quality of greenhouse vegetables for fresh consumption. Crit Rev Plant Sci 24(3):227–247, ISSN 0735–2689
Henson R (2008) The rough guide to climate change, 2nd edn. Penguin Books, London, p 384
Hertog MLATM, Rudell DR, Pedreschi R, Schaffer RJ, Geeraerd AH, Nicolai BM, Ferguson I (2011) Where systems biology meets postharvest. Postharvest Biol Technol 62:223–237
Hodges DM (2003) Postharvest oxidative stress in horticultural crops. Food Products Press, New York. ISBN 1-56022-962-4
Hodges DW, Forney CF, Wismer WV (2001) Antioxidant responses in harvested leaves of two cultivars of spinach differing in senescence rates. J Am Soc Hortic Sci 126:611–617
Hodges DM, Lester GE, Munro KD, Toivonen PMA (2005) Oxidative stress: importance for postharvest quality. HortScience 39(5):924–929, ISSN 0018–5345
Högy P, Fangmeier A (2009) Atmospheric CO_2 enrichment affects potatoes: 2 tuber quality traits. Eur J Agron 30:85–94
Houston J (2006) Evaporation in the Atacama Desert: an empirical study of spatiotemporal variations and their causes. J Hydrol 330:402–412
Hussain A, Qarshi IA, Nazir H, Ullah I (2012) Plant Tissue Culture: Current Status and Opportunities. In: Leva A, Rinaldi LMR (eds) Recent advances in plant *in vitro* culture. InTech, Rijeka, pp 1–28, doi.org/10.5772/52760
Imahori Y (2012) Postharvest stress treatments in fruits and vegetables. In: Ahmad P, Prasad MNV (eds) Abiotic stress responses in plants: metabolism, productivity and sustainability. Springer, New York, pp 347–358. doi:10.1007/978-1-4614-0634-1

IPCC (2001) Working group II: impacts, adaptations and vulnerability. http://www.grida.no/climate/ipcc_tar/wg2/005.html. Accessed 13.03.09.

IPCC (2007) In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) The physical science basis. contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, 996p

Jaganath IB, Crozier A (2008) Overview of health-promoting compounds in fruit and vegetables. In: Tomás-Barberán FA, Gil MI (eds) Improving the health-promoting properties of fruit and vegetable products, Woodhead publishing series in food science, technology and nutrition no. 157. Woodhead Publishing Limited/CRC Press LLC, Cambridge, pp 3–37

James C (2008) Global status of commercialized biotech/GM crops. ISAAA Brief No. 39, Ithaca, NY, p 243

Jobling J (2002) Postharvest management of fruit and vegetables. Good Fruit and Vegetables Magazine, January 2002, Melbourne, Australia, Sydney Postharvest Laboratory, Sydney Postharvest Laboratory Information Sheet. www.postharvest.com.au/. 22 Aug 2012

Kader AA (1992) Postharvest biology and technology: an overview. In: Kader AA (ed) Postharvest technology of horticultural crops. UC Publication No. 3311. University of California, Division of Agriculture and Natural Resources, Oakland, pp 15–20

Kader AA (1993) Postharvest handling. In: Preece JE, Read PE (eds) The biology of horticulture – an introductory textbook. Wiley, New York, pp 353–377

Kader AA (1995) Maturity, ripening, and quality relationships of fruit-vegetables. ISHS Acta Horticult 434:249–256, Strategies for Market Oriented Greenhouse Production

Kader AA, Saltveit ME (2003) Atmosphere modification. In: Bartz JA, Brecht JK (eds) Postharvest physiology and pathology of vegetables, 2nd edn. Marcel Dekker, Inc, New York, pp 229–246. ISBN 0-8247-0687-0

Kalnay E, Cai M (2003) Impact of urbanization and land-use change on climate. Nature 423:528–531

Kanellis AK, Tonutti P, Perata P (2009) Biochemical and molecular aspects of modified and controlled atmospheres. In: Yahia E (ed) Modified and controlled atmospheres for storage, transportation and packaging of horticultural commodities. CRC Press, Boca Raton, pp 553–567. ISBN 978-1-4200-6957-0

Karathanos VT, Bakalis S, Kyritsi A, Rods PS (2006) Color degradation of beans during storage. Int J Food Prop 9:61–71

Kaur L, Singh J, Singh N, Ezekiel R (2007) Textural and pasting properties of potatoes (*Solanum tuberosum* L.) as affected by storage temperature. J Sci Food Agric 87:520–526

Kinay P, Yildiz F, Sen F, Yildiz M, Karacali I (2005) Integration of pre- and postharvest treatments to minimize *Penicilium* decay of Satsuma mandarins. Postharvest Biol Technol 37:31–36

Kitinoja L, Gorny J (1998) Post-harvest technology for fruits and vegetables Produce marketers: economic opportunities. Quality and Food Safety by, Department of Pomology, University of California, Davis. A joint publication of UC Post harvest Outreach Program and Punjab Horticultural Post harvest technology Centre, USAID/ACE

Kitinoja L, Kader AA (2002) Small-scale postharvest handling practices: a manual for horticultural crops, 4th edn, Postharvest horticulture series no. 8E. University of California, Davis Postharvest Technology Research and Information Center, Davis

Kovacs E, Keresztes A (2002) Effect of gamma and UV-B/C radiation on plant cells. Micron 33:199–210

Lamikanra O, Imam S, Ukuku D (2005) Produce degradation: pathways and prevention. Taylor & Francis, Boca Raton, p 677

Lloyd J, Farquhar GD (2008) Effects of rising temperatures and [CO_2] on the physiology of tropical forest trees. Philos Trans R Soc Biol Sci 363:1811–1817

Lo Scalzo R, Bianchi G, Genna A, Summa C (2007) Antioxidant properties and lipidic profile as quality indexes of cauliflower (*Brassica oleracea* L. var. *botrytis*) in relation to harvest time. Food Chem 100:1019–1025

Lopez G, Larrigaudière C, Girona J, Behboudian MH, Marsal J (2011) Fruit thinning in 'conference' pear grown under deficit irrigation: implications for fruit quality at harvest and after cold storage. Sci Hortic 129(1):64–70, ISSN 0304–4238

Lurie S, Zhou HW, Lers A, Sonego L, Alexandrov S, Shomer I (2003) Study of pectin esterase and changes in pectin methylation during normal and abnormal peach ripening. Physiol Plant 119(2):287–294

Mauzerall DL, Wang X (2001) Protecting agricultural crops from the effects of tropospheric ozone exposure: reconciling science and standard setting in the United States, Europe, and Asia. Annu Rev Energy Environ 26:237–268

McKay CP, Friedmann EI, Gomez-Silva B, Caceres-Villanueva L, Andersen DT, Landheim R (2003) Temperature and moisture conditions for life in the extreme arid region of the Atacama Desert: four years of observations including the El Nino of 1997–1998. Astrobiology 3:393–406

Miedes E, Lorences EP (2004) Apple (*Malus domestica*) and tomato (*Lycopersicum esculentum*) fruits cellwall hemicelluloses and xyloglucan degradation during *Penicillium expansum* infection. J Agric Food Chem 52(26):7957–7963

Miller AR (2003) Harvest and handling injury: physiology, biochemistry, and detection. In: Bartz JA, Brecht JK (eds) Postharvest physiology and pathology of vegetables, 2nd edn. Marcel Dekker, Inc, New York, pp 177–208. ISBN 0-8247-0687-0

Moretti CL, Mattos LM, Calbo AG, Sargent SA (2010) Climate changes and potential impacts on postharvest quality of fruit and vegetable crops: a review. Food Res Int 43:1824–1832. doi:10.1016/j.foodres.2009.10.013

Nag PK, Nag A, Ashtekar SP (2007) Thermal limits in men in moderate to heavy work in tropical farming. Ind Health 45:107–117

Negi PS, Handa AK (2008) Structural deterioration of the produce: the breakdown of cell wall components. In: Paliyath G, Murr DP, Handa AK, Lurie S (eds) Postharvest biology and technology of fruits, vegetables, and flowers, vol 978-0-8138-0408-8/2008, 1st edn. Wiley-Blackwell Publishing, Ames. ISBN 978-0-8138-0408-8/2008

Pailly O, Tison G, Amouroux A (2004) Harvest time and storage conditions of "Star Ruby" grapefruit (*Citrus paridisi* Macf.) for short distance summer consumption. Postharvest Biol Technol 34:65–73

Paliyath G, Murr DP (2008a) Common fruits, vegetables, flowers, and their quality characteristics. In: Paliyath G, Murr DP, Handa AK, Lurie S (eds) Postharvest biology and technology of fruits, vegetables, and flowers, 1st edn. Wiley-Blackwell Publishing, Ames. ISBN 978-0-8138-0408-8/2008

Paliyath G, Murr DP (2008b) Biochemistry of fruits. In: Paliyath G, Murr DP, Handa AK, Lurie S (eds) Postharvest biology and technology of fruits, vegetables, and flowers, 1st edn. Wiley-Blackwell Publishing, Ames. ISBN 978-0-8138-0408-8/2008

Paliyath G, Murr DP, Handa AK, Lurie S (2008) Postharvest biology and technology of fruits, vegetables, and flowers, 1st edn. Wiley-Blackwell Publishing, Ames. ISBN 978-0-8138-0408-8/2008

Pech J-C, Bernadac A, Bouzayen M, Latche A (2005) Use of genetic engineering to control ripening, reduce spoilage, and maintain quality of fruits and vegetables. In: Ben-Yehoshua S (ed) Environmentally friendly technologies for agricultural produce quality. Taylor & Francis, Boca Raton, pp 397–438

Plaza P, Usall J, Texido-Vinas T (2003) Control of green and blue mold by curing on oranges during ambient and cold storage. Postharvest Biol Technol 28:195–198

Prasanna V, Prabha TN, Tharanathan RN (2007) Fruit ripening phenomena–an overview. Crit Rev Food Sci Nutr 47(1):1–19

Rapaka VK, Faust JE, Dole JM, Runkle ES (2007) Effect of time of harvest on postharvest leaf abscission in Lantana (*Lantana camara* L. "Dallas Red") unrooted cuttings. HortScience 42:304–308

Rathinasabapathi B (2004) Survival of Salmonella Montevideo on tomato leaves and mature green tomatoes. J Food Prot 67:2277–2279

Rico D, Martin-Diana AB, Frيas JM, Henehan GTM, Barry-Ryan C (2007) Effect of ozone and calcium lactate treatments on browning and textura properties of fresh-cut lettuce. J Sci Food Agric 86:2179–2188

Robertson A, Tirado C, Lobstein T, Jermini M, Knai C, Jensen J, Ferro-Luzzi A, James WPT (2004) Food and health in Europe: a new basis for action, European series, no. 96. WHO Regional Publications, Copenhagen

Rolle RS (2006) Processing of fruits and vegetables for reducing postharvest losses and adding value. In: Postharvest management of fruit and vegetables in the Asia-Pacific Region. Food and Agriculture Organization of the United Nations Agricultural and Food Engineering Technologies Service, Rome

Rubio MC, Gonzalez MC, Minchin FR, Webb KJ, Arrese-Igor C, Ramos J, Becana B (2002) Effects of water stress on antioxidant enzymes of leaves and nodules of transgenic alfalfa overexpressing superoxide dismutase. Physiol Plant 115:531–540

Ruoyi K, Zhifang Y, Zhaoxin L (2005) Effect of coating and intermittent warming on enzymes, soluble pectin substances and ascorbic acid of *Prunus persica* (cv. Zhonghuashoutao) during refrigerated storage. Food Res Int 38(3):331–336

Sage RF, Kubien D (2007) The temperature response of C_3 and C_4 photosynthesis. Plant Cell Environ 30:1086–1106

Sala JM, Lafuente MT (2004) Antioxidant enzymes activities and rind staining in "Navelina" oranges as affected by storage relative humidity and ethylene conditioning. Postharvest Biol Technol 31:277–285

Sams CE, Conway WS (2003) Preharvest nutritional factors affecting postharvest physiology. In: Bartz JA, Brecht JK (eds) Postharvest physiology and pathology of vegetables, 2nd edn. Marcel Dekker, Inc, New York, pp 161–176. ISBN 0-8247-0687-0

Sargent SA, Ritenour MA, Brecht JK (2000) Handling, cooling, and sanitation techniques for maintaining postharvest quality. University of Florida, Coop Exten Serv, HS719. Available online at: http://www.gladescropcare.com/postharvest quality.pdf. Accessed 21 June 2010

Schick JL, Toivonen PMA (2002) Reflective tarps at harvest reduce stem browning and improve fruit quality of cherries during subsequent storage. Postharv Biol Technol 25:117–121

Sfakiotakis E, Chlioumis G, Gerasopoulos D (2005) Preharvest chilling reduces low temperature breakdown incidence of kiwifruit. Postharvest Biol Technol 38:169–174

Shamaila M (2005) Water and its relation to fresh produce. In: Lamikanra O, Imam S, Ukuku D (eds) Produce degradation: pathways and prevention. Taylor & Francis, Boca Raton, pp 267–291

Shao XF, Tu K, Zhao YZ, Chen L, Chen YY, Wang H (2007) Effects of pre-storage heat treatment on fruit ripening and decay development in different apple cultivars. J Hort Sci Biotechnol 82(2):297–303

Shibairo SI, Upadhyaya MK, Toivonen PMA (2002) Changes in water potential, osmotic potential, and tissue electrolyte leakage during mass loss in carrots stored under different conditions. Sci Hortic 95:13–21

Simkin AJ, Zhu C, Kuntz M, Sandmann G (2003) Light–dark regulation of carotenoids biosynthesis in pepper (*Capsicum annum*) leaves. J Plant Physiol 160:439–443

Simson SP, Straus MC (2010) Post-harvest technology of horticultural crops. Oxford Book Company/Mehra Offset Press, Delhi

Sinha NK (2011) Handbook of vegetables and vegetable processing. Wiley-Blackwell Publishing, Ames. ISBN 978-0-8138-1541-1

Sirivatanapa S (2006) Packaging and transportation of fruits and vegetables for better marketing. In: Postharvest management of fruit and vegetables in the Asia-Pacific Region. Food and Agriculture Organization of the United Nations Agricultural and Food Engineering Technologies Service, Rome

Smith RC (2010) Vegetable maturity dates, yields and storage. H-912 (Revised). NDSU Extension Service, North Dakota State University Fargo, ND 58108. http://www.ag.ndsu.edu/pubs/plantsci/hortcrop/h912.pdf/30.12.2012

Solovchenko AE, Avertcheva OV, Merzlyak MN (2006) Elevated sunlight promotes ripening-associated pigment changes in apple fruit. Postharvest Biol Technol 40:183–189

Stine SW, Song I, Choi C, Gerba CP (2005) Effect of relative humidity on preharvest survival of bacterial and viral pathogens on the surface of cantaloupe, lettuce and bell peppers. J Food Prot 68:1352–1358
Surendranathan KK (2005) Postharvest biotechnology of fruits with special reference to banana – perspective and scope. Indian J Biotechnol 4:39–46
Tian SP, Jiang AL, Xu Y, Wang YS (2004) Responses of physiology and quality of sweet cherry fruit to different atmospheres in storage. Food Chem 87:43–49
Toivonen PMA (2005) Postharvest storage procedures and oxidative stress. HortScience 39(5):938–942, ISSN 0018–5345
Toivonen PMA (2009) Benefits of combined treatment approaches to maintaining fruit and vegetable quality. Fresh Prod 3(1):58–64, ISSN 1749–4788
Toivonen PMA (2011) Postharvest physiology of vegetables. In: Hui YH, Sinha N, Ahmed J, Evranuz EÖ, Siddiq M (eds) Handbook of vegetables and vegetable processing. Wiley-Blackwell Publishing, Ames, pp 199–220. ISBN 978-0-8138-1541-1
Toivonen PMA, Hodges DM (2011) Abiotic stress in harvested fruits and vegetables. In: Shanker AK, Venkateswarlu B (eds) Abiotic stress in plants – mechanisms and adaptations. InTech Europe, pp 39–58. ISBN 978-953-307-394-1
Tzortzakisa N, Borlanda A, Singletona I, Barnes J (2007) Impact of atmospheric ozone-enrichment on quality-related attributes of tomato fruit. Postharvest Biol Technol 45(3):317–325
Vicente AR, Manganaris GA, Sozzi GO, Crisosto CH (2009) Nutritional quality of fruits and vegetables. In: Florkowski WJ, Prussia SE, Shewfelt SL, Brueckner B (eds) Postharvest handling: a systems approach, Food Science and Technology Series. Academic Press, Elsevier Inc., New York, USA, pp 57–106
Watkins CB (2003) Postharvest physiological disorders of fresh crops. In: Thomas B, Murphy DJ, Murray BG (eds) Encyclopedia of applied plant sciences. Elsevier Ltd, San Diego. ISBN 978-0-12-227050-5
Whitmore JS (2000) Drought management on farmland. Kluwer Academic Publishers, Dordrecht. ISBN 0-7923-5998-4
Witkowska I, Woltering EJ (2010) Pre-harvest light intensity affects shelf-life of fresh-cut lettuce. Acta Horticulturae 877:223–227, ISSN 0567–7572
Wszelaki AL, Mitcham EJ (2000) Effects of super atmospheric oxygen on strawberry fruit quality and decay. Postharvest Biol Technol 20:125–133
Yetisir H, Caliskan ME, Soylu S, Sakar M (2006) Some physiological and growth responses of watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai] grafted onto *Lagenaria siceraria* to flooding. Envrion Exp Bot 58:1–8

Herbicides: History, Classification and Genetic Manipulation of Plants for Herbicide Resistance

Sharad Vats

Abstract Weeds have known to affect human activities including agriculture since ages. Looking at the global scenario the major contributors of crop loss are weeds, followed by animals and pathogens. World War II started the 'chemical era' for the development of herbicide. Herbicides are used to kill weeds and are still the largest product type accounting for 47.6 % of global pesticide sales followed by insecticide (29.4 %), fungicide (17.5 %) and others (5.5 %). Herbicides have been classified in various ways but classification based on site of action of herbicide is comparatively better as herbicide resistance management can be handled more properly and effectively. Commonly used herbicides globally are generally broad spectrum and non-selective which restricts their use in arable lands. Thus there is need to modify the crops genetically so that the crop plants remain unaffected by herbicide application. Different strategies have been used either individually or in combination to develop transgenic plants. Reports suggest that the global area covered by herbicide-resistant transgenic plant outnumbers the area covered by any other genetically modified plants. However, inadvertent use of herbicides has helped in evolution of resistant weeds. This problem is now a subject of discussion worldwide. Integrated herbicide management is important to avoid selection pressure for the evolution of resistance. Transgenic plants have really helped the mankind in increasing the crop yield but use of transgenic plant is still a debatable topic which needs meticulous research, observations and experimentations for a final statement either in favour or against.

Keywords Weeds • Herbicides • Transgenic plants • Resistant weeds • Integrated herbicide management

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1 Introduction

1.1 Weeds and Other Pests: A Serious Issue

Weeds can be defined as plants which are undesirable, persistent, damaging and interfere with growth of other crop plants (Fig. 1) thus affecting human activities, agriculture, natural processes and economy of the country. These plants influence the produce of farmers in several ways detailed below:

- Compete for light, moisture and nutrients affecting quality and quantity of produce
- Interfere with and damage harvesting equipment
- Harbours pests and diseases
- Toxic properties of weeds cause health problems to humans and animals
- Contaminate aquatic resources
- Interferes and adversely affects natural ecosystem.

Human race got familiar to weeds since they started to cultivate crops around 10000 B.C (Hay 1974) and almost simultaneously these unwanted plants were recognized as a problem. This initiated the conflict between mankind and weeds. The agriculture in tropics is maximally affected by weeds still they are comparatively underestimated crop pests. Out of the total annual loss of agricultural produce in India, weeds share almost 37 % followed by insects, diseases and other pests (Yaduraju 2006; Fig. 2). Looking at the global scenario the major contributors of crop loss are again weeds, followed by animals and pathogens (Oerke 2006; Fig. 3).

Fig. 1 Luxuriant growth of weeds

Fig. 2 Loss of agricultural produce in India by different pests

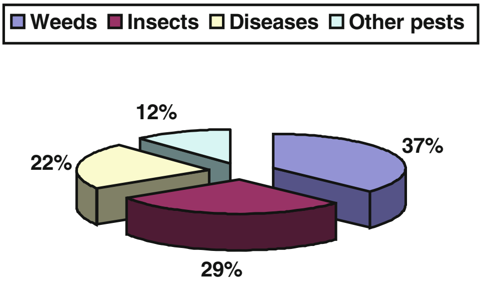
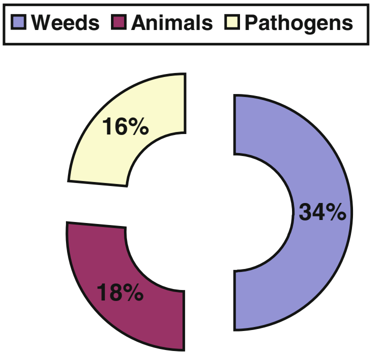


Fig. 3 World-wide crop loss by different pests



Bridges (1992) reported that weeds cause crop loss in United States to an estimated value of $6 billion per annum. Later he added that impact of weeds on the US economy exceeds $20 billion annually (Bridges 1994). Annual loss of about Rs. 1,980 crores to Indian agriculture is caused by weeds, which is higher than cumulative losses caused by insects, pests, and diseases (Yadav and Malik 2005). Soil deterioration in terms of nutrient depletion is another problem. It has been reported that weeds deplete nutrients from soil to the level of 11.0, 3.0 and 10.0 kg/ha of N, P_2O_5 and K_2O respectively (Gautham and Mishra 1995). The ever-increasing world population adds yet another bitter taste to this problematic situation. The world population has increased from 3.5 billion in 1970 to 8 billion in 2011 and is estimated to rise to 9 billion people, which means that the food production should increase by 70 % (FAO 2009). This requires intensified farming which will put further pressure on depleting ground water in the major agricultural producing regions of the USA, India, Pakistan and China (FAO 2011). Weeds are added threat to this issue. Berca stated that 'weeds eat the food of about 1 billion inhabitants' (Pacanoski 2007). The obvious risk of increased pest introductions due to various factors viz., climate change, increased global trade and increased human mobility cannot be ruled out, which poses serious threat to future crop loss.

1.2 Herbicides, the Highest Selling Pesticide

One of the solutions to the aforesaid problem is the use of pesticides. Pesticide is a rather wider term used to define substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest/s. Under United States law, a pesticide is also intended for use as a plant regulator, defoliant, or desiccant. Pesticides include herbicides for destroying unwanted vegetation (weeds), insecticides for controlling a wide variety of insects, fungicides used to prevent molds and mildew, disinfectants for preventing the spread of bacteria, and substances used to control mice and rats. According to United States Environmental Protection Agency (USEPA) pests include various organisms like insects, mice and other animals, weeds, fungi, microorganisms such as bacteria and viruses and prions (USEPA 2013).

A study revealed that that market value of global pesticide sales in 2008 increased to $40,475,000,000. Europe has the maximum share (31.7 %) followed by Asia, Latin America, North America, and Africa (Fig. 4). Herbicide is still the largest product type (Fig. 5), accounting for 47.6 % of global pesticide sales followed by Insecticide (29.4 %), Fungicide (17.5 %) and others (5.5 %) (CLS 2000). Global sales forecast is estimated to

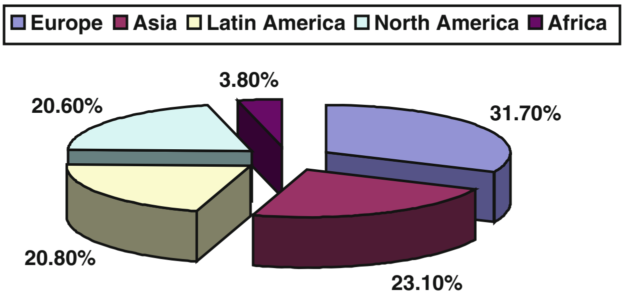


Fig. 4 Global pesticide sales in different regions of the world

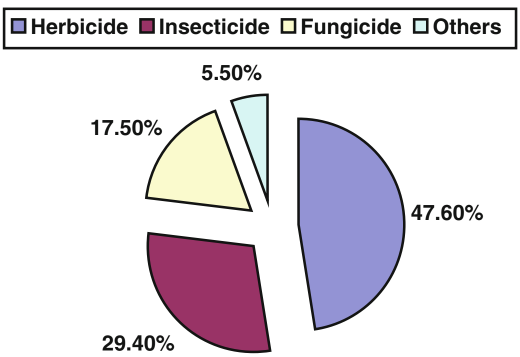


Fig. 5 Global sales of pesticide

reach $59 billion in 2016, according to *World Agricultural Pesticides,* a new study from The Freedonia Group (http://www.farmchemicalsinternational.com/article/30890/global-demand-for-agricultural-pesticides-to-approach-59-billion-in-2016).

1.3 History of Herbicides

History of herbicides starts with the advent of agriculture. In early days of farming humans had to spent good amount of their energy to remove weeds from arable lands so that suitable conditions are provided for the optimum growth of desired crops. Simultaneously the thought about weed management or control started to occupy minds of the then farmers.

Hay (1974) gave six stages in evolution of weed control:

1. 10000 B.C. – Removing weeds by hand
2. 6000 B.C. – The use of primitive hand tools to till the land and destroy weeds
3. 1000 B.C. – Animal-powered implements like harrows
4. 1920 A.D. – Mechanically-powered implements like cultivators, blades, harrows, finger-weeders, rotary-hoes, rod-weeders, etc.
5. 1930 A.D. – Biological control, and
6. 1947 A.D. – Chemical control, with the commercial development of organic herbicides such as 2, 4-D and MCPA.

Initially man used to remove weeds by hand. Around 6000 B.C. primitive hand tools replaced the use of bare hands to destroy weeds. Then it was the era of the use of animals like oxen and horses to pull harrows (tools) in 1000 B.C. The early Romans (164 B.C.) used salt to destroy the agriculture in Carthage. English farmers in sixteenth century used salt to selectively kill thistle in wheat fields and remove weeds from garden paths (Lowery 1987).

Use of chemicals as herbicide is not new. Chemicals, in crude form, such as oil wastes, rock salts, crushed arsenical ores, copper salts, and sulfuric acid have been in use for weed eradication from railway tracks, car roads and timber yards (Green et al. 1987). These herbicides used to destroy all the plants and can be categorized as non-selective. Also, the treated plot of land remained toxic to the plants for good period of time. Unfortunately these chemicals could not be used in arable lands because of the adverse effect on crop plants. Thus, application of selective herbicide that specifically kill only weeds came into existence.

Bolley in the United States, Schultz in Germany and Bonnett in France initiated research on way back in 1900 (Klingman et al. 1982) and concluded that inorganic compounds and solutions of copper salts selectively control broadleaf weeds in cereals.

When plant growth regulators were discovered nobody would have believed that these chemicals can be a probable herbicide. Plant growth was one the most important topics of study for the early Botanists. Darwin from England, Boysen-Jensen from Denmark did the famous experiment on oat coleoptile and phototropism.

It was Went who found that the chemical present in oat coleoptile is an active growth regulator (Went and Thimann 1937). This gave an impetus to research in this area and now growth regulators as herbicides is one the key areas of research. Went once stated that, "When I worked 25 years ago with the growth hormone (2,4-D), I had many wild ideas about what it might do once it was available in large quantities, but I never dreamed that it would lead to the development of weed killers. This is an excellent example, how fundamental research may lead to the solution of very practical problems" (Andersen 1991).

The chemical synthesis of 2, 4-dichlorophenoxyacetic acid (2, 4-D) was described by Pokorny in 1941. Thereafter other salts and esters of 2, 4-D was developed. This growth regulator came in prime news during World War II when scientists from United States and England initiated research on plant growth regulators. This actually started the 'chemical era' for the development of herbicides. Templeman and Sexton in 1940s reported that phenoxyacetic acids were toxic to dicotyledonous, but not monocotyledonous plants. Later, 2, 4-D came in commercial use in U.S.A. and MCPA for use in Europe from 1947 (Rao 2000). Farmers use 2, 4-D as a selective killer of broadleaf dicot plants but not monocots (grass species).

DNOC (dinitro compound), a contact herbicide, was used in France during 1993 for selective weed control. It acted against annual weeds without damaging cereals appreciably. DNOC is not translocated in plants. It was not effective against perennial weeds because their root system remained unaffected, which could later develop shoots. DNOC and other dinitro compounds played a big part in increasing food production during World War II as herbicides (Cremlyn 1991). Success in the use of these herbicides persuaded the scientists and industries as well to put money and mind in herbicides related researches, which is still in progress.

2 Classification

Herbicides are classified/grouped in various ways e.g. according to the chemical family, activity, method of application, site of action or timing of application.

2.1 Classification Based on Translocation

2.1.1 Systemic/Translocated

These herbicides are extensively translocated in the plant through its vascular system along with water, nutrients and other materials from site of absorption to sites of action. Systemic herbicides are more effective on perennial weeds than contact herbicides. Unlike contact herbicides which are fast acting, systemic herbicides require longer time period (days or weeks) to kill weeds. Glyphosate and glufosinate are nonselective systemic herbicides. 2,4-D and dicamba are examples of selective systemic herbicides.

2.1.2 Non-systemic/Contact

These herbicides kill only the portion of plant tissue that is in contact. These are not translocated through the plant. Uniform spray coverage and particle size are essential for adequate application. They are less effective on perennial plants, which are able to regrow from rhizomes, roots or tubers. Repeated application of contact herbicide is needed to kill regrowth of underground plant parts. These are comparatively fast acting herbicides e.g. bromoxynil and bentazon are contact herbicides.

2.2 Classification Based on Time of Application

2.2.1 Preplant

Preplant herbicides are nonselective herbicides applied to soil before planting and gets mechanically incorporated into the soil. The objective for incorporation is to prevent dissipation through photodecomposition and/or volatility. The herbicides kill weeds as they grow through the herbicide treated zone. Volatile herbicides have to be incorporated into the soil before planting the pasture. Agricultural crops grown in soil treated with a preplant herbicide include tomatoes, corn, soybeans and strawberries. Soil fumigants like metam-sodium and dazomet are in use as preplant herbicides.

2.2.2 Preemergence

Preemergence herbicides are applied before the weed seedlings emerge through the soil surface. Herbicides do not prevent weeds from germinating but they kill weeds as they grow through the herbicide treated zone by affecting the cell division in the emerging seedling. Dithopyr and Pendimethalin are Preemergence herbicides. Weeds that have already emerged before application or activation are not affected by pre-herbicides as their primary growing point escapes the treatment.

2.2.3 Postemergence

These herbicides are applied after weed seedlings have emerged through the soil surface and generally require multiple applications for adequate control. They can be foliar or root absorbed, selective or nonselective, contact or systemic. Liquid formulations of herbicides are more effective than granular formulations. Application of these herbicides is avoided during rain because the problem of being washed off to the soil makes it ineffective. 2,4-D is a selective, systemic, foliar absorbed Postemergence herbicide.

2.3 Classification Based on Method of Application

2.3.1 Soil Applied

Herbicides applied to the soil are usually taken up by the root or shoot of the emerging seedlings and are used as preplant or preemergence treatment. Several factors influence the effectiveness of soil-applied herbicides. Weeds absorb herbicides by both passive and active mechanism. Herbicide adsorption to soil colloids or organic matter often reduces its amount available for weed absorption. Positioning of herbicide in correct layer of soil is very important, which can be achieved mechanically and by rainfall. Herbicides on the soil surface are subjected to several processes that reduce their availability. Volatility and photolysis are two common processes that reduce the availability of herbicides. Many soil applied herbicides are absorbed through plant shoots while they are still underground leading to their death or injury. Thiocarbamates (e.g. EPTC) and dinitroanilines (e.g. trifluralin) are soil applied herbicides.

2.3.2 Foliar Applied

These are applied to portion of the plant above the ground and are absorbed by exposed tissues. These are generally postemergence herbicides and can either be translocated (systemic) throughout the plant or remain at specific site (contact). External barriers of plants like cuticle, waxes, cell wall etc. affect herbicide absorption and action. Glyphosate, 2,4-D and dicamba are foliar applied herbicide.

2.4 Classification Based on Specificity

2.4.1 Selective Herbicides

They control or suppress certain plants without affecting the growth of other plants species. Selectivity may be due to translocation, differential absorption, physical (morphological) or physiological differences between plant species. 2,4-D, mecoprop, dicamba control many broadleaf weeds but remains ineffective against turfgrasses.

2.4.2 Non-selective Herbicides

These herbicides are not specific in acting against certain plant species and kill all plant material with which they come into contact. They are used to clear industrial sites, waste ground, railways and railway embankments. Paraquat, glufosinate, glyphosate are non-selective herbicides.

2.5 Classification Based on Site of Action

Herbicides are often classified according to their site of action, because as a general rule, herbicides within the same site of action class will produce similar symptoms on susceptible plants. Classification based on site of action of herbicide is comparatively better as herbicide resistance management can be handled more properly and effectively.

Weed Science Society of America (WSSA) was developed by Retzinger and Mallory-Smith (1997). They proposed herbicide classification according to site of action with a view that it would help in dealing with herbicide resistance management. In order to differentiate herbicides with the same site of action each class was given a group number. The International Herbicide Resistance Action Committee (HRAC) also published a classification system based on letters for each group (Schmidt 1998). Mallory-Smith and Retzinger (2003) updated the classification and also included some herbicides listed in the *Weed Science Society of America 2002 Herbicide Handbook* but not sold in North America. Adequate changes were done to align this classification in accordance with classification published by HRAC (Table 1). Basic structures of some herbicides have been given in Fig. 6.

Table 1 Classification of herbicides according to site of action

Group	Site of action	Chemical family	Common name	Trade name[a]
1(A)	Inhibitors of acetyl CoA carboxylase (ACCase)	Aryloxyphenoxy propionate	Clodinafop	Discover (USA), Horizon (Canada)
			Cyhalofop-butyl	Clincher (registration pending)
			Diclofop	Hoelon, Various
			Fenoxaprop	Whip, Acclaim, Various
			Fluazifop-P	Fusilade 2000, Fusilade DX
			Haloxyfop	Not sold in North America
			Propaquizafop	Not sold in North America
			Quizalofop-P	Assure
		Cyclohexanedione	Alloxydim	Not sold in North America
			Butroxydim	Not sold in North America
			Clethodim	Select, Prism
			Cycloxydim	Not sold in North America
			Sethoxydim	Poast, Poast Plus
			Tralkoxydim	Achieve

(continued)

Table 1 (continued)

Group	Site of action	Chemical family	Common name	Trade name[a]
2(B)	Inhibitors of acetolactate synthase (ALS), also called acetohydroxyacid synthase (AHAS)	Imidazolinone	Imazamethabenz	Assert
			Imazamox	Beyond, Raptor
			Imazapic	Cadre, Plateau
			Imazapyr	Arsenal
			Imazaquin	Scepter, Image
			Imazethapyr	Pursuit
		Pyrimidinylthio-benzoate	Bispyribac-sodium	Not sold in North America
			Pyrithiobac	Staple
			Pyribenzoxim	Not sold in North America
		Sulfonylamino-carbonyltriazolinone	Flucarbazone-sodium	Everest
			Propoxycarbazone	Olympus, Attribute
		Sulfonylurea	Amidosulfuron	Not sold in North America
			Azimsulfuron	Not sold in North America
			Bensulfuron	Londax
			Chlorimuron	Classic
			Chlorsulfuron	Glean, Telar
			Cinosulfuron	Not sold in North America
			Cyclosulfamuron	Not sold in North America
			Ethametsulfuron	Muster
			Ethoxysulfuron	Not sold in North America
			Flazasulfuron	Not sold in North America
			Flupyrsulfuron-methyl-sodium	Not sold in North America
			Foramsulfuron	Not sold in North America
			Halosulfuron	Permit, Battalion
			Iodosulfuron	Not sold in North America
			Mesosulfuron	Osprey
			Metsulfuron	Ally, Escort
			Nicosulfuron	Accent
			Primisulfuron	Beacon
			Prosulfuron	Peak, Exceed

(continued)

Table 1 (continued)

Group	Site of action	Chemical family	Common name	Trade name[a]
			Pyrazosulfuron-ethyl	Not sold in North America
			Rimsulfuron	Titus
			Sulfometuron	Oust
			Sulfosulfuron	Maverick, Maverick Pro
			Thifensulfuron	Pinnacle, Harmony
			Triasulfuron	Amber Logran
			Tribenuron	Express
			Trifloxysulfuron sodium	Enfield
			Triflusulfuron	Debut, Safari, Upbeet
		Triazolopyrimidine	Cloransulam-methyl	First Rate
			Diclosulam	Strongarm
			Florasulam	Primus
			Flumetsulam	Broadstrike
3(K1)	Inhibitors of microtubule assembly	Dinitroaniline	Benefin	Balan
			Ethalfluralin	Sonalan
			Oryzalin	Surflan
			Pendimethalin	Prowl, Stomp
			Prodiamine	Barricade (only sold to distributors, fertilizer mix)
			Trifuralin	Treflan, Various
		Pyridine	Dithiopyr	Dimension
			Thiazopyr	Visor
		None	DCPA	Dacthal
4(O)	Synthetic auxins	Phenoxy	2,4-D	Various
			2,4-DB	Various
			Dichlorprop, 2,4-DP	Various
			MCPA	Various
			MCPB	Various
			Mecoprop, PP	Various
		Benzoic acid	Dicamba	Various
		Carboxylic acid	Clopyralid	Reclaim, Stinger, Various
			Fluroxypyr	Starane
			Picloram	Tordon, Grazon, Various
			Triclopyr	Garlon, Various
		Quinaline carboxcylic acid	Quinclorac (dicots)	Drive, Facet, Paramount

(continued)

Table 1 (continued)

Group	Site of action	Chemical family	Common name	Trade name[a]
5(Cl)	Inhibitors of photosynthesis at photosystem II site A	Phenyl-carbamate	Desmedipham	Betanex
			Phenmedipham	Spin-Aid
		Pyridazinone	Pyrazon	Pyramin
		Triazine	Ametryn	Evik
			Atrazine	AAtrex, Various
			Cyanazine	Bladex
			Desmetryn	Not sold in North America
			Prometon	Pramitol
			Prometryn	Caparol, Various
			Propazine	Milo Pro
			Simazine	Princep, Various
			Simetryu	Not sold in North America
			Terbumeton	Not sold m North America
			Terbuthylazine	Not sold in North America
			Trietazine	Not sold in North America
		Triazinone	Hexazinone	Velpar
			Metamitron	Not sold in North America
			Metribuzin	Sencor, Lexone
		Triazolinone	Amicarbazone	BAY MKH 3586, BAY 314666
		Uracil	Bromacil	Hyvar X
			Terbacil	Sinbar
6(C3)	Inhibitors of photosynthesis at photosystem II site B	Benzothiadiazole	Bentazon	Basagran, Various
		Nitrile	Bromoxynil	Buctril, Brominal, Various
			Ioxynil	Various
		Phenyl-pyridazine	Pyridate	Lentagran, Tough
7(C2)	Inhibitors of photosynthesis at photosystem II site A; different binding behavior from group 5	Amide	Propanil	Propanil, Stam, Various
		Urea	Chlorotoluron	Not sold in North America
			Dimefuron	Not sold in North America
			Diuron	Karmex, Direx
			Fluometuron	Cotoran, Meturon
			Isoproturon	Not sold in North America

(continued)

Table 1 (continued)

Group	Site of action	Chemical family	Common name	Trade name[a]
			Linuron	Lorox, Linex
			Methibenzuron	Not sold m North America
			Metoxuron	Not sold in North America
			Monolinuron	Not sold in North America
			Siduron	Tupersan
			Tebuthiuron	Spike, Preflan
8(N)	Inhibitors of lipid synthesis; not ACCase inhibition	Thiocarbamate	Butylate	Genate+
			Cycloate	Ro-Neet
			EPTC	Eptam, Eradicane
			Esprocarb	Not sold in North America
			Molinate	Ordram
			Pebulate	Tillam, Edge
			Prosulfocarb	Boxer, Defi, Arcade
			Thiobencarb	Bolero
			Triallate	Avadex, Fargo
			Vernolate	Vernam
		None	Bensulide	Prefar, Betasan, Various
9(G)	Inhibitor of 5-enolypyruvyl-shikimate-3-phosphate synthase (EPSP)	None	Glyphosate	Roundup, Touchdown, Various
10(H)	Inhibitor of glutamine synthetase	None	Glufosinate	Ignite, Liberty, Rely
11(F3)	Inhibitors of carotenoid biosynthesis (unknown target)	Triazole	Amitrole	Amitrol T, Amizol
			Aclonifen	Not sold in North America
12(F1)	Inhibitors of the phytoene desaturase (PDS)	Pyridazinone	Norflurazon	Zorial, Evital, Solicam
		Pyridinecarboxamide	Diflufenican	Not sold in North America
			Picolinafen	Not sold in North America
		Other	Beflubtamid	UBH-820

Adapted with permission from Weed Science Society of America; Mallory-Smith and Retzinger (2003)

[a] For manufacturer see Weed Science Society of America Herbicide Handbook 8th edition and www.greenbook.net

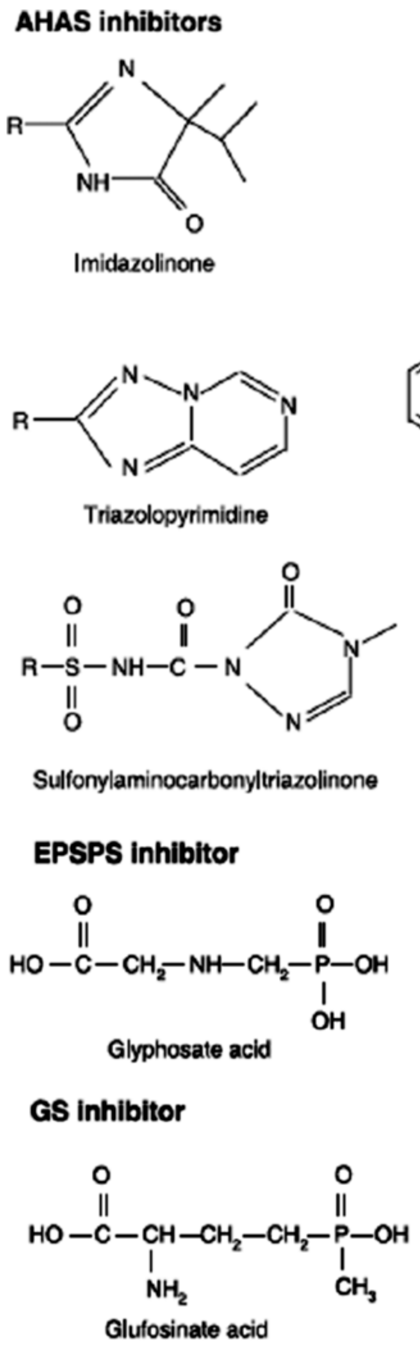


Fig. 6 Basic molecular structure of herbicides that inhibit important enzymes of plant metabolism. R, R1 and R2 are additional groups attached to the basic structure (Adapted from Tan et al. 2006)

3 Herbicide Resistant Transgenic Plants

There is no doubt about the fact that herbicides effectively control weeds that help in getting high yields and good quality crops. Herbicides that are effective and commonly used worldwide are generally broad spectrum and non-selective. This restricts their use in arable lands. Another associated factor is excessive use of herbicides, which leads to selection of resistant weeds (discussed later). The above facts necessitated the development of herbicide resistant transgenic plant (HRTP). Herbicides generally target essential metabolic processes in plants e.g. photosynthesis, mitosis or amino acid biosynthesis. These processes are common in both crops and weeds. Biotechnological approaches have been used to engineer crops tolerant to herbicides. Commercial viability of these engineered crops has urged a section of scientists/companies globally to develop HRTP.

According to a report during 170.3 million hectares of biotech crops were grown worldwide in 2012, which was 10.3 millions more from 160 million hectares in 2011 with an annual growth rate of 6 %. Comparing the data from the year 1996 an incredible increase of 100-fold has been observed in hectares of land covered by biotech crops (James 2012). In 2010 in USA 93 % of all soybeans, 78 % of all cotton and 70 % of all maize varieties were herbicide-resistant (Pollegioni et al. 2011). Also, there has been significant rise in transgenic herbicide resistant crops since 1996 as compared to the rise in Insect resistant transgenic crops (Bt Crops). The global area covered by HRTP is almost 100 million hectares which outnumbers the insect resistant transgenic crops (Bt Crops) (James 2012; Fig. 7).

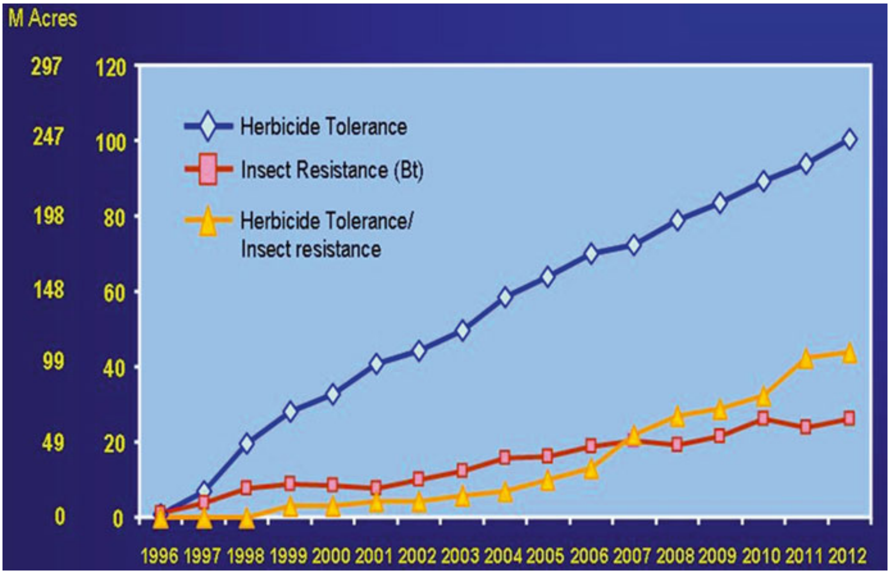


Fig. 7 Global area of biotech crops by trait, 1996–2012 (Million Hectares, Million Acres; Adapted with permission from ISAAA; James 2012)

3.1 Glyphosate Tolerance

Glyphosate (N-phosphonomethyl-glycine) is most widely used non-selective, foliar applied, broad spectrum herbicide (Malik et al. 1989). It was developed by the Monsanto Co. and introduced to world of agriculture in 1974. Glyphosate inhibits the enzyme enolpyruvyl shikimate-3-phosphate synthase (EPSPS) (EC 2.5.1.19) which is a key enzyme in the biosynthesis of aromatic amino acids (Fig. 8). The enzyme is

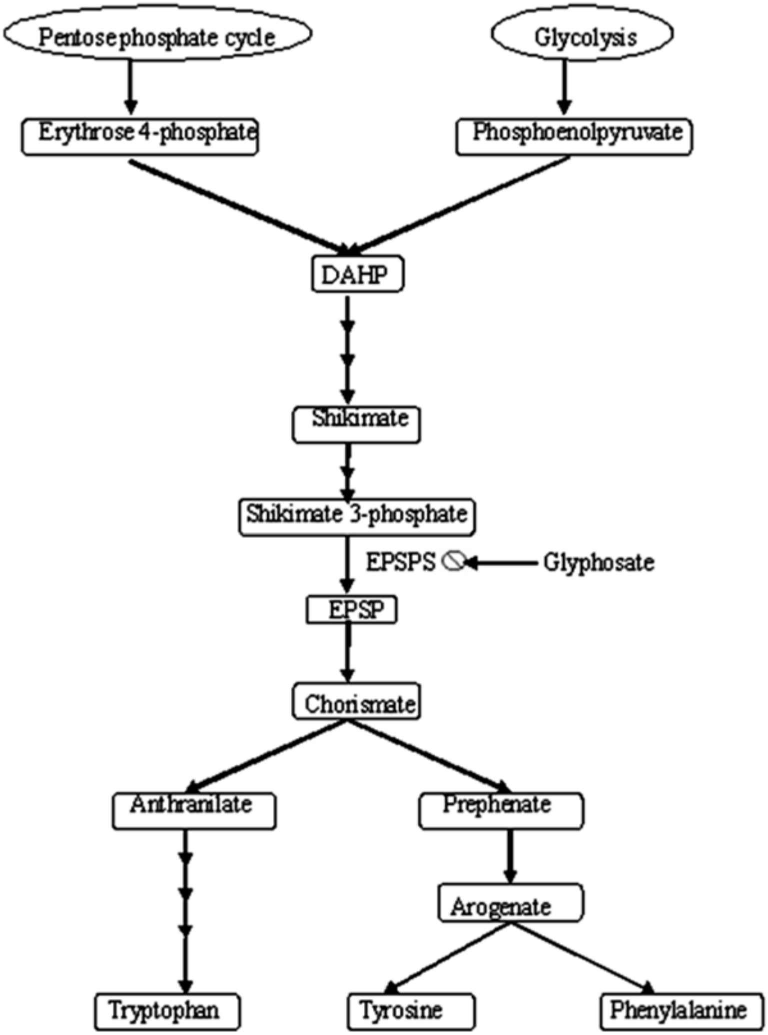


Fig. 8 Biosynthetic pathway of amino acids and site of action of Glyphosate. *DAHP* 3-deoxy-D-arabino-heptulosonate 7- phosphate, *EPSPS* Enolpyruvyl shikimate-3-phosphate synthase, *EPSP* 5-enolpyruvylshikimate-3-phosphate

nuclear-encoded and is involved in the biosynthesis of plastid-localized Shikimic Acid Pathway (SAP) (Steinrucken and Amrhein 1980; Gruys and Sikorski 1999). EPSPS uses two substrates phosphoenol pyruvate (PEP) and shikimate-3-phosphate (S3P) to form EPSP (5-enolpyruvylshikimate-3-phosphate). Glyphosate competitively interferes with the binding of PEP to the active site of EPSPS forming a stable but noncovalent ternary complex with the enzyme and S3P (Marzabadi et al. 1996; McDowell et al. 1996). The X-ray crystallographic structures of the enzyme, ternary complex of S3P-PEP-enzyme and S3P-glyphosate-enzyme confirm that glyphosate occupies the position of PEP (Stallings et al. 1991; Schönbrunn et al. 2001).

SAP leads to the formation of aromatic amino acids (primary metabolites) Tyrosine, Phenylalanine and Trptophan together with tetrahydrofolate, ubiquinone and vitamins K and E (Haslam 1993; Franz et al. 1997). These aromatic amino acids serve as precursors for synthesis of myriads of secondary metabolites including lignin, flavonoids and alkaloids (Herrmann 1995). Moreover, almost 35 % of the dry weight plant mass is represented by aromatic molecules derived from the SAP (Franz et al. 1997), which further details about the importance of the pathway. SAP is present in plants and microorganisms, and absent in mammals, birds, fish, insects and reptiles. Also, the active site of the EPSPS is highly consistent in higher plants and thus, glyphosate affects a broad spectrum of weeds indiscriminately. Use of glyphosate is a low cost and low environmental impact strategy for weed control. One of the major reasons for glyphosate being cost effective is the expiry of the patent on the molecule in 2000 bringing the entry of generic producers. 'Glyphosate is a one in a 100-year discovery that is as important for reliable global food production as penicillin is for battling diseases' (Powles 2010). Application of glyphosate is almost inevitable to save plants from adverse action of weeds. The problem arises when it is used in arable land. Being specific to EPSPS it also adversely affects crop plants, which hinders its use by farmers. Transgenics has solved this problem to a great extent and is the choice of the hour.

3.1.1 Overproduction of EPSP Synthase

One of the earliest approaches to develop glyphosate tolerance in plants was overexpression of EPSPS. *Petunia* cultures were stepwise grown in increased amount of glyphosate. This helped in selection of cultures wherein the EPSPS production was quite higher than normal. It was concluded that the increased production was not due to increased expression of EPSPS gene but due to gene amplification (up to 20 copies). Overproduction of EPSPS retains sufficient amount of EPSPS that is not inhibited by glyphosate to carry on normal plant metabolism. High levels of EPSPS mRNA eased their isolation and further construction of cDNA for this gene to be reintroduced in plants. EPSPS cDNA was fused with CaMV 35S promoter and *nos* terminator sequence in vector pMON506 and introduced into *Petunia* using *Agrobacterium.* One of the biggest advantages of this technique was it avoided the problem of protein targeting (use of transit peptide), which has to be dealt with carefully while introducing prokaryotic genes that lack organelle specific transit peptides. Plant EPSPS cDNA possess transit peptide and the resulting transgenic plant showed 40 times increase in EPSPS activity together with tolerance to glyphosate at a concentration twofold to fourfold higher than required to kill wild plants in field conditions. Nafzinger et al (1984) reported the presence of 52-fold glyphosate tolerant carrot cells after adaptation to 25 mM glyphosate. Also, there was 12-fold increase in EPSPS synthase activity. Similar results were shown in other plant cell cultures (Steinrucken et al. 1986; Dyer et al. 1988). It was observed that EPSPS gene amplification was a stable phenotype even when the selected culture is grown for a period in a media devoid of glyphosate.

3.1.2 Expression of an Insensitive Form of the Target Enzyme

Two types of EPSPS have been identified (Barry and Padgette 1992). Type I EPSPS is present in plants and bacteria and is naturally sensitive to glyphosate whereas type II EPSPS is present in some forms of bacteria, tolerant of glyphosate (Della-Cioppa et al. 1987; Stalker et al. 1985; Funke et al. 2006). Analogs of glyphosate (>1,000) have been tried to see the effect against EPSPS but minor changes in the structure of glyphosate resulted in significant lower efficiency (Franz et al. 1997). It can be concluded that glyphosate is not a mere analog of PEP, but it somehow appears to mimic an intermediate state of PEP (Pollegioni et al. 2011). The first report about mutant gene of EPSPS was studied on *Salmonella typhimurium.* The organism was treated with ethyl methanesulfonate and mutant selection was done at *aroA* locus. The mutant gene conferred high resistance to glyphosate when introduced in *Escherichia coli* (Comai et al. 1983). Later Comai et al. (1985) introduced modified *aro A* gene in tobacco and produced first herbicide tolerant transgenic plant with decreased affinity for glyphosate without much affecting the kinetics of the enzyme. It was actually a single site mutation with an amino acid substitution of a proline for serine which provided limited tolerance. Transgenic tomato was developed using *Agrobacterium tumefaciens* having a binary vector with two neomycin phosphotransferase (NPT) II genes and a mutant *aro*A gene. Tomato plant was found to be tolerant to glyphosate at concentrations of 0.84 kg active ingredient/ha (Fillatti et al. 1987). Single site mutation (Gly96Ala) in EPSPS coding gene in *Klebsiella pneumoniae* and *E. coli* showed resistance to glyphosate but the enzyme had very low affinity for PEP and poor catalytic efficiency (Sost and Amrhein 1990; Eschenburg et al. 2002). The low level of tolerance in the above experiments appeared because of the fact that prokaryote gene did not possess plastid transit peptide sequence and therefore the expressed gene product was not transported to chloroplast, which is its actual site of action. With the evolution in the field of recombinant DNA technology scientists thought of clubbing the transit peptide sequence to the mutant EPSPS gene or directly expressing the gene in plastid to overcome the problem.

A hybrid EPSPS gene was constructed by fusion of the C-terminal end of a mutated *aro A* gene from *E. coli* to the N-terminal end of the *Petunia* EPSPS cDNA sequence containing the transit peptide sequence. Glyphosate tolerance in transgenic tobacco increased from 0.01 to 1.2 mM/L. the experiment thus validated the strategy and concluded the feasibility of expression of prokaryotic genes incorporated into the plant nuclear genome, giving appropriate promoter and termination signals. However, the expression of prokaryotic genes in transgenic plant is often not a success, which was seen in the case of *E. coli* and *S. typhimurium* and requires comprehensive modification to obtain high expression. Herbicide tolerant *A. tumefaciens* strain CP4 has low Km for PEP and is resistant to glyphosate. The CP4-EPSPS enzyme consists of a single polypeptide with 455 amino acids. The amino acid sequence has been reported to be 48.5–59.3 % similar and 23.3–41.1 % identical to native EPSPS of plants and bacteria (Padgette et al. 1996; Dill 2005). This gene with CaMV 35S promoter and chloroplast transit peptide sequence from *Petunia* and *Arabidopsis* has been incorporated in glyphosate tolerant Roundup Ready crops of Monsanto (Soybean, cotton and oilseed rape). *PpAroA1* gene from *Pseudomonas putitda* was optimized for transformation of tobacco (*PparoA1* optimized) and fused with chloroplast transit peptide. This gene was used to transform tobacco using *Agrobacterium*-mediated transformation. The transformed plant conferred tolerance against glyphosate and proposes novel and good candidate for HRTP (Yan et al. 2011). Rahnella aquatilis strain GR20 isolated from the rhizosphere of grape in glyphosate contaminated vineyards showed presence of *AroA* gene which tolerated higher levels of glyphosate as compared to *AroA* gene from *E. coli.* Transgenic tobacco plants transformed with *AroA* gene from *R. aquatilis* were more resistant to glyphosate at a concentration of 5 mM than that of *AroA* gene from *E. coli.* Substitution mutation showed greater sensitivity to glyphosate than the wild type *R. aquatilis* EPSPS (Peng et al. 2012).

Maize EPSPS transgene obtained with double mutations (T102I and P106S) using site directed mutagenesis approach was used in a maize plant to achieve commercial tolerance to glyphosate (Lebrun et al. 2003; Dill 2005; Pline-Srnic 2006). This transgenic event is known as GA21. As a result, the transformed plant with the inserted CP4-EPSPS or the modified maize EPSPS transgene has an alternative EPSPS that is less or insensitive to glyphosate compared to endogenous EPSPS. Consequently, the transformed plant has a normal synthesis of EPSP even though glyphosate inhibits the endogenous EPSP synthase and kills the non-transformed plant.

The above research justifies the feasibility of the use of resistant EPSPS but one of the major problems with the approach is that the mutant enzyme not only has lower (increased Km, the Michaelis – Menten constant) affinity for the competitive inhibitor but also for substrate. Thus, glyphosate resistant genes from various sources have been tested for their efficiency in different plants.

3.1.3 Detoxification of Glyphosate

Another strategy for glyphosate tolerance is expression of enzymes that detoxify (break down) the herbicide. Detoxification is provided by *gox* gene isolated from *Ochrobactrum anthropi* (earlier *Achromobacter* sp.) strain LBAA. The gene encodes for glyphosate oxidoreductase, which degrades glyphosate to glyoxylate and

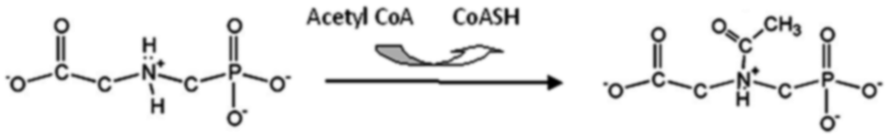


Fig. 9 N-acetylation of glyphosate

AMPA (aminomethylphosphonic acid): HOOC–CH_2–NH–CH_2–PO_3H_2 → HOOC–CHO + NH_2–CH_2–PO_3H_2; and helps the organism to thrive on glyphosate as a carbon or phosphorous source (Barry et al. 1992; Komoba and Sandermann 1992; Padgette et al. 1996; Dill 2005). Transgenic oilseed rape with the above gene has shown good tolerance in field condition. Monsanto use *A. tumefaciens* CP4 EPSPS gene together with *gox* for canola. This approach is quite useful because apart from imparting glyphosate tolerance to the plant it also avoids higher level of herbicide accumulation, which can be harmful. This shows integration of two strategies for HRTP. Transgenic canola and maize expressing both these genes are now grown commercially (Saroha et al. 1998).

N-acetylation of glyphosate converts it into non-toxic form N-acetylglyphosate by enzyme glyphosate *N*-acetyltransferase (GAT) is yet another approach to achieve detoxification (Fig. 9). Directed evolution was used to enhance enzyme activity. Microbial cultures were screened for maximum enzyme activity. Unfortunately the kinetic properties were not sufficient to develop glyphosate resistant transgenic organisms. *Gat* gene from *Bacillus licheniformis* was subjected to repeated DNA shuffling. Eleven iterations of DNA shuffling improved enzyme efficiency by almost four orders from 0.87 to 8,320/mM/min, which was nearly 10,000-fold higher Kcat/Km improvement over parental enzyme. Glyphosate tolerance was observed when the gene was engineered into *E. coli*, *Arabidopsis*, maize and tobacco (Castle et al. 2004). This study added another milestone in the field of science wherein improvement of enzymes with useful but insufficient activities can be achieved by applying directed evolution until the desired activity is gained.

3.2 Glufosinate Tolerance

Glufosinate is usually applied as ammonium salt (glufosinate ammonium) and also called as Phosphinothricin (PPT). It is a post emergence non-selective herbicide and inhibits the enzyme glutamine synthetase (E.C. 6.3.1.2) by binding to it irreversibly (Wild and Manderscheid 1984; Fig. 10). The enzyme occurs in different isoforms displaying specific properties. Leaves have two isoforms and root generally contains one isoform. The root isoform was found to be more sensitive to PPT than the chloroplast isoform in both *Triticum aestivum* and *Sinapis alba* (Wild and Manderscheid 1984; Manderscheid and Wild 1986). Glutamine synthetase plays an essential role in nitrogen metabolism by catalyzing the condensation

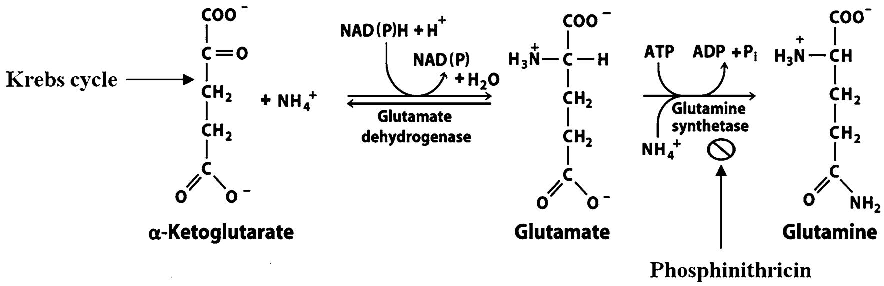


Fig. 10 Biosynthetic pathway of Glutamine and site of action of Phiosphinothricin

of glutamate and ammonia to form glutamine. PPT, an analogue of glutamate, leads to deficiency of important amino acid glutamine, which affects translational process (Tachibana et al. 1986). There is accumulation of ammonia as well, which indicates that transamination of glyoxylate to glycine in photorespiration is adversely affected. Accumulation of glyoxylate leads to inhibition of RuBP-carboxylate and fixation of CO_2. Deficiency of Calvin cycle intermediates occurs due to the disruption of photorespiration (Sauer et al. 1987). Under atmospheric condition (400 ppm CO_2, 21 % O_2) the herbicide causes inhibition of photosynthesis. However, conditions (1,000 ppm CO_2, 2 % O_2) wherein photorespiration cannot take place the inhibition of photosynthesis is comparatively very low. This reveals that inhibition of photosynthesis is based on inhibition of a part of photorespiratory process (Wendler et al. 1990). It has been suggested that ammonia accumulation cause phytotoxicity. However, in a study it was observed that application of almost 40 times the standard level (10 mM) of ammonium nitrate had no effect on *Medicago sativa* callus culture though there was 27 times increase in endogenous ammonia. Thus, it can be concluded that cellular accumulation of ammonia cannot be the sole cause but one of the causes of phytotoxicity in plants after glufosinate application (Krieg et al. 1990).

PPT is derived from a natural product. Bialaphos, which is a natural tri-peptide (PPT-Ala-Ala) produced by at least two *Streptomyces hygroscopicus* species as an extracellular product (Bayer et al. 1972). It can be directly used as a herbicide and has been commercially used as Herbiace (Meiji Seika). Proteolytic cleavage of Ala residues gives active from of L-PPT (Kondo et al. 1973; Fig. 11). The uptake of PPT is generally through leaves and is dependent on various aspects like species of plant, environmental factors and rate of application. Unlike glyphosate, the translocation of PPT in comparatively limited in plants. Basta is trade name of PPT which was marketed by Hoechst (German Company). Series of company take over and mergers took place and Basta is now owned by Bayer CropScience. Bayer has also come out with different formulation of PPT and is marketed with the brand name Liberty. Likewise the transgenic PPT tolerant crops are called as LibertyLink crops.

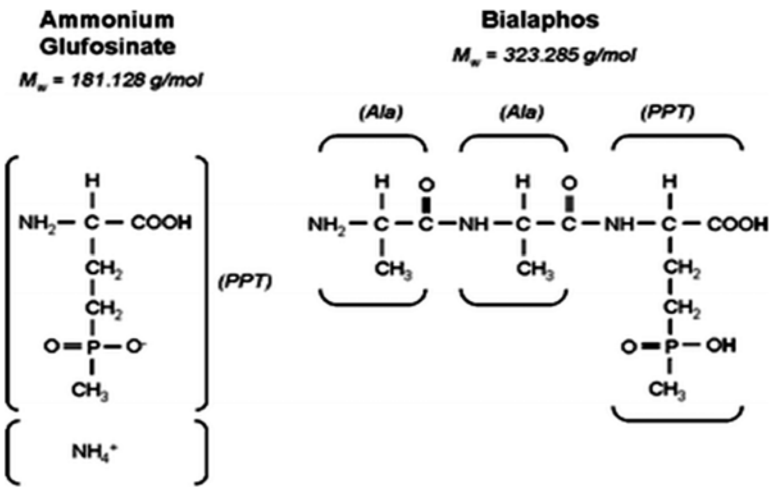


Fig. 11 Chemical structures of ammonium glufosinate and bialaphos highlighting their similarity (Adapted from Montague et al. 2007)

Since Bialaphos is a natural compound therefore the host organism must be having some detoxification mechanism to alleviate physiological problems associated with PPT. In the same lead two genes *bar* and *pat* were identified in *Streptomyces hygroscopicus* and *S. viridichromogeses* (Wohlleben 2000), respectively. They code for enzyme phosphinothricin acetyltransferase (PAT). The enzyme acetylates the free NH_2 group of PPT and prevents it from binding to glutamine synthetase (Thompson et al. 1987). This approach was used to develop PPT tolerant transgenic crop by Plant Genetic Systems (subsequently acquired by AgrEvo) under the contract from Hoechst (Slater et al. 2008). Several transgenic crops have been developed by introducing *bar* gene under the control of 35S promoter. Both *PAT* and *BAR* enzymes are quite similar in their activity with comparable molecular weights, and show immuno-cross-reactivity to their respective antisera (Wehrmann et al. 1996). Tobacco was first plant to be engineered for glufosinate tolerance using *bar* gene, which gave tolerance to glufosinate without any adverse effect on flowering or seed set (DeBlock et al. 1987). Thereafter several plants have been engineered which include corn, rice, wheat, sugarbeet, oilseed rape, alfalfa, cotton, lettuce, sugarcane, cassava and dry beans (Gordon-Kamm et al. 1990; Christou et al. 1991; Vasil et al. 1992; D'Halluin et al. 1992; Cobb 1992; Keller et al. 1997; Mohapatra et al. 1999; Falco et al. 2000; Sarria et al. 2000; Aragao et al. 2002). USFDA (United States food and drug administration) have permitted the commercial production of glufosinate tolerant cotton, sugar beet, maize canola and soybeans. Commercial production of glufosinate tolerant crops has been allowed in almost eight countries worldwide (Dhingra and Daniell 2004).

3.3 2,4-D Tolerance

As discussed earlier growth regulator herbicides like 2,4-D still remains one of the most widely used herbicides since its introduction in 1940s (Marth and Mitchell 1944; Mitchell et al. 1944). They selectively act against dicot plants by mimicking the activity of auxin. In order to explore gene products/genes that could degrade 2,4-D several studies were done on soil bacteria. There are few reports which details about plasmid based genes that could metabolize the growth regulator (Don and Pemberton 1981).

Gene *tfdA* from *Alcaligenes eutrophus* JMP134 (soil bacteria) is present in the plasmid (pJP4) which codes for an enzyme was earlier referred to as 2,4-dichlorophenoxyacetate monooxygenase (DPAM) (Don et al. 1985; Harker et al. 1989; Perkins and Lurquin 1988; Pieper et al. 1989) but later it was shown that it is α-ketoglutarate-dependent dioxygenase that converts α-ketoglutarate to succinate and carbon dioxide concomitant with the conversion of 2, 4-D to 2, 4-DCP (2,4-dichlorophenol), which is almost 100 times less phytotoxic than 2,4-D (Lyon et al. 1989), and glyoxylate (Fukumori and Hausinger 1993). It is the first step in the bacterial 2, 4-D degradative pathway. Transgenic tobacco was made using gene (*tfdA*) fused with plant expression sequence and was delivered in plant using *Agrobacterium*. Increased tolerance was observed both in tissue culture and field condition (Streber and Willmitzer 1989). Bayley et al. (1992) engineered 2, 4-D resistance in cotton using 35S promoter fused *tfdA* gene. The engineered plant tolerated three times the field application of 2, 4-D. Tolerant cotton verities have been released in Australia (Mulwa and Mwanza 2006). In another study transgenic tobacco was made targeting the expression of tfdA to either meristematic tissues or chloroplast-containing tissues. The promoters were taken from *Pisum sativum* plastocyanin gene (*petE*) and an *Arabidopsis thaliana* histone gene (*H4A*). The transgenic plant showed comparatively less resistance (Last and Llewellyn 1999).

3.4 Engineering Tolerance Against Acetolactate Synthase Inhibitors

Acetolactate synthase (ALS)/Acetohydroxyacid synthase (AHAS) is a key enzyme in the biosynthesis of branched chain amino acids. It catalyzes two reactions: the condensation of two pyruvate molecules to acetolactate and formation of 2-acetohydroxybutyrate from pyruvate and 2-ketobutyrate (Bender 1985; Singh and Shaner 1995). Acetolactate is precursor of leucine and valine whereas 2-acetohydroxybutyrate leads to the formation of isoleucine (Fig. 12). The whole sequence of events of this nuclear encoded is carried out in chloroplast (Schulze-Siebert et al. 1984; Vaughn and Duke 1991; Saari and Mauvais 1996; Reade and Cobb 2002). The branched chain amino acids regulate the activity of ALS (feedback inhibition) (Stidham and Singh 1991; Shaner et al. 1996). On the other hand, the product

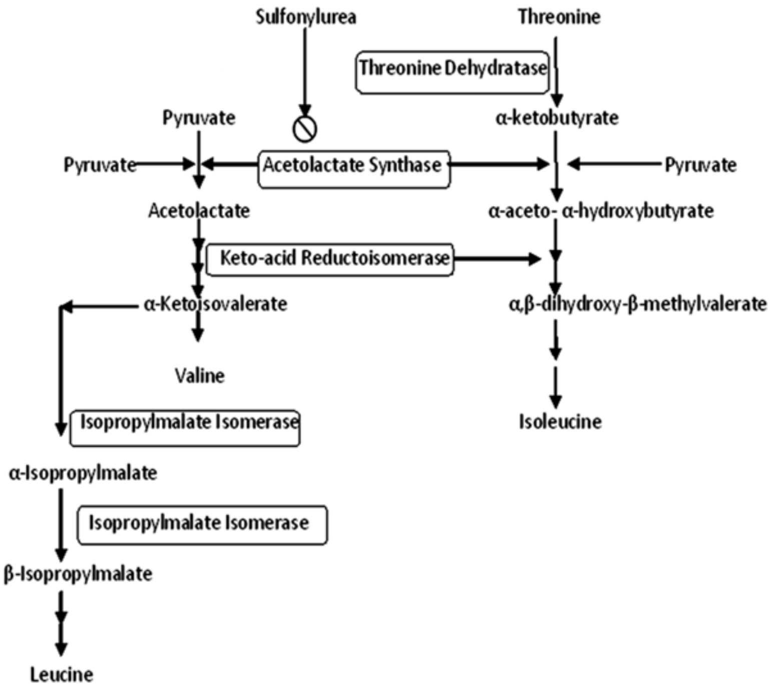


Fig. 12 Biosynthesis of leucine, valine and Isoleucine and site of action of Sulfonylurea

amino acids function as feedback regulators of the activity of ALS. Five classes of ALS inhibiting herbicides (sulfonylureas, imidazolinones, pyrimidinylthio(or oxy)-benzoates, triazolopyrimidines, and sulfonylamino-carbonyltriazolinones) have been commercialized (Vencill 2002; Mallory-Smith and Retzinger 2003).

Almost 30 commercial herbicides have been identified from sulfonylureas. Each class of herbicide is unique as they bind to ALS at different site which are overlapping (Ott et al. 1996). The inhibition of ALS by imidazolinones is uncompetitive with respect to pyruvate (Stidham and Singh 1991; Shaner and Singh 1997). ALS has been studied comprehensively in terms of kinetics and genetics (Shimizu et al. 2002). It has been postulated that ALS specific herbicides binds to enzyme at the entry site for ALS substrate or substrate access channel (Pang et al. 2003). This inhibits the activity of the enzyme and leads to the deficiency of branched chain amino acids. Finally there is death of susceptible species of plant due to starvation for branched chain amino acids (Shaner and Singh 1992). Thus the herbicides can also be considered as growth inhibitors that, which act faster than glyphosate.

The ALS consists of large subunits having catalytic activity and small regulatory subunits (Tan et al. 2005). Each catalytic subunit is a polypeptide with almost 670 amino acids. Five commonly occurring mutations in the large subunit gene of ALS at codon 122, 197, 205, 574, and 653 (with reference to *Arabidopsis thaliana* (L)

Heynh) have been shown to confer tolerance against ALS-inhibiting herbicides (Tranel and Wright 2002; Christoffers et al. 2004). The amino acids of these codons are distributed over the entire primary structure of ALS but in quaternary structure they are folded in the adjacent area (Ott et al. 1996). This area presents the binding site of the enzyme inhibitors. Mutation in codon 574 encodes for ALS resistant to all families of ALS inhibitors (Tan et al. 2005) whereas mutation at codon 197 expresses ALS that is more tolerant to sulfonylureas than imidazolinones. Mutation at codons 122, 205, and 653 results in imidazolinones-resistant ALS.

Plant transformation using Oligonucleotide-mediated gene manipulation was tried to develop an imidazolinone resistant maize line (Zhu et al. 2000). This methodology has been efficiently used in DNA repair and gene therapy experiments in mammalian systems (Yoon et al. 1996). An oligonucleotide was predicted by researchers at Pioneer Hi-Bred international having combination of DNA and RNA bases, with 32-base section having nearly exact homology to the target sequence of maize gene (ALS) with a single mismatch base at the point of desired mutation. Particle bombardment method was used to deliver chimeric oligonucleotide into the target cells. Homologous recombination of the oligonucleotide with the endogenous homologous sequence resulted in a direct change in the targeted gene. Specifically, there was a change from Ser621 (encoded by AGT) to Asn621 (encoded by AAT), which conferred resistance to herbicide Lightning (a mixture of imazathapyr and imazapyr) in the regenerated plants. Nine independently transformed cells were tested for Lightning tolerance. Highly resistant plant had AGT → AAT mutation and susceptible plants had unaltered ALS gene (Slater et al. 2008).

Imidazolinone resistance allele (*imr1*) from GH90 mutant line of *A. thaliana* was expressed in tobacco under the control of 35S promoter showed selective imidazolinone resistance. ALS gene having both the *imr1* mutation and the *csr1* mutation conferred tolerance to both sulfonylurea and imidazolinone herbicides after engineering in transgenic tobacco. The study concludes that multiple mutations in ALS gene confer tolerance to more than one herbicide in tested transgenic plant (Hattori et al. 1992). Transgenic cotton was raised in similar way to provide tolerance to multiple herbicides (Rajasekaran et al. 1996). Molecular modeling has been used to aid development of herbicide resistant transgenic plants. Transgenic tobacco was raised using this approach that conferred high tolerance to imidazolinone (Ott et al. 1996). The study opened new horizon in transgenics that utilized molecular modeling and molecular biology. This approach can be vital for future development for ecologically conducive sustainable agricultural systems. ALS mutant gene from chlorsulfuron tolerant *A. thaliana* (Haughn and Somerville 1986) was engineered in garlic which tolerated 3 mg/l of chlorsulfuron (Park et al. 2002). Commercially triasulfuron and metsulfuron-methyl herbicide tolerant flax is grown in Canada and the US. Cotton with a mutant form of ALS insensitive to sulfonylureas is grown in the US. Florigene Inc. has developed sulfonylurea resistant carnations grown in Australia and the European Union (Dhingra and Daniell 2004).

Imidazolinone-tolerant maize and canola have been reported to have Ser653Asn and trp574leu gene mutation whereas wheat and rice has only Ser653Asn mutation. The tolerant sunflower has ala205val mutation of ALS gene. Apart from the above commercialized imidazolinone-tolerant crops several other crops have been studied for the same trait (Tan et al. 2005). All mutated and imidazolinone-tolerant ALS genes are semidominant and therefore crop tolerance is enhanced with gene dosage (Tan et al. 2006). Four imidazolinone herbicide active ingredients (imazamox, imazethapyr, imazapyr, and imazapic) have been registered on imidazolinone-tolerant plants, which can be applied singly, in combination with other imidazolinones, or in combination with other herbicides for a season-long weed control.

The regulatory policies for commercialization of transgenic crops are quite diverse (McHughen and Smyth 2008). For instance, there is general acceptance of induced mutations in plant breeding whereas similar regulation is not imposed on transgenic crops (Parry et al. 2009). As a result mutation strategies are being supported and the use of transgenics discouraged. *Arabidopsis* substitution mutant csr1-2 (Ser653Asn) was compared with wild-type plants using microarray analysis. Complete absence of pleiotropic effects was observed which can be attributed to the mutation at the transcriptional level showing that imidazolinones specifically act on ALS as their only target (Manabe et al. 2007). In a recent study transgenic plant was created by replacing *csr1-2* gene by native *csr1* gene and absence of pleiotropic effects. Moreover, the resistance and biomass accumulation in the presence of imazapyr was found to be more than *csr1-2* substitution mutant (Schnell et al. 2012). Distinctive properties of imidazolinones, glyphosate and glufosinate have been compared in Table 2.

3.5 Engineering Tolerance Against Photosynthesis Inhibitors

Photosynthesis is one of the most essential processes in plants. This process is carried out in chloroplast and photosystem I (PS I) and photosystem II (P SII) are important components of the same. Photosynthesis being a prime process can be a soft target for herbicides. Some herbicides act on the electron transport chain of PS II and others act by diverting the electron flow at PS I (Dodge 1991).

PSII is a multisubunit protein-pigment having D1 protein as one the components. D1 complex acts as an apoprotein for Qb (the secondary quinone electron acceptor) in PS II which mediates electron flow towards plastoquinone pool within the thylakoid membrane (Dodge 1991). The D1 protein is encoded by *psbA* gene located in chloroplast genome. It is a 32-kDa polypeptide of PS II having a high turnover rate that is light dependent. The D-1 protein predicted a hydrophobic nature (Zurawski et al. 1982) with five transmembrane helices (Trebst 1987). Herbicides targeting the PS II electron flow chain bind to the Qb site of the D1 protein thereby blocking the binding of plastoquinone, an electron acceptor at this stage of the photosynthetic electron transport chain.

S-triazines type herbicide like atrazine and chemically unrelated ureas like diuron inhibit photosynthesis by targeting D1 polypeptide, which adversely affects the transport of plastoquinone (mobile electron carrier) (Devine et al. 1993). This leads

Table 2 Comparison between three herbicides belonging to different chemical families

Inhibitor	Imidazolinones	Glyphosate	Ghifosinate
Herbicide			
Target enzyme	AHAS	EPSPS	GS
Enzyme inhibition with respect to substrate	Uncompetitive	Uncompetitive. competitive	Competitive
Use rate (g ae/ha)	20–1,700	160–4,200	320–1,560
Application method	Foliar, soil	Foliar	Foliar
Application timing to weeds	Pre- emergence, post-emergence	Post-emergence	Post-emergence
Weed control activity	Broad spectrum	Non-selective	Non-selective
Acute rat oral toxicity (LD_{50} mg/kg)	>5,000	5,600	1,910
Tolerant crop			
Mechanism of tolerance	Tolerant AHAS	Tolerant EPSPS, detoxification of glyphosate	Detoxification of glufosinate
Method of development	Mutagenesis or selection	Genetically engineered	Genetically engineered
Foreign gene inserted	No	Yes	Yes
Foreign gene source	None	*Agrobacterium* sp. strain CP4, *Ochrobactrum anthropi* strain LBAA	*Streptomyces hygroscopicus*, *S. viridochromogenes*
Modified or inserted target-site gene	Variant AHAS gene	CP4-EPSPS or modified maize EPSPS gene	None
Inserted metabolism gene	None	*gox* gene	*bar* or *pat* gene
Organism classification	Non-transgenic	Transgenic	Transgenic

Adapted from Tan et al. (2006)

to the disruption of electron flow between the PS I and PS II and as a result the formation of end product of light reaction (NADPH) is reduced. This affects the carbon fixation. There is also reduction in beta-carotene that exposes chloroplasts to oxidative damage (Dhingra and Daniell 2004).

Some plants have inherent capacity to resist PS II inhibitors through metabolic detoxification or decreased uptake and translocation. Sequence analysis of *psbA* gene from *Amaranthus hybridus*, a resistant weed, revealed three mutations. Two were silent mutation and the third was Ser264Gly mutation (McIntosh and Hirschberg 1983). This mutation affects the stromal side of the protein and lowers the binding of *s*-triazine herbicides. There is associated impaired photosynthesis which affects plant growth and plant vigor is also compromised (McCloskey and Holt 1990). Sequence analysis of other atrazine resistant plants showed conserved sequence as well as single substitution mutation at same position in the polypeptide (Goloubinoff et al. 1984; Barber 1987). *Abutilon* has been reported to possess detoxification as mode of resistance (Andersen and Gronwald 1987). A substitution mutation Ser264Ala in *Chlamydomonas rheinhardii* in *psbA* gene conferred resistance against herbicide (Erickson et al. 1985). Another resistance has been reported against diuron because of mutation Ile219Val. The above mutants showed varying degree of cross resistance against different PS II inhibiting herbicide. Atrazine tolerant transgenic tobacco has been reported with transformation of nuclear genome with mutant *psbA* gene having transit peptide sequence.

The herbicide bromoxynil acts as a electron flow inhibitor and an uncoupler (Sanders and Pallett 1985). Dicot plants are mostly affected by bromoxynil and therefore it is used to control broadleaf weeds selectively among monocot crops. Gene (*bxn*) coding for nitrilase enzyme from a soil bacteria *Klebsiella ozaenae* (Stalker and McBride 1987; Stalker et al. 1988) was introduced in cotton using *Agrobacterium* transformation to yield bromoxynil tolerant plant (Stalker et al. 1996). The enzyme detoxifies bromoxynil to nontoxic benzoic acid. Detoxification process takes place in cytoplasm before bromoxynil reaches its site of action within chloroplast. This gene has been used to provide considerable tolerance to other plants like oilseed rape, tobacco and potato (Freyssinet et al. 1989). Calgene Inc and Aventis Crop Sciences Inc has developed bromoxynil resistant canola (Mulwa and Mwanza 2006).

4 Resistant Weeds

It is true that evolution of science helps various communities but the saying 'excess of everything is bad' fits in this context as well. The introduction of herbicides in the field of agriculture was quite handy for farmers. Herbicides helped them to combat unwanted weeds and simultaneously to increase the crop yield. However, these advantages were accompanied with inadvertent disadvantage of occurrence of resistant weeds varieties. Several workers have reviewed herbicide resistance (Devine and Eberlein 1997; Devine and Preston 2000; Preston and Mallory-Smith 2001; Smeda and Vaughn 1997).

There is an inherent ability of some weeds or crop biotype to survive adverse effect of herbicide/s to which the original population was susceptible. These weeds are termed as resistant weeds. Biotype refers to a group of plants within a species that has specific biological traits, because of genetic variation, that is not common to the population as a whole. 'Survival of the fittest/Natural selection' holds true in this regard. Resistant weeds survive the application of herbicides unlike the susceptible ones and gradually their population increases causing damage to crop plants and their yield.

Cross resistance is term used when following exposure to a herbicide, a weed population evolves resistance to herbicides from chemical classes to which it has never been exposed. Cross resistance has been observed in *Lolium rigidum* in Australia which evolved resistance to the ACCase inhibitor diclofop and exhibited cross-resistance to selective ALS inhibitors sulfonylureas and imidazolinones (Heap and Knight 1986; Christopher et al. 1991; Holt 1993). On the other hand weed biotype resistant to more than one herbicide with different target sites to which a population has been exposed is called as multiple resistance. For instance during 1985 in Great Britain resistance to chlortoluron [N′-(3-chloro- 4-methylphenyl)-N,N-dimethylurea] was reported in biotypes of *Alopecurus myosuroides* Huds. (black-grass or slender foxtail) (Kemp et al. 1990; Moss and Cussans 1985). Later these biotypes were observed to exhibit multiple resistance to other chemicals with different mode of action (Moss 1990).

Herbicide resistance was observed in 1957 against 2,4-D in Hawaii (Hilton 1957), however, the first confirmed report was against triazine herbicide in *Senecio vulgaris* (common groundsel) in 1970 (LeBaron and Gressel 1982; Ryan 1970; Radosevich and Appleby 1973). Latest reports suggest that currently 217 species (129 dicots and 88 monocots) of herbicide resistant weeds globally. Resistance has shown against 21 out of 25 known herbicide sites of action which includes 148 different herbicides. Data suggest that there has been almost exponential rise in the resistant weed varieties from 1980 (Heap 2013; Fig. 13).

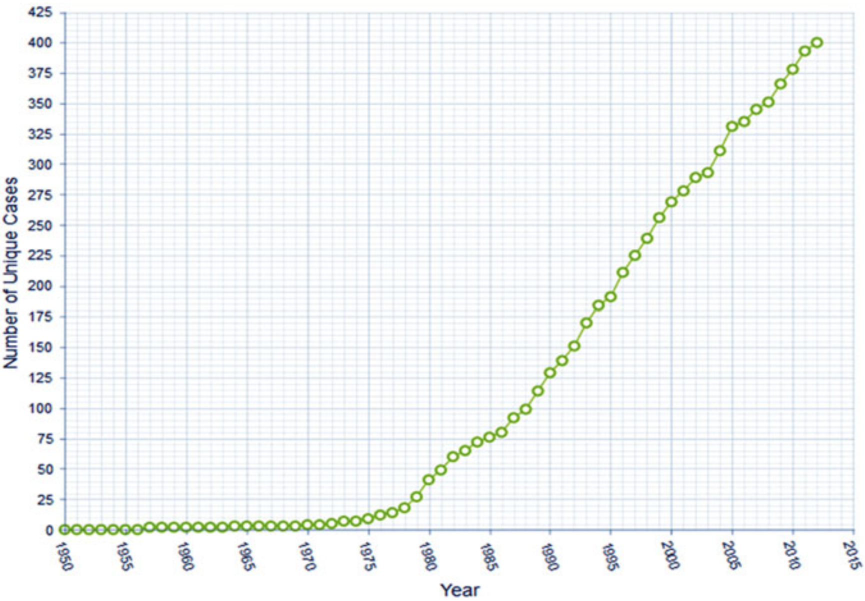


Fig. 13 Increase in resistant weeds globally (Adapted with permission from Heap 2013)

4.1 Mechanisms of Resistance

Herbicide resistance is the inherent ability of a species to survive and reproduce following exposure to a dose of herbicide normally lethal to its wild type. There can be various ways by which weeds develop resistance against herbicide/s.

4.1.1 Morphological Barrier

The herbicide is not taken readily by weed due to various morphological barriers like increased wax coat on cuticle, hairy epidermis and reduced leaf area. These barriers either do not allow herbicides to get to its site of action or accumulate up to the lethal dose.

4.1.2 Compartmentalization or Sequestration

Sometimes herbicides are sequestered in different locations of the cell or tissue away from the site of action e.g. lipid rich glands or oil bodies might be the site of immobilization of lipophilic herbicide (Stegink and Vaughn 1988). Sequestration of paraquat ahead of chloroplast has been reported (Bishop et al. 1987; Vaughn et al. 1989). Compartmentalization of herbicide in cell vacuole which inhibits its access to the site of action also provides resistance to weeds.

4.1.3 Detoxification

Resistant weed biotype has been observed to possess enzymes that detoxify the herbicide. The herbicide is converted into less harmful products through biochemical process before it reaches the site of action. Resistance shown by *Alopecurus myosuroides* against chlorotoluron and isoproturon (Kemp and Caseley 1991; Kemp et al. 1990) and Biotypes *of Lolium rigidum* against atrazine and chlorotoluron has been attributed to their rapid degradation possibly catalyzed by cytochrome P-450 monooxygenases (Holt 1993).

4.1.4 Altered Target Site

Resistant weeds show altered site of action of herbicide because of which the weeds remains unsusceptible to herbicide. This has been attributed to genetic changes that lead to formation of enzymes/proteins with some changes. This renders the enzyme functional but resistant against the herbicide. *Lactuca sativa* biotype has been observed to possess modified ALS enzyme (target for sulfonylurea herbicides) which is resistant to sulfonylurea (Eberlin et al. 1999).

4.1.5 Overproduction of Target Enzyme

Enhanced production of target enzyme dilutes the effect of the herbicide. The applied amount of herbicide targets the enzyme normally but left over extra amount of enzyme helps in fulfilling the needed metabolic activity.

4.2 Strategies for Weed Management

4.2.1 Minimal Reliance on Herbicides

Herbicides must be used whenever it is essential. Indiscriminate use of the same results in resistance. If possible, conventional ways of eradicating weeds must be used.

4.2.2 Use of Herbicides Mixture and Herbicide Rotation

Use of same type of herbicide on each application helps in selection and evolution of resistant weeds. In order to avoid the problem herbicides must be used in rotation. Also, mixture of herbicide with different site of action can avoid the problem as it broadens the spectrum of activity.

4.2.3 Crop Rotation

This helps in breaking up the weed life cycle. Alternating crops of different seasons signify tilling of the land at different times each year, which destroys resistant and susceptible weeds. Crop rotation leads to simultaneous rotation of herbicides and management practices.

4.2.4 Vigilance After Herbicide Application

Monitoring the field after herbicide application helps in targeting and destroying left over weeds using alternative methods. This minimizes proliferation of probable resistant weeds.

4.2.5 Use Herbicides with Short Residual Life

Application of herbicides with a long residual life enhances the selection pressure for resistant weeds. Increasing the dose of herbicide extends its residual period in the soil. Thus, use of herbicides with short residual life should be used.

4.2.6 Sanitation of Equipments

Cleanliness of equipments used in agriculture is a must to restrict the spread of resistant biotypes. Dissemination of resistant varieties is prevented if equipments are cleaned thoroughly before moving them from infested fields to clean fields.

4.2.7 Integrated Weed Control Measures

Various weed management practices viz., biological, mechanical and cultural must be combined as and when needed.

Herbicide resistance in weeds is now a subject of discussion worldwide. The rate with which resistant weed biotypes are occurring is quite alarming. Continuous and indiscriminate use of same herbicides has complicated the situation. Over reliance on herbicides should be avoided. Integrated herbicide management is important to avoid selection pressure for the evolution of resistance. There are certain benefits of resistant weeds as it helps scientists to explore and understand the resistance mechanism and use the same in crop plants. However, this needs issue needs to be dealt with thoroughly and effectively.

5 Transgenic Plants: A Debatable Topic

Like the two sides of coin every technology is also associated with its benefits and problems. Use of transgenic plant is a highly debatable topic worldwide. There is still no consensus whether these engineered plants should be a part of our daily life. Scientific reports both in favour and against transgenic plants are available. One of the issues that need to be addressed is gene flow from herbicide tolerant crops to weeds. There can be two ways by which this can happen:

1. Fertilization of related weeds by pollen from herbicide tolerant crops may produce tolerant hybrids. Introgression of herbicide tolerant trait into weeds may occur by backcrossing of these hybrids with weeds, which is essential for a stable incorporation of genes.
2. There can be horizontal transfer of genes by biological means (e.g. viruses) leading to spread of herbicide tolerant trait into plant species.

Apart from the above two issues the herbicide tolerant crops may become a volunteer weed outside the cultivation area and may form a self-sustaining weed population.

The possible solutions to the above problems are:

1. Create Herbicide tolerant plants that are male sterile-Plants which produce non-functional pollen. Another approach can be to develop genetically modified plants in such a way that the pollen is devoid of introduced resistant gene (Daniell et al. 1998; Daniell 1999a, b; Gressel 1999). Thus, there cannot be any problem of gene flow.

2. Another approach can be to create buffer zone/buffer strip around fields where genetically modified are grown (Huang et al. 1999; Daniell 1999a, b). Genetically modified crop is surrounded by a zone of same crop which is unmodified. It is assumed that resistant gene transfer to weeds by wind-blown pollen would not take place beyond the buffer zone. The actual width of buffer zone is still debatable. Moreover, large buffer zone is practically not feasible.

References

Andersen RN (1991) The north central weed control conference: origin and evolution. North Central Weed Sci. Soc, Champaign. 206 p

Andersen RN, Gronwald JW (1987) Non-cytoplasmic inheritance of atrazine – tolerance in velvet leaf (Abutilon theophrasti). Weed Sci 35:496–498

Aragao FJL, Vianna GR, Albino MMC, Rech EL (2002) Transgenic dry bean tolerant to the herbicide glufosinate ammonium. Crop Sci 42:1298–1302

Barber J (1987) Photosynthetic reaction centres: a common link. Trends Biochem Sci 12:321–326

Barry G, Padgette SR (1992) Glyphosate tolerant 5-enolpyruvylshikimate-3-phosphate synthases. World Patent, WO 92/04449

Barry G, Kishore G, Padgette S, Taylor M, Kolacz K, Weldon M, Re D, Eichholtz D, Fincher K, Hallas L (1992) Inhibitors of amino acid biosynthesis: strategies for imparting glyphosate tolerance to crop plants. In: Singh BK, Flores HE, Shannon JC (eds) Biosynthesis and molecular regulation of amino acids in plants. American Society of Plant Physiologists, Rockville, pp 139–145

Bayer E, Gugel K, Hagele K, Hagenmaier H, Jessipow S, Koning W, Zahner H (1972) Stoffwechselprodukte von mikroorganismen. Phosphinothricin und Phospinothricyl-alanyl-alanin. Helv Chimica Acta 55:224–239

Bayley C, Trolinder N, Ray C, Morgan M, Quisenberry JE, Ow DW (1992) Engineering 2,4-D resistance in cotton. Theor Appl Genet 83:645–649

Bender DA (1985) Amino acid metabolism, 2nd edn. John Wiley & Sons, Chichester

Bishop T, Powles SB, Comic G (1987) Mechanism of paraquat resistance in *Hordeumg laucum*. II. Paraquat up take and translocation. Aust J Plant Physiol 14:539–547

Bridges DC (1992) Crop losses due to weeds in the United States. Weed Sci Soc Am, Champaign, p 403

Bridges DC (1994) Impact of weeds on human endeavours. Weed Technol 8:392–395

Castle LA, Siehl DL, Gorton R, Patten PA, Chen YH, Bertain S, Cho HJ, Duck N, Wong J, Liu D, Lassner MW (2004) Discovery and directed evolution of a glyphosate tolerance gene. Science 304:1151–1154

Christoffers MJ, Nandula VK, Mengistu LW, Messersmith CG (2004) Altered herbicide target sites: implications for herbicide-resistant weed management. In: Inderjit (ed) Weed biology and management. Kluwer, Dordrecht, pp 199–210

Christopher JT, Powles SB, Liljegren DR, Holtum JAM (1991) Cross resistance to herbicides in annual ryegrass (*Lolium rigidum*) II. Chlorsulfuron resistance involves a wheat-like detoxification system. Plant Physiol 95(1):036–043

Christou OP, Ford TL, Kofro M (1991) Production of transgenic rice (*Oryza sativa* L.) plants from agronomically important indica and japonica varieties via electric discharge particle acceleration of exogenous DNA into immature zygotic embryos. Nat Biotechnol 9:957–962

Cobb A (1992) The transfer of herbicide resistance to crops. In: Herbicides and plant physiology. Chapman & Hall, London/New York, pp 145–151

Comai L, Sen LC, Stalker DM (1983) An altered aroA gene product confers resistance to the herbicide glyphosate. Science 22:370–371

Comai L, Facciotti D, Niatt WR, Thompson G, Rose RE, Stalker DM (1985) Expression in plants of a mutant *aroA* gene from *Salmonella typhinurium* confers tolerance to glyphosate. Nature 317:741–744

Commission of Life Sciences (2000) The future role of pesticides in US agriculture. Committee on the future role of pesticides in US agriculture, Board on agriculture and natural resources and Board on environmental studies and toxicology, National Research Council. National Academy Press, Washington, DC, p 332

Cremlyn RJ (1991) Agrochemicals: preparation and mode of action. Wiley, Great Britain

D'Halluin K, Bossut M, Mazur B, Leeman J, Botterman J (1992) Transformation of sugarbeet (*Beta vulgaris* L.) and evaluation of herbicide resistance in transgenic plants. Nat Biotechnol 10:309–314

Daniell H (1999a) GM crops: public perception and scientific solutions. Trends Plant Sci 4(12): 467–469

Daniell H (1999b) New tools for chloroplast genetic engineering. Nat Biotechnol 17(9):855–856

Daniell H, Datta R, Varma S, Gray S, Lee SB (1998) Containment of herbicide resistance through genetic engineering of the chloroplast genome. Nat Biotechnol 16(4):345–348

DeBlock M, Botterman J, Vandewiele M, Dockx J, Thoen C, Gossele V, Movva NR, Thompson C, Montagu MV, Leemans J (1987) Engineering herbicide resistance in plants by expression of a detoxifying enzyme. EMBO J 6:2513–2518

Della-Cioppa G, Bauer SC, Taylor ML, Rochester DE, Klein BK, Shah DM, Fraley RT, Kishore GM (1987) Targeting a herbicide resistant enzyme from Escherichia coli to chloroplasts of higher plants. Nat Biotechnol 5:579–584

Devine MD, Eberlein CV (1997) Physiological, biochemical and molecular aspects of herbicide resistance based on altered target sites. In: Roe RM, Burton JD, Kuhr RJ (eds) Herbicide activity: toxicology, biochemistry and molecular biology. IOS, Amsterdam, pp 159–185

Devine MD, Preston C (2000) The molecular basis of herbicide resistance. In: Cobb AH, Kirkwood RC (eds) Herbicides and their mechanisms of action. CRC, Boca Raton, pp 72–104

Devine MD, Duke SO, Fedtke C (1993) Physiology of herbicide action. Prentice Hall, Englewood Cliffs

Dhingra A, Daniell H (2004) Engineering herbicide resistance pathways in plastids. In: Daniell H, Chase CD (eds) Molecular biology and biotechnology of plant organelles. Kluwer Academic Publishers, Dordrecht, pp 491–512

Dill GM (2005) Glyphosate-resistant crops: history, status and future. Pest Manag Sci 61:219–224

Dodge AD (1991) Photosynthesis. In: Kirkwood RC (ed) Target sites for herbicide action. Plenum Press, New York, pp 1–28

Don RH, Pemberton JM (1981) Properties of six pesticide degradation plasmids isolated from *Alcaligenes paradoxus* and *Alcaligenes eutrophus*. J Bacteriol 145:681–686

Don RH, Weightman AJ, Knackmuss HJ, Timmis KN (1985) Transposon mutagenesis and cloning analysis of the pathways for degradation of 2,4-dichlorophenoxyacetic acid and 3-chlorobenzoate in *Alcaligenes eutrophus* JMP134. J Bacteriol 161:85–90

Dyer WE, Weller SC, Bressan R, Hermann K (1988) Glyphosate tolerance in tobacco (*Nicotiana tabacum* L.). Plant Physiol 88:661–666

Eberlin CV, Guittieri MJ, Berger PH, Fleming JK, Mallory-Smith CA, Thil DC, Baerg RJ, Belkmap WR (1999) Physiological consequences of mutation for ALS-inhibitor resistance. Weed Sci 47:383–392

Erickson JM, Rahire M, Rochaix JD, Mets L (1985) Herbicide resistance and cross-resistance: changes at three distinct sites in the herbicide-binding protein. Science 228(4696):204–207

Eschenburg S, Healy ML, Priestman MA, Lushington GH, Schonbrunn E (2002) How the mutation glycine96 to alanine confers glyphosate insensitivity to 5-enolpyruvyl shikimate-3-phosphate synthase from Escherichia coli. Planta 216:129–135

Falco MC, Tulmann-Neto A, Ulian EC (2000) Transformation and expression of a gene for herbicide resistance in a Brazilian sugarcane. Plant Cell Rep 19:1188–1194

FAO (2009) How to feed the world in 2050. FAO, Rome

FAO (2011) The state of the world's land and water resources for food and agriculture (SOLAW): managing systems at risk. fao.org/nr/solaw/solaw-home/en/. Accessed July 2013

Fillatti JJ, Kiser J, Rose R, Comai L (1987) Efficient transfer of a glyphosate tolerance gene into tomato using a binary *Agrobacterium tumefaciens* vector. Biotechnology 5:726–730

Franz JE, Mao MK, Sikorski JA (1997) Glyphosphate's molecular mode of action. In: Glyphosate: a unique global herbicide. American Chemical Society, Washington, DC, pp 521–642

Freyssinet G, Leroux B, Lebrun M, Pelissier B, Sailland A, Pallett KE (1989) Transfer of bromoxynil resistance into crops. Paper presented at the Brighton Crop Protection Conference, Farnham

Fukumori F, Hausinger RP (1993) Alcaligenes eutrophus JMP134 "2,4-dichlorophenoxyacetate monooxygenase" is an alpha-ketoglutarate-dependent dioxygenase. J Bacteriol 175(7): 2083–2086

Funke T, Han H, Healy-Fried ML, Fischer M, Schonbrunn E (2006) Molecular basis for the herbicide resistance of roundup ready crops. Proc Natl Acad Sci U S A 103:13010–13015

Gautham KC, Mishra JS (1995) Problems prospects and new approaches in weed management. Pestic Info 21:7–19

Goloubinoff P, Edelman M, Hallick RB (1984) Chloroplast-coded atrazine resistance in *Solanum* nigrum: psbA loci from susceptible and resistant biotypes are isogenic except for a single codon change. Nucleic Acids Res 12(24):9489–9496

Gordon-Kamm WJ, Spencer TM, Mngano ML, Adams TR, Daines RJ, Start WG, O'Brian JV, Chambers SA, Adams WR Jr, Willets NG, Rice TB, Mackey CJ, Krueger RW, Kausch AP, Lemaux PG (1990) Transformation of maize cells and regeneration of fertile transgenic plants. Plant Cell 2:603–618

Green MB, Hartley GS, West TF (1987) Chemicals for crop improvement and pest management, 3rd edn. Pergamon Press, Oxford, p 207

Gressel J (1999) Tandem constructs: preventing the rise of superweeds. Trends Biotechnol 17(9):361–366

Gruys KJ, Sikorski JA (1999) Inhibitors of tryptophan, phenylalanine, and tyrosine biosynthesis as herbicides. In: Singh B (ed) Plant amino acids. Marcel Dekker, Inc., New York, pp 357–384

Harker AR, Olsen RH, Seidler RJ (1989) Phenoxyacetic acid degradation by the 2,4-dichlorophenoxyacetic acid (TFD) pathway of plasmid pJP4: mapping and characterization of the TFD regulatory gene, tfdR. J Bacteriol 171:314–320

Haslam E (1993) Shimikic acid: metabolism and metabolites. John Wiley and Sons Inc, Chichester

Hattori J, Rutledge R, Labbe H, Brown D, Sunohara G, Miki B (1992) Multiple resistance to sulfonylureas and imidazolinones conferred by an acetohydroxy acid synthase gene with separate mutations for selective resistance. Mol Gen Genet 232:167–173

Haughn GW, Somerville C (1986) Sulfonylurea resistant mutants of *Arabidopsis thaliana*. Mol Gen Genet 204:430–434

Hay JR (1974) Gain to the grower from weed science. Weed Sci 22(5):439–442

Heap I (2013) The international survey of herbicide resistant weeds. Available www.weedscience.org. Accessed on July 2013

Heap IM, Knight R (1986) The occurrence of herbicide cross-resistance in a population of annual ryegrass, *Lolium rigidum*, resistant to diclofop-methyl. Aust J Agric Res 37:149–156

Herrmann KM (1995) The shikimate pathway: early steps in the biosynthesis of aromatic compounds. Plant Cell 7:907–919

Hilton HW (1957) Herbicide tolerant strain of weeds. Hawain Sugar Planters Association Annual Reports, p 69

Holt JS (1993) Mechanisms and agronomic aspects of herbicide resistance. Annu Rev Plant Physiol Plant Mol Biol 44:203–229

http://www.farmchemicalsinternational.com/article/30890/global-demand-for-agricultural-pesticides-to-approach-59-billion-in-2016. Accessed July 2013
Huang F, Buschman LL, Higgins RA, McGaughey WH (1999) Inheritance of Resistance to Bacillus thuringiensis Toxin (Dipel ES) in the European Corn Borer. Science 284(5416):965–967
James C (2012) Global status of commercialized biotech/GM crops for 2012, ISAAA brief no. 44. ISAAA, Ithaca
Keller G, Spatola L, McCabe D, Martinell B, Swain W, John ME (1997) Transgenic cotton resistant to herbicide bialaphos. Transgenic Res 6:385–392
Kemp MS, Caseley JC (1991) Synergists to combat herbicide resistance. In: Caseley JC, Cussans GW, Atkin RK (eds) Herbicide resistance in weeds and crops. Butterworth-Heinemann, Oxford, pp 279–292
Kemp MS, Moss SR, Thomas TH (1990) Herbicide resistance in *Alopecurus myosuroides*. In: Green MB, LeBaron HM, Moberg WK (eds) Managing resistance to agrochemicals: from fundamental research to practical strategies. ACS Books, Washington, DC, pp 376–393
Klingman GC, Ashton FM, Noordhoff LJ (1982) Weed science: principles and practices. John Wiley & Sons, New York, p 449
Komoba D, Sandermann H Jr (1992) Plant metabolism of herbicides with C–P bonds: phosphinothricin. Pestic Biochem Physiol 43:95–102
Kondo Y, Shomura T, Ogawa Y, Tsuroka T, Watanabe K, Totukawa T, Suzuki T, Moriyama T, Yoshida J, Inouye S, Niida T (1973) Isolation and physicochemical and biological characterization of SF-1293 substances. Sci Rep Meiji Seika Kaisha 13:34–43
Krieg LC, Walker MA, Senaratna T, McKersie BD (1990) Growth, ammonia accumulation and glutamine synthetase activity in alfalfa (*Medicago sativa* L.) shoots and cells treated with phosphinothricin. Plant Cell Rep 9:80–83
Last DI, Llewellyn DJ (1999) A detoxification gene in transgenic *Nicotiana tabacum* confers 2,4-D tolerance. Weed Sci 47:401–404
LeBaron HM, Gressel J (eds) (1982) Herbicide resistance in plants. Wiley, New York
Lebrun M, Sailland A, Freyssinet G, Degryse E (2003) Mutated 5-enolpyruvylshikimate-3-phosphate synthase, gene coding for said protein and transformed plants containing said gene. US Patent 6,566,587
Lowery RF (1987) Granular formulations and application. In: McWhorter CG, Gebhardt MR (eds) Methods of applying herbicides, WSSA monograph 4. Weed Sci. Soc. Amer, Champaign, pp 165–176. 358 pp
Lyon BR, Llewellyn DJ, Huppatz JL, Dennis ES, Peacock WJ (1989) Expression of a bacterial gene in transgenic tobacco confers resistance to the herbicide 2,4-dichlorophenoxyacetic acid. Plant Mol Biol 13:533–540
Malik J, Barry G, Kishore G (1989) The herbicide glyphosate. Biofactors 2:17–25
Mallory-Smith CA, Retzinger EJ Jr (2003) Revised classification of herbicides by site of action for weed resistance management strategies. Weed Technol 17:605–619
Manabe Y, Tinker N, Colville A, Miki B (2007) CSR1, the sole target of imidazolinone herbicide in Arabidopsis thaliana. Plant Cell Physiol 48:1340–1358
Manderscheid R, Wild A (1986) Studies on the mechanism of inhibition by phosphinothricin of glutamine synthetase isolated from *Triticum aestivum* L. J Plant Physiol 123:135–142
Marth PC, Mitchell JW (1944) 2,4-Dichlorophenoxyacetic acid as a differential herbicide. Bot Gaz 106:224–232
Marzabadi MR, Gruys KJ, Pansegrau PD, Walker MC, Yuen HK, Sikorski JA (1996) An EPSP synthase inhibitor joining shikimate 3-phosphate with glyphosate: synthesis and ligand binding studies. Biochemistry 35:4199–4210
McCloskey WB, Holt JS (1990) Triazine resistance in S*enecio vulgaris* parental and nearly isonuclear backcrossed biotypes is correlated with reduced productivity. Plant Physiol 92:954–962

McDowell LM, Schmidt A, Cohen ER, Studelska DR, Schaefer J (1996) Structural constraints on the ternary complex of 5-enolpyruvylshikimate 3-phosphate synthase from rotational-echo doubleresonance NMR. J Mol Biol 256:160–171

McHughen A, Smyth S (2008) US regulatory system for genetically modified [genetically modified organism (GMO), rDNA or transgenic] crop cultivars. Plant Biotechnol J 6:2–12

McIntosh L, Hirschberg J (1983) Molecular basis of herbicide resistance in *Amaranthus hybridus*. Science 222:1346–1349

Mitchell JW, Davis FF, Marth PG (1944) Turf weed control with plant growth regulators. Golfdom 18:34–36

Mohapatra U, McCabe MS, Power JB, Schepers F, Van der Arend A, Davey MR (1999) Expression of the bar gene confers herbicide resistance in transgenic lettuce. Transgenic Res 8:33–44

Montague A, Ziauddin A, Lee R, Ainley WM, Strommer J (2007) High-efficiency phosphinothricin-based selection for alfalfa transformation. Plant Cell Tissue Org Cult 91(1):29–36

Moss SR (1990) Herbicidec ross-resistancein slender foxtail (*Alopecurus myosuroides*). Weed Sci 38:492–496

Moss SR, Cussans GW (1985) Variability in the susceptibility of *Alopecurus myosuroides* (black-grass) to chlortoluron and isoproturon. Asp Appl Biol 9:91–98

Mulwa RMS, Mwanza LM (2006) Biotechnology approaches to developing herbicide tolerance/selectivity in crops. Afr J Biotechnol 5(5):396–404

Nafzinger ED, Widholm JM, Steinrucken HC, Kilmer JL (1984) Selection and characterization of a carrot line tolerant to glyphosate. Plant Physiol 76:571–574

Oerke EC (2006) Crop losses to pests. J Agric Sci 144:31–43

Ott K, Kwagh J, Stockton GW, Sidorov V, Kakefuda G (1996) Rational molecular design and genetic engineering of herbicide resistant crops by structure modeling and site-directed mutagenesis of acetohydroxyacid synthase. J Mol Biol 263:359–368

Pacanoski Z (2007) Herbicide use: benefits for society as a whole- a review. Pak J Weed Sci Res 13(1–2):135–147

Padgette SR, Re DB, Barry GF, Eichholtz DE, Delannay X, Fuchs RL, Kishore GM, Fraley RT (1996) New weed control opportunities: development of soybeans with a Roundup Ready TM gene. In: Duke SO (ed) Herbicide resistant crops. CRC Press, Boca Raton, pp 53–84

Pang SS, Guddat LW, Duggleby RG (2003) Molecular basis of sulfonylurea herbicide inhibition of acetohydroxyacid synthase. J Biol Chem 278:7639–7644

Park MY, Yi NR, Lee HY, Kim ST, Kim M, Park JH, Kim JK, Lee JS, Cheong JJ, Choi YD (2002) Generation of chlorsulfuron-resistant transgenic garlic plants (*Allium sativum* L.) by particle bombardment. Mol Breed 9:171–181

Parry MAJ, Madjwick PJ, Bayon C, Tearall K, Hernandez-Lopez A, Baudo M, Rakszegi M, Hamada W, Al-Yassin A, Ouabbou H, Labhilili M, Phillips AL (2009) Mutation discovery for crop improvement. J Ext Bot 60:2817–2825

Peng R-H, Tian Y-S, Xiong A-S, Zhao W, Fu X-Y, Han H-J, Chen C, Jin X-Y, Yao Q-H (2012) A Novel 5-Enolpyruvylshikimate-3-Phosphate Synthase from *Rahnella aquatilis* with significantly reduced glyphosate sensitivity. PLoS One 7(8):e39579. doi:10.1371/journal.pone.0039579

Perkins EJ, Lurquin PF (1988) Duplication of a 2,4-dichlorophenoxyacetic acid monooxygenase gene in *Alcaligenes eutrophus* JMP134(pJP4). J Bacteriol 170:5669–5672

Pieper DH, Engesser DH, Knackmuss HJ (1989) Regulation of catabolic pathways of phenoxyacetic acids and phenol in *Alcaligenes eutrophus* JMP 134. Arch Microbiol 151:365–371

Pline-Srnic W (2006) Physiological mechanisms of glyphosate resistance. Weed Technol 20:290–300

Pollegioni L, Schonbrunn E, Siehl D (2011) Molecular basis of glyphosate resistance-different approaches through protein engineering. FEBS J 278(16):2753–2766

Powles SB (2010) Gene amplification delivers glyphosate- resistant weed evolution. Proc Natl Acad Sci U S A 107:955–956

Preston C, Mallory-Smith CA (2001) Biochemical mechanisms, inheritance, and molecular genetics of herbicide resistance in weeds. In: Powles SB, Shaner DL (eds) Herbicide resistance and world grains. CRC, Boca Raton, pp 23–60

Radosevich SR, Appleby AP (1973) Relatives susceptibility of two common groundsel (*Senecio vulgaris* L.) biotypes to 6 s-triazines. Agron J 65:553–555

Rajasekaran K, Grula JW, Hudspeth RL, Pofelis S, Anderson DM (1996) Herbicide-resistant Acala and Coker cottons transformed with a native gene encoding mutant forms of acetohydroxyacid synthase. Mol Breed 2:307–319

Rao VS (2000) Principles of weed science, 2nd edn. CRC Press, New Hampshire

Reade JPH, Cobb AH (2002) Herbicides: modes of action and metabolism. In: Naylor REL (ed) Weed management handbook. Blackwell Science, Oxford, pp 134–170

Retzinger EJ, Mallory-Smith C (1997) Classification of herbicides by site of action for weed resistance management strategies. Weed Technol 11:384–393

Ryan GF (1970) Resistance of common groundsel to simazine and atrazine. Weed Sci 18:614–616

Saari LL, Mauvais CJ (1996) Chapter 8, Sulfonylurea herbicide-resistant crops. In: Duke SO (ed) Herbicide resistant crops. CRC Press, Boca Raton, pp 127–142

Sanders GE, Pallett KE (1985) *In vitro* activity and binding characteristics of the hydroxybenzonitriles in chloroplasts isolated from *Matricaria inodora* and *Viola arvensis*. Pestic Biochem Physiol 24:317–325

Saroha MK, Sridhar P, Malik VS (1998) Glyphosate-tolerant crops: genes and enzymes. J Plant Biochem Biotechnol 7:65–72

Sarria R, Torres E, Angel F, Chavarriaga P, Roca WM (2000) Transgenic plants of cassava (*Manihot esculenta*) with resistance to basta obtained by *Agrobacterium*-mediated transformation. Plant Cell Rep 19:339–344

Sauer H, Wild A, Ruhle W (1987) The effect of phosphinothricin (glufosinate) on photosynthesis II. The causes of inhibition of photosynthesis. Z Naturforsch 42c:270–278

Schmidt RR (1998) Classification of herbicides according to mode of action. Bayer Ag, Leverkusen, p 8

Schnell J, Labbé H, Kovinich N, Manabe Y, Miki B (2012) Comparability of imazapyr-resistant Arabidopsis created by transgenesis and mutagenesis. Transgenic Res 21(6):1255–1264

Schönbrunn E, Eschenburg S, Shuttleworth WA, Schloss JV, Amrhein N, Evans JNS, Kabsch W (2001) Interaction of the herbicide glyphosate with its target enzyme 5-enolpyruvylshikimate 3-phosphate synthase in atomic detail. Proc Natl Acad Sci U S A 98:1376–1380

Schulze-Siebert D, Heineke D, Scharf H, Schultz G (1984) Pyruvate derived amino acids in spinach chloroplasts – synthesis and regulation during photosynthetic carbon metabolism. Plant Physiol 76:465–471

Shaner DL, Singh BK (1992) How does inhibition of amino acid biosynthesis kill plants? In: Singh B, Flores H, Shannon J (eds) Biosynthesis and molecular regulation of amino acids in plants. American Society of Plant Physiologists, Rockville, pp 174–183

Shaner DL, Singh BK (1997) Acetohydroxyacid synthase inhibitors. In: Roe RM (ed) Herbicide activity: toxicology, biochemistry and molecular biology. Ios Press, Washington, DC, pp 69–110

Shaner DL, Bascomb NF, Smith W (1996) Imidazolinone-resistant crops: selection, characterization, and management. In: Duke SO (ed) Herbicide-resistant crops: agricultural, environmental, economic, regulatory, and technical aspects. CRC Lewis, New York, pp 143–157

Shimizu T, Nakayama I, Nagayama K, Miyazawa T, Nezu Y (2002) Acetolactate synthase inhibitors. In: Bger P, Wakabayashi K, Hirai K (eds) Herbicide classes in development. Springer, Berlin, pp 1–41

Singh BK, Shaner DL (1995) Biosynthesis of branched chain amino acids: from test tube to field. Plant Cell 7:935–944

Slater A, Scott NW, Fowler MR (2008) Plant biotechnology: the genetic manipulation of plants, 2nd edn. Oxford University Press, Oxford

Smeda RJ, Vaughn KC (1997) Mechanisms of resistance to herbicides. In: Sjut V (ed) Molecular mechanisms of resistance to agrochemicals, Chemistry of plant protection, vol. 13. Springer, Berlin/Heidelberg, pp 79–123

Sost D, Amrhein N (1990) Substitution of Gly-96 to Ala in the 5- enolpyruvylshikimate-3-phosphate synthase of *Klebsiella pneumoniae* results in a greatly reduced affinity for the herbicide glyphosate. Arch Biochem Biophys 282:433–436

Stalker DM, McBride KE (1987) Cloning and expression in *Escherichia coli* of a *Klebsiella ozaenae* plasmid-borne gene encoding a nitrilase specific for the herbicide bromoxynil. J Bacteriol 169:955–960

Stalker DM, Hiatt WR, Comai L (1985) A single amino acid substitution in the enzyme 5-enolpyruvylshikimate-3-phosphate synthase confers resistance to the herbicide glyphosate. J Biol Chem 260:4724–4728

Stalker DM, Malyj LD, McBride KE (1988) Purification and properties of a nitrilase specific for the herbicide bromoxynil and corresponding nucleotide-sequence analysis of the *bxn* gene. J Biol Chem 263:6310–6314

Stalker DM, Kiser JA, Baldwin G, Coulombe B, Houck CM (1996) Cotton weed control using the BXN system. In: Duke SO (ed) Herbicide resistant crops. Lewis, Boca Raton, pp 93–105

Stallings WC, Abdel-Meguid SS, Lim LW, Shieh H, Dayringer HE, Leimgruber NK, Stegeman RA, Anderson KS, Sikorski JA, Padgette SR, Kishore GM (1991) Structure and topological symmetry of the glyphosate target 5-enopyruvylshikimate-3-phosphate synthase: a distinctive protein fold. Proc Natl Acad Sci U S A 88:5046–5050

Stegink SJ, Vaughn KC (1988) Norflurazon SAN-9789 reduced abscisic acid levels in cotton seedlings: a glandless isoline is more sensitive than it's glanded counterpart. Pest Biochem Physiol 31:269–275

Steinrucken H, Amrhein N (1980) The herbicide glyphosate is a potent inhibitor of 5-enolpyruvylshikimic acid-3-phosphate synthase. Biochem Biophys Res Commun 94:1207–1212

Steinrucken H, Schutz A, Amrhein N, Porter C, Fraley R (1986) Overproduction of 5-enolphyruvylshikimate-3-phosphate synthase in glyphosate tolerant *Petunia* hybrida cell line. Arch Biochem Biophys 244:169–178

Stidham MA, Singh BK (1991) Imidazolinone-acetohydroxyacid synthase interactions. In: Shaner DL, O'Conner SL (eds) The imidazolinone herbicides. CRC Press, Boca Raton, pp 71–90

Streber WR, Willmitzer L (1989) Transgenic tobacco plants expressing a bacterial detoxifying enzyme are resistant to 2,4-D. Nat Biotechnol 7:811–816

Tachibana K, Watanabe T, Sekizawa Y, Takematsu T (1986) Accumulation of ammonia in plants treated with bialaphos. J Pestic Sci 11:33–37

Tan S, Evans RR, Dahmer ML, Singh BK, Shaner DL (2005) Imidazolinone- tolerant crops: history, current status and future. Pest Manag Sci 61:246–257

Tan S, Evans R, Singh B (2006) Herbicidal inhibitors of amino acid biosynthesis and herbicide-tolerant crops. Amino Acids 30:195–204

Thompson CJ, Movva RN, Tizard R, Crameri R, Davies JE, Lauwereys M, Botterman J (1987) Characterization of the herbicide-resistance gene *bar* from *Streptomyces hygroscopicus*. EMBO J 9:2519–2523

Tranel PJ, Wright TR (2002) Resistance of weeds to ALS-inhibiting herbicides: what have we learned? Weed Sci 50:700–712

Trebst A (1987) The three-dimensional structure of the herbicide binding niche on the reaction centre polypeptides of photosystem II. Z Naturforsch 42c:742–750

USEPA (2013) United States Environmental Protection Agency. http://www.epa.gov/pesticides/about/. Accessed July 2013

Vasil V, Castillio AM, Fromm M, Vasil IK (1992) Herbicide resistant fertile transgenic wheat plants obtained by microprojectile bombardment of regenerable embryonic callus. Nat Biotechnol 10:667–674

Vaughn KC, Duke SO (1991) Biochemical basis of herbicide resistance. Chem Plant Protect 7:141–169

Vaughn KC, Vaughan MA, Camilleri P (1989) Lack of cross resistance of paraquat-resistant hairy fleabane (*Conyza bonariensis*) to other toxic oxygen generators indicates enzymatic protection is not the resistance mechanism. Weed Sci 37:5–11

Vencill WK (ed) (2002) Herbicide handbook. Weed Science Society of America, Lawrence

Wehrmann A, Van Vliet A, Opsomer C, Botterman J, Schulz A (1996) The similarities of *bar* and *pat* gene products make them equally applicable for plant engineers. Nat Biotechnol 14:1274–1278

Wendler C, Barniske M, Wild A (1990) Effect of phosphinothricin (glufosinate) on photosynthesis and photorespiration of C_3 and C_4 plants. Photosynth Res 24(1):55–61

Went FW, Thimann KV (1937) Phytohormones. The Macmillan Co., New York, p 294

Wild A, Manderscheid R (1984) The effect of phosphinothricin on the assimilation of ammonia in plants. Z Naturforsch 39c:500–504

Wohlleben W (2000) Nucleotide sequence of phosphinothricin-*N*-acetyl- transferase gene from *Streptomyces viridochromogenes*, TueH94, and its expression in *Nicotiana tabacum*. Gene 70:25–37

Yadav A, Malik RK (2005) Herbicide resistant *Phalaris minor* in wheat–a sustainability issue. Resource book. Department of Agronomy and Directorate of Extension Education, CCSHAU, Hisar, p 152

Yaduraju NT (2006) Herbicide resistant crops in weed management. In: The extended summaries, golden jubilee national symposium on conservation agriculture and environment, Banaras Hindu University, Banaras, 26–28 October, pp 297–298

Yan H-Q, Chang S-H, Tian Z-X, Zhang L, Sun Y-C, Li Y, Wang J, Wang Y-P (2011) Novel AroA from *Pseudomonas putida* confers tobacco plant with high tolerance to glyphosate. PLoS One 6(5):e19732. doi:10.1371/journal.pone.0019732

Yoon K, Cole-Strauss A, Kmiec EB (1996) Targeted gene correction in mammalian cells mediated by a chimeric RNA/DNA oligonucleotide. Proc Natl Acad Sci U S A 93:2071–2076

Zhu T, Mettenburg K, Peterson DJ, Tagliani L, Baszczynski CL (2000) Engineering herbicide-resistant maize using chimeric RNA/DNA oligonucleotide. Nat Biotechnol 18:555–558

Zurawski G, Bonhert H-J, Whitfield PR, Bottomley W (1982) Nucleotide sequence of the gene for the 32000-M, thylakoid membrane protein from *Spinacia oleracea* and *Nicotiana debneyi* predicts a totally conserved primary translation product of Mr 38950. Proc Natl Acad Sci U S A 79:7699–7703

Nitrogen-Fixing Plant-Microbe Symbioses

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Abstract The symbiotic conversion of atmospheric nitrogen into nitrogenous compounds assimilated by higher organisms is an essential part of the global N cycle and a supporting pillar of agricultural practices. The nitrogen fixing symbiosis is not confined only to leguminous plants but also found some other plant families. The symbiotic relationship between plants and rhizobia is initiated by the release of flavonoids into the rhizosphere by the plant root. The release of flavonoids are sensed by the rhizobial transcriptional regulator *NodD*, which leads to symbioses-specific responses such as the release of Nod factors. The enzymes involved in the synthesis of basic Nod factor structure are encoded by the *nodABC* genes, conserved in almost all types of rhizobia. The perception of Nod factor by the plant is mediated by a receptor-like kinase, which induces intracellular calcium oscillations and leads to the deformation of root hairs through the restructuring of the cytoskeleton, leading to the formation of an infection thread. Although rhizobia are capable of synthesizing their own amino acids in the free living stage, within the infection thread rhizobia are dependent on the plant host for amino acids and other compounds.

Rhizobia were initially classified on the basis of their morphological, physiological characteristics and dominantly on host plant that they nodulate but after the invention of molecular techniques their molecular characteristics were taken into consideration. Thus, rhizobial taxonomy was repeatedly revised and refined. Currently, about 145 species of rhizobia have described from the genera *Azorhizobium*, *Allorhizobium*, *Agrobacterium*, *Bradyrhizobium*, *Ensifer* and *Rhizobium* and the taxonomic status of some genera and species has been revised. Root nodulating beta-rhizobia from different legumes have only been described

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recently, but the molecular evidence showed that they are existed as legume symbionts for 50 million years. Different species of beta-rhizobia contain common nodulation genes like *nodABC*, *nodD*, *nifH*, and these genes are very similar to the symbiotic genes of traditional rhizobia.

Phylogenetic analyses are the basis to understanding the evolutionary history of individual genes or entire genomes of microorganisms. Initially, the bacterial phylogenies were reconstructed on the basis of morphological and physiological characteristics of the cell. Later, sequence analysis and chemical content analysis were introduced to further improve phylogenies. Sequencing of 16S rRNA genes not only contribute significantly to the elimination of plasmid-borne characteristics from rhizobial taxonomy, but also helped in the identification of beta and gamma rhizobia. Nowadays, the sequencing of housekeeping genes, DNA profiling and the application of DNA arrays have become standard methods in bacterial taxonomy.

The legume-rhizobia association contributes significantly to the symbiotic biological nitrogen-fixing process, but other microbes such as cyanobacteria, endophytic bacteria, or *Frankia* sp. also form nitrogen-fixing associations of various degrees of intimacy with plants. Of course, the main benefit of legume cultivation comes from their ability to fix nitrogen in symbiosis with rhizobia but others are also beneficial for plants growth and survival in different environments. Aside from its biological benefit, biological nitrogen fixation is also critical for agriculture due to its impact on farming cost, sustainable land use, soil quality, and mitigation of greenhouse gas emissions. The present review covers the mechanism of legume-rhizobium symbiosis, rhizobial taxonomy, and non-rhizobial symbiotic nitrogen fixation processes by cyanobacteria, endophytic diazotrophs and *Frankia* sp.

Keywords Endophytic diazotrophs • *Frankia* • Nod factor • Phylogenetic analysis • *Rhizobia* • *Rhizobial* taxonomy

1 Introduction

The conversion of atmospheric nitrogen into nitrogenous compounds assimilated by higher organisms is an essential part of the global nitrogen cycle and a supporting pillar of agricultural practices. The two major pathways for nitrogen fixation in nature are the reduction of N_2 to ammonium by the nitrogenase enzymes of prokaryotes and, to a much lesser extent, the oxidation of N_2 to nitrogen oxides through lightning strike and photo-oxidation in the upper atmosphere. A turning point in the global nitrogen economy was the development of the industrial Haber-Bosch process, by which nitrogen is catalytically reduced to ammonia using hydrogen under conditions of high pressure and temperature. The production of nitrogen fertilizer through the Haber-Bosch process has been a major driver behind the increases in agricultural output and subsequent human population growth since the middle of the twentieth century. Billions of people are directly dependent on industrial nitrogen fixation for their food security and it has been estimated that up to a third of the human population is dependent on the Haber-Bosch process (Wolfe 2001). As the Haber-Bosch process is an energy-intensive reaction and is largely dependent on hydrogen derived from fossil fuels, viable alternatives for ensuring a steady supply of fixed nitrogen for agriculture and thus maintaining food security in a post-petroleum economy are necessary to support the large and continuously-growing human population.

Although only prokaryotes are capable of biological nitrogen fixation, a variety of plants have evolved intricate mutualistic symbioses with nitrogen-fixing bacteria to take advantage of this unique ability. Nitrogen-fixing symbiosis is particularly prevalent in leguminous plants belonging to the family *Fabaceae*. As a consequence of this symbiosis, members of this family are essentially independent of nitrogen fertilization, and the cultivation of legumes such as beans, lentils, or peas has been widespread since early agriculture to provide high-protein crops on relatively poor or exhausted soils. Although other plants also form nitrogen-fixing associations, the rhizobia-legume symbiosis is agriculturally the most relevant, and has been studied the most intensively.

2 The Rhizobia-Legume Symbiosis

2.1 Legume Taxonomy and Nodulation

The term 'legume', although not phylogenetically valid, is commonly used to describe members of the plant family *Fabaceae*. This family comprises the subfamilies *Caesalpinioideae*, *Mimosoideae* and *Papilionoideae*, which are sometimes given the status of closely-related families like *Caesalpiniaceae*, *Mimosaceae* and *Fabaceae*. Molecular evidence does however suggest that the *Caesalpinioideae* are paraphyletic within the (monophyletic) *Fabaceae* (Doyle and Luckow 2003).

Fabaceae are the third largest family of flowering plants after *Orchidaceae* and *Asteraceae* and comprises of about 700 genera and 2,000 species (O'Brian et al. 2009). It is extremely diverse plant family, ranging from small annual herbs to large trees, and is widespread in most habitats, from aquatic to desert environments. Nodulation, although widespread in this family, is not a general characteristic for legumes and also not restricted to family *Fabaceae*. Nodules are also found in nine other families in addition to legumes (Soltis et al. 1995). However, most of Papilionoidae genera and Mimosoidae genera are thought to form nitrogen-fixing nodules (Sprent 2001), making legumes by far the most important group of nodulated plants. The closest relatives of the legumes, such as *Polygalaceae*, *Surianaceae* and *Quillajaceae* do not show nodulation. Thus, it has been concluded that nodulation have been acquired independently in different legume groups (Doyle and Luckow 2003). Nonetheless, nodules from within the Papilionoideae also exhibit extensive diversity regarding morphology, anatomy and chemistry (Sprent 2001).

Nodulation is a complex event, and there is no simple explanation for its evolutionary origin (Doyle 1998; Sprent 2001; Doyle and Luckow 2003). It is assumed that the origin of nitrogen-fixing symbioses is not confined to a particular plant group, but rather originated independently in various ancestors (Doyle and Luckow 2003). Nevertheless, the events that lead to establishing a successful symbiosis between the two partners are broadly similar in all rhizobia-legume symbioses. The nitrogen-fixing symbiosis is predominant in leguminous plants belonging to the family *Fabaceae*, but is also found in other nine plant families and the nodulation has originated independently in various ancestors.

2.2 Establishing Symbiosis

The symbiotic relationship between nitrogen-fixing bacteria and plants depends on an extensive exchange of information between the two partners. The core features of this exchange of signals are conserved; subtle differences in signalling ensure that only specific bacterium-plant combinations lead to a successful symbiosis. The culminating of this interaction is the formation of a root nodule, an organ dedicated to creating a suitable environment for symbiotic nitrogen fixation.

Inter-kingdom communication is imitated by the release of flavonoids into the rhizosphere by the plant root (Peters et al. 1986; Djordjevic et al. 1987). As different plant species produce different flavonoid variants, this step serves as the first means of symbiont selection, as a given set of flavonoids is only recognised by a the subset of rhizobia that are adapted to infect the host producing them. Specificity of recognition is determined by the specificity of the constitutively expressed flavonoid-binding transcriptional regulator NodD, which serves as a master regulator for symbiosis genes (Fellay et al. 1995). Thus, the symbiotic association between plants and rhizobia is initiated by the release of flavonoids into the rhizosphere by plant root which may trigger the rhizobial transcriptional regulator NodD for this mutualistic alliance.

2.3 Bacterial Attachment

In the initial stages of infection, rhizobia attach to the root hair surface in a process that may involve a number of different mechanisms. The initial loose contact is mediated by means of adhesion proteins on the bacterial cell surface that recognize specific determinants of the plant cell wall. The molecular basis for the initial attachment may differ according to the symbiotic partners involved, adding another layer of selection specificity. For example, *Bradyrhizobium japonicum* attaches to the root hair surface of soybean via a unipolarly located lactose-binding lectin on the bacterial cell surface (Loh et al. 1993; Ho et al. 1994). Conversely, plant-derived lectins on the root hair surface have also been shown to bind to sugar moieties on the rhizobial cell surface (Calvert et al. 1978). In addition to these host-specific interactions, rhizobia are also able to associate with the roots of non-host legumes and also non-legumes, and even invade the intracellular spaces of the root cortex (Schloter et al. 1997).

The general unspecific adhesion of rhizobia to plant root hairs has been proposed to be dependent secreted calcium-binding proteins common to many rhizobia (Smit et al. 1992). The initial protein-mediated attachment of the bacterial cells to the root hair is then strengthened by the synthesis of cellulose fibrils by the rhizobia (Smit et al. 1992; Ausmees et al. 1999). Specific rhizobial attachment to legume root surface is mediated by several factors contributed by both mutualistic partners and the rhizobial calcium-binding protein also plays a role in non-specific attachment.

2.4 Nod Factor Synthesis and Signalling

The perception of a suitable flavonoid by the NodD regulator induces the rhizobia to respond by synthesizing specific host-directed signal molecules known as Nod factors. The core structure of a Nod factor is that of a lipo-chito-oligosaccharides consisting of four or five β-1,4 linked *N*-acetyl-glucosamine residues with a fatty acid residue replacing the *N*-acetyl group at the non-reducing end. To generate host-specificity, this basic structure is subject to variation, and certain rhizobial strains may synthesize a number of different varieties (Perret et al. 2000; Cullimore et al. 2001). Specificity determinants may be the identity of the fatty acyl group or further modifications at other locations of the oligosaccharide. For example, the pea, vetch, and lentil symbiont *R. leguminosarum* bv. *viciae* produces four different Nod factors consisting of a chito-oligosaccharide backbone with either a $C_{18:1}$ or $C_{18:4}$ acyl moiety, and the presence of absence of an O-acetyl group at the non-reducing end (Spaink et al. 1991). As plants only recognize Nod factor variants produced by compatible rhizobial strains, Nod factors are another key nodulation specificity determinant, and their regulation and biosynthesis has been extensively studied.

The constitutively expressed regulator NodD binds to the *nod* box, a conserved DNA sequence located upstream of nodulation operons, and activates transcription upon flavonoid binding, including the Nod factor biosynthesis genes (Rossen et al. 1985; Spaink et al. 1989; Schlaman et al. 1992). The enzymes involved in the synthesis of the basic Nod factor structure are encoded by the *nodABC* genes, which are conserved in all rhizobia. NodC is responsible for the synthesis of the *N-acetyl-glucosamine* backbone (Atkinson and Long 1992; Roche et al. 1996; Kamst et al. 1997), which is deacetylated at the non-reducing end by the action of the deacetylase NodB (John et al. 1993). The deacetylated Nod factor precursor is then acylated by *NodA*, with the exact identity of the fatty acid residue determined by the specificity of *NodA* (Atkinson et al. 1994; Rohrig et al. 1994; Roche et al. 1996), which is thus the first specificity determinant in Nod factor biosynthesis. Further modification of the basic Nod factor structure, such as the addition of sulphate, methyl or other residues to the backbone or the desaturation of the acyl residue, may be carried out by further strain-specific enzymes (Perret et al. 2000; Cullimore et al. 2001).

The sensitivity of legumes to an appropriate Nod factor is extremely high, and specific responses are induced at nano molar concentrations (Heidstra et al. 1994). The perception of Nod factor leads to a de-differentiation and activation of cell division in cells of the inner cortex, forming the nodule primordium (Oldroyd et al. 2011). The perception of a suitable flavonoid by the NodD regulator causes rhizobia to respond by synthesizing specific host-directed signal molecules known as Nod factors. The enzymes involved in the synthesis of the basic Nod factor structure are encoded by the *nodABC* genes, which are conserved in all rhizobia. The perception of Nod factors leads to de-differentiation and activation of cell division in cells of the inner cortex, leading to the formation of the nodule primordium.

2.5 Plant Signalling Pathway

Recognition on Nod factor by the plant initiates a well-researched signalling pathway. Several Nod factor receptors like NFR1, NFR5, LYK3, NFP, SYM37 and SYM10 have been identified in various legume species like *L. japonicas*, *Glycine max*, *M. truncatula* and *Pisum sativum* (Kouchi et al. 2010). Nod factors are recognized by receptor-like kinases with extracellular LysM domains (NFR1/NFR5), leading to intracellular oscillations of calcium ion concentrations with a participation of a symbiotic receptor kinase and components of the nuclear envelope and nuclear pore (Fig. 1). Calcium oscillations trigger a response via a calcium and calmodulin-dependent protein kinase, which phosphorylates the CYCLOPS protein. Several downstream transcription factors (NSP1, NSP2, ERN1 and NIN) regulate Nod factor-induced gene expression and induce nodule morphogenesis (Kouchi et al. 2010).

2.6 Infection Thread Formation

Nod factor perception also induces root hairs deformation via a restructuring of the cytoskeleton (Sieberer et al. 2005). In the presence of attached rhizobia, the altered direction of reinitiated tip growth causes a curling of the root hairs (Heidstra et al. 1994; Esseling et al. 2003), leading to an entrapment of attached rhizobia within the curls. With the exception of a few species such as peanut, in which rhizobia invade the root cortex by spreading through the intercellular spaces, the invasion of the root by the rhizobia originates from within these infection pockets.

The root hair cell wall poses a significant barrier to entry that has to be surmounted for successful infection. An obvious mechanism to achieve this is the use of hydrolytic enzymes to erode the cell wall sufficiently to bacteria entry. However, in order to avoid excessive degradation of the cell wall and destruction of the root

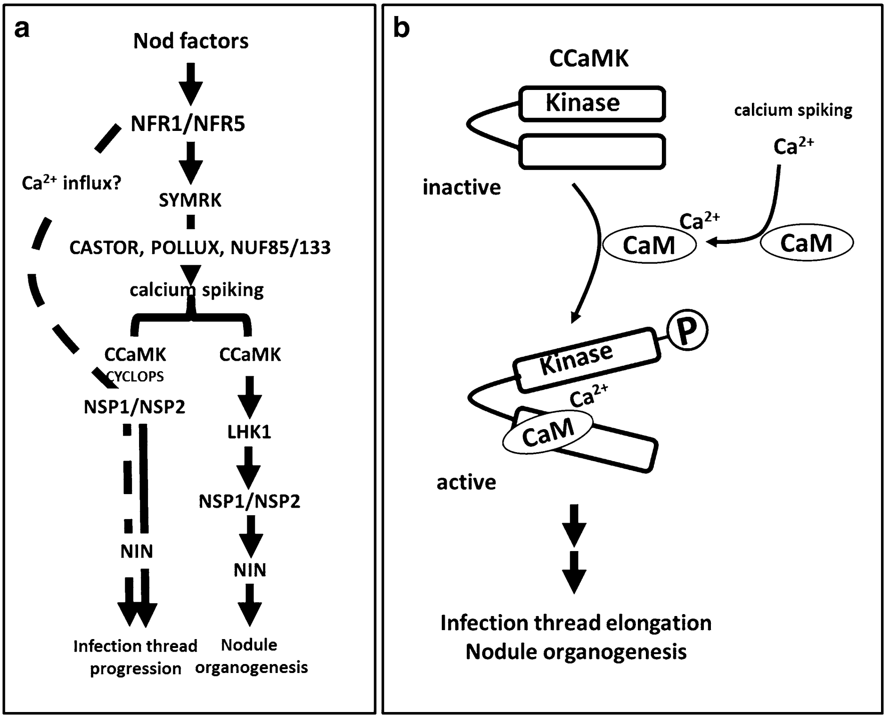

Fig. 1 Proposed symbiotic signaling pathways for legume nodulation. (**a**) The gene cascades involved in early symbiotic signaling. Rhizobial Nod factor induces Nod factor receptors to generate signals; one leads into the common symbiosis pathway (CSP, *solid line*) and the rest (*dotted line*) is prerequisite for successful infection. (**b**) Ca^{2+} signaling and calmodulin (CaM)-dependent protein kinase activation are main players in infection thread formation and growth

hair the activity of such enzymes has to be controlled and localized within the infection pocket. In some rhizobia cell associated pectinolytic and cellulolytic enzymes have been identified (Finnie et al. 1997, 1998; Krehenbrink and Downie 2008) that may be responsible for the erosion of root cell walls surrounding rhizobial cells (Mateos et al. 2001). Conversely, rhizobial factors have been reported to induce the production of a plant-derived polygalacturonase in plants (Palomares et al. 1978).

After the rhizobia have passed the cell wall, a tubular invagination of the cell wall termed the infection thread is laid down by the plant at the point of invasion (Fig. 2a). As the entry of non-symbiotic or even pathogenic bacteria into the infection thread could have disastrous consequences, the plant mounts a number of defence responses, including an increase in reactive oxygen species that have to be overcome by rhizobia (Santos et al. 2001; Jones et al. 2008; Krehenbrink et al. 2011). Successful bacteria also have to be able to utilize the nutrients present within the infection thread for growth (Gage 2002). Entry and growth within the infection thread are therefore also critical checkpoints for symbiont selection. The infection thread elongates further down the root hair and passes through the cortex to reach the meristematic cells of the nodule primordium. Bacteria follow the elongation of the infection thread by growing within it by binary fission. Once the infection thread has reached the nodule primordium, membrane vesicles that contain the rhizobial cells are pinched off and released into the host cell (Fig. 2a). At no point do the rhizobia themselves cross the host plasma membrane and enter the cytoplasm.

The Nod factor perception by a plant receptor-like kinase induces intracellular calcium oscillations and leads to root hair deformation via a restructuring of the cytoskeleton, leading to an entrapment of attached rhizobia within the roots and the formation of an infection thread.

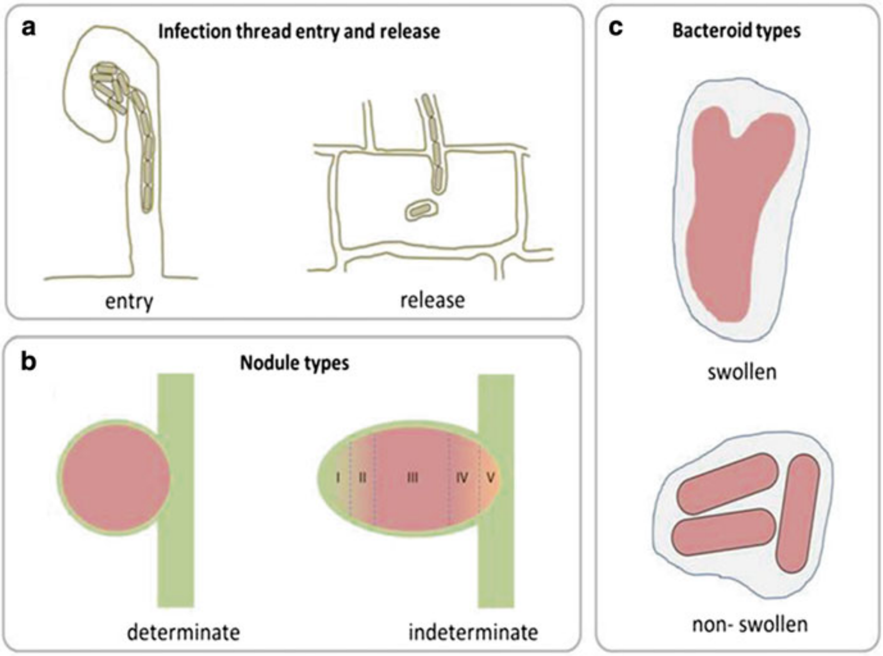

Fig. 2 Morphological features of the rhizobia-legume symbiosis. (**a**) Entry (*left*) and release (*right*) of rhizobia. Attached rhizobia are trapped in an infection pocket by a curling root hair and grow along the forming infection thread. Upon reaching the nodule primordium, bacterial cells enveloped in a plant derived membrane are pinched off into the plant cytoplasm. (**b**) Determinate (*left*) and indeterminate (*right*) nodules. Determinate nodules do not contain an apical meristem and have no developmental gradient. Both are present in indeterminate nodules. (**c**) Swollen and non-swollen bacteroids. Both types of bacteroid are surrounded by the plant-derived peribacteroid membrane. Swollen bacteroids are larger than free-living cells, pleiotropic in shape, and often branched. Non-swollen bacteroids are of a similar size and shape as free-living rhizobia

2.7 The Nodule Creates an Environment Suitable for Nitrogen Fixation

After release into the nodule primordium, rhizobia differentiate into bacteroids that are surrounded by the plant-derived peribacteroid membrane (Terpolilli et al. 2012). At the centre of a mature nodule, a microaerobic environment is created by a combined effort of the bacteria and the plant, allowing the oxygen-sensitive bacterial nitrogenase complex to efficiently fix atmospheric nitrogen. As well as being oxygen-sensitive, rhizobial nitrogen fixation also requires large amounts of ATP, which is supplied most efficiently by aerobic respiration, which in turn is oxygen-dependent (Downie 2005). A key role in solving this apparent paradox is played by the plant protein leghaemoglobin, which, similar to animal haemoglobins, serves as an oxygen store by reversibly binding molecular oxygen. However, unlike animal haemoglobin, leghaemoglobin exhibits a very rapid rate of oxygen binding and a comparatively slow rate of release (Appleby 1984). This high affinity for oxygen and the fact that leghaemoglobin accumulates to millimolar concentrations provides a buffering system for oxygen capable of maintaining a free oxygen concentration in the nanomolar range (Appleby 1984). Bacteroids are able to respire effectively at these low oxygen concentrations by expressing an alternative cytochrome oxidase with a very high oxygen affinity (Preisig et al. 1996), while the leghaemoglobin buffer provides a sufficiently high oxygen flux to keep oxygen levels constant.

After release into the nodule primordium, the rhizobia begin to fix atmospheric nitrogen at the centre of mature nodule. As the key enzyme, nitrogenase, is oxygen-sensitive, a microaerobic environment is created within the nodule by the rhizobia and the plant to protect it from damage. The protein leghaemoglobin serves as an oxygen store by exhibiting a very rapid rate of oxygen binding and a comparatively slow rate of release.

2.7.1 Nodule Types

Depending on whether or not the nodule meristem remains active after complete nodule differentiation or not, nodule types are classified as determinate or indeterminate (Terpolilli et al. 2012). In the former, the nodule meristem ceases activity relatively early in development, leading to nearly spherical nodules. Nodules of this type are for example formed by plants of the phaseoloid tribe such as beans and *Lotus japonicus*. In indeterminate nodules the apical nodule meristem remains active throughout the lifetime of the nodule, indeterminate nodules are therefore usually of an elongate shape. Plants that form indeterminate nodules include plants of the galegoid clade such as peas and *Medicago trucatula*.

Indeterminate nodules are also characterized by five distinct zones: (i) the apical meristem, (ii) the infection zone, where bacteria are released into the plant cell from infection threads, (iii) the nitrogen fixation zone with fully differentiated bacteroids, (iv) the senescence zone, and (v) the zone where free-living rhizobia from old infection threads re-infect the nodules as saprophytes (Vasse et al. 1990; Timmers et al. 2000). Determinate nodules lack any discernible zonation, and only contain a continuous nitrogen fixation zone (Fig. 2b). Depending on the plant, legume nodules may be determinate or indeterminate. In determinate nodules the meristem ceases activity at early stage of development, whereas it remains active through the lifetime of the nodule in indeterminate nodules.

2.7.2 Bacteroid Types

Besides these gross morphological differences, determinate and indeterminate nodules also differ at the level of bacteroid differentiation. The symbiosomes of determinate nodules usually contain a small number of bacteroids that are of a similar shape and size as free-living bacteria, while symbiosomes from indeterminate nodules usually contain a single swollen and pleomorphic bacteroid (Fig. 2c). The correlation between nodule type and bacteroid differentiation is however only a general rule and not absolute, and in some plants determinate nodules may contain swollen bacteroids, and vice versa (Oono and Denison 2010). Bacteroids in indeterminate nodules also undergo extensive end reduplication, resulting in high chromosome counts (n = 24 in *S. meliloti*), while determinate bacteroids do not undergo such an end reduplication (Mergaert et al. 2006). Indeterminate bacteroids also exhibit increased membrane permeability and are terminally differentiated and cannot revert to the free-living state. This is not the case in determinate bacteroids, which can de-differentiate into the free-living state upon nodule injury or senescence (Mergaert et al. 2006). The extent of bacteroid differentiation is not predetermined by the bacterial partner. *R. leguminosarum* bv. *viciae* usually infects the indeterminate nodules of pea and vetch and undergoes end reduplication, but this is not the case if it is modified to be able to colonize determinate *L. japonicus* nodules by introducing additional *nod* genes (Downie et al. 1985). The fact that the plant can induce its symbiont to terminally differentiate to effectively become a nitrogen-fixing organelle and give up its independent viability demonstrates the extent to which the plant is in control of the symbiosis.

Transcriptomic analysis of the indeterminate nodules from *M. truncatula* revealed a large number of nodule specific cysteine-rich defensin-like peptides containing 60–90 amino acids that colocalize with bacteroids and are absent from the determinate nodulator *L. japonicus* (Mergaert et al. 2003; Alunni et al. 2007; Kereszt et al. 2011). Expression of nodule cysteine-rich peptides from *M. truncatula* in *L. japonicus* led to enlarged and permeable bacteroids that closely resembled swollen bacteroids from *M. truncatula* nodules. Addition of nodule cysteine-rich peptides to free-living *S. meliloti* induced a similar endoreduplication, membrane permeabilization, and loss of viability as that observed in swollen bacteroids (de Velde et al. 2010). Nodule cysteine-rich peptides therefore appear to be directly responsible for controlling the fate of the bacterial symbiont within the nodule.

The adenosine triphosphate-binding cassette superfamily transporter BacA has been identified as being essential for bacteroid development (Glazebrook et al. 1993;

Tan et al. 2009) and BacA mutants have an increased resistance to antimicrobial peptides like bleomycin and Bac7 and a decreased resistance detergents and vancomycin (Marlow et al. 2009; Karunakaran et al. 2010). Although BacA is also involved in the modification of *S. meliloti* lipid A with very long-chain fatty acids, biosynthetic very long-chain fatty acid mutants are capable of full symbiosis with alfalfa exhibit a normal resistance to antimicrobial; peptides, demonstrating that the absence of very long-chain fatty acid lipid A is not the cause of the observed BacA phenotype (Ferguson et al. 2006).

R. leguminosarum bv. *viciae bacA* mutant is incapable of nitrogen fixation with its indeterminate host, but a bacA mutant of *R. leguminosarum* bv. *phaseoli* is capable of normal nitrogen fixation with its determinate host *P. vulgaris*, which lacks nodule cysteine-rich peptides (Oldroyd et al. 2011). As BacA modulates sensitivity to antimicrobial peptides and only affects the nodulation phenotype on plant that produce nodule cysteine-rich peptides, it is thought that BacA is directly or indirectly involved in mediating the response of the bacteria to nodule cysteine-rich peptides (Terpolilli et al. 2012). As BacA causes bacteria to differentiate into a terminally differentiated bacteroid that is unable to return to a free-living state in the presence of nodule cysteine-rich peptides, it would at first glance seem that the loss of bacA would be selected for in the nodule to allow the bacteria to escape terminal differentiation. However, although the presence of BacA is required for the differentiation of rhizobia into a non-viable state (Haag et al. 2011). Therefore, rather than being the cause of terminal differentiation, BacA has a pivotal role in protecting the bacteria from the toxic effect of the nodule cysteine-rich peptides (de Velde et al. 2010; Wang et al. 2010; Haag et al. 2011), in effect commuting a death sentence into life imprisonment. The exact mechanism by which BacA achieves this remains unknown, but may involve a substantial restructuring of both the inner and outer bacterial membrane (Karunakaran et al. 2010; Oldroyd et al. 2011).

Bacteriods in indeterminate nodules undergo extensive endo-reduplication, whereas bacteroids of determinate nodules do not. Transcriptomic analysis of the indeterminate nodules from *M. truncatula* revealed a large number of nodule-specific cysteine-rich defensin-like peptides consisting of 60–90 amino acids that colocalize with bacteroids and are absent from the determinate nodulator *L. japonicus*.

3 Symbiotic Auxotrophy

Once the rhizobia have entered the infection thread they become dependent on the plant host for the provision of nutrients (Gage 2002), and the ability to utilize the mix of carbon sources present within the infection thread and nodule may play a role in symbiont selection to some extent. The dependence on the plant partner may become even more severe after bacteroid differentiation. Although capable of synthesizing its own amino acids in the free-living state, some rhizobia such as *R. leguminosarum* shut down branched chain amino acid biosynthesis after differentiation and rely on amino acids supplied by the plant (Terpolilli et al. 2012). In addition to this facultative symbiotic auxotrophy, the nitrogen fixation activity of the bacteroid is also dependent on homocitrate provided by the plant. Free-living diazotrophs such as *Azotobacter vinelandii* are able to synthesize homocitrate as an essential component of the nitrogenase Fe-molybdenum cofactor from 2-oxoglutarate and acetyl-coenzyme A via the homocitrate synthase NifV (Zheng et al. 1997), but this enzyme is absent from most rhizobia. Instead, homocitrate is synthesized by the plant homocitrate synthase FEN1 and transferred to the bacteroid for incorporation into Fe-molybdenum cofactor. FEN1 is only expressed in nodules infected with rhizobia. FEN1 mutant plants are still infected by rhizobia, but the bacteroids are unable to fix nitrogen, and in contrast to wild type nodules homocitrate is not present in the *FEN1* mutant. When either FEN1 or *nifV* from *A. vinelandii* is expressed in *M. loti*, nitrogen fixation again becomes possible in a FEN1 plant mutant (Hakoyama et al. 2009).

As nitrogenase activity is exquisitely sensitive to oxygen, it is not surprising that the transcription of the genes involved in nitrogen fixation is only up regulated under low oxygen conditions. The exact mechanism by which this takes place however varies between species, but up regulation of nitrogen fixation is usually accompanied by a cessation of ammonia assimilation into amino acids within the bacteroid, which is in stark contrast to free-living diazotrophs (Terpolilli et al. 2012). Plants infected with bacteria carrying mutations in *gltB* (glutamate oxoglutarate amidotransferase) and *aldA* (alanine dehydrogenase) that are unable to assimilate ammonia exhibit normal nitrogen fixation (Mulley et al. 2011). They have suggested that bacteroids directly supply the plant with unassimilated ammonia. Instead, ammonia assimilation into amino acids takes place within the plant, which then returns some of the nitrogen back to the bacteroid in the form of amino acids (Patriarca et al. 2002; Oldroyd et al. 2011). Within the infection thread rhizobia become dependent on the plant host for nutrients and other compounds. Although rhizobia are capable of synthesizing their own amino acids in the free living stage, some rhizobia, like *Rhizobium leguminosarum*, cease to synthesize amino acid at the bacteroid stage.

3.1 Rhizobial Taxonomy

Our understanding of bacterial taxonomy is not fixed, but rather subject to change with the development of new ideas and new insights. To accommodate these new insights, it is necessary to adapt the taxonomy, and subsequently changes have occurred in the classification, nomenclature and identification of bacterial strains. As for eukaryotes there are different levels of bacterial taxonomy such as phylum, family, genus, species and sub-species (Brenner et al. 2005).

Rhizobia are well known for their capacity to form nodules with legumes. At the very beginning of bacterial classification (Bergey et al. 1923), rhizobia were described as gram-negative, aerobic, non-spore forming nodule-forming bacteria. Nodulation capacity was the main distinguishing criterion, and non-nodulating bacteria with the same morphological characteristics were not included in rhizobia. Later, nodulation host range and behavior on different growth media were used to further distinguish between different rhizobial strains. Based on growth rate, the rhizobia has been classified as fast or slow growing (Young 1996). It had been shown that some rhizobia contain extra-chromosomal symbiotic genes on plasmids, and these plasmids are transferable between rhizobia and among other bacteria by transformation and conjugation. Although the transferable nature of plasmids and plasmid-borne genes had been well known to the Scientific community, for a long time plasmid-borne characteristics such as nodulation and pathogenicity were dominant characters in rhizobial taxonomy (Zurkowski and Lorkiewicz 1976). For example, four rhizobial species such as *R. leguminosarum*, *R. phaseoli*, *R. trifoli* and *R. meliloti* were included on the first published valid list of bacterial species, with the taxonomy dominated by the nodulation host (Skerman et al. 1980).

In 1975, the International Committee of Systematic Bacteriology has proposed a minimum standard for describing new species. Following the code of bacterial nomenclature, Jordan (1982) used numerical taxonomy, DNA hybridization, guanine cytosine content, serological response, extracellular composition, carbohydrate utilization pattern, metabolism type, bacteriophage and antibiotic susceptibility, protein composition and bacteroid type to propose the new genus *Bradyrhizobium* in the family *Rhizobiacae* and rearranged previously-described species. Three previously-described species such as *R. leguminosarum*, *R. trifoli* and *R. phaseoli* were combined into a single species named as *R. leguminosarum*. Other two species, *R. meliloti* and *R. loti*, remained in the genus *Rhizobium*, but all slow-growing rhizobia were transferred to the genus *Bradyrhizobium* (Jordan 1984).

Initially, rhizobial taxonomy was dominated by its nodule formation capacity. As science progressed, morphological, physiological, and molecular characteristics were also taken into consideration, and rhizobial taxonomy was repeatedly revised and refined.

3.1.1 Taxonomy of Alpha-Rhizobia

As a consequence of the definition in terms of function (nodulation) rather than taxonomy, the group '*Rhizobia*' contains diverse species of bacteria and is not a monophyletic group of bacteria. For a long time it was assumed that all rhizobia came from the alpha proteobacteria, but recently a number of genera have been described from beta and also gamma proteobacteria.

The international sub-committee on the taxonomy of *Rhizobium* and *Agrobacterium* is responsible for curating the taxonomy of root-nodulating rhizobia (http://edzna.ccg.unam.mx/rhizobial-taxonomy/node/4). According to this, 145 species from alpha-rhizobia from the genera *Rhizobium* (Frank 1889), *Azorhizobium* (Dreyfus et al. 1988), *Bradyrhizobium* (Jordan and Allan 1974), *Ensifer* (Chen et al. 1988; Scholla and Elkan 1984), *Agrobacterium* (Conn 1942), and *Allorhizobium* (de Lajudie et al. 1998) have been described (Gyaneshwar et al. 2011).

Rhizobium

To describe the nodule forming bacteria of the legume tribe *viciae*, Frank (1889) proposed the name *Rhizobium*, with *Rhizobium leguminosarum* as the type species. In this proposal, all nodule-forming bacteria are known as rhizobia, with *Rhizobium* as the first genus of nodule-forming bacteria. There are two synonyms such as *Phytomyxa* and *Rhizobacterium* for this genus (http://edzna.ccg.unam.mx/rhizobial-taxonomy/node/4). It is a very diverse genus with more than seven sub-lineages described by Tian et al. (2010) and an additional two sub-lineages described by Rashid et al. (2014). Currently, it has 65 valid published species. Different species have been isolated from a wide range of host like *Pisum*, *Lathyrus*, *Phaseolus*, *Trifolium*, *Lupinus*, *Lens*, *Vicia*, *Galega*, *Stylosanthes*, *Acacia*, *Macroptilium*, *Medicago*, *Sesbania*, *Coronilla*, *Hedysarum*, *Indigofera* and *Astragalus* (Willems 2006).

Bradyrhizobium

Following the proposal of the International Committee on Bacterial Taxonomy, Jordan (1982) first proposed the genus *Bradyrhizobium* and included it in Bergey's Manual on Systematic Bacteriology. This genus showed very slow growth on yeast extract mannitol agar medium, with 1 mm colony diameter reached within 5–7 days at 25–30 °C. It produces an alkaline reaction in mineral salt-mannitol medium. At present it contains 15 valid published species.

Agrobacterium

Conn (1942) proposed the new genus *Agrobacterium* to describe plant hypertrophy-causing bacteria and later Skerman et al. (1980) included this genus in the approved list of bacteria. Although this genus was described on the basis of phytopathological properties, not all species are pathogenic. For example, *Agrobacterium tumefaciens* forms tumours and *Agrobacterium rhizogen*es forms hairy roots, but *Agrobacterum radiobacter* is not pathogenic. The others three bacterial species in this proposed genus are pathogenic for very specific hosts only, such as *Agrobacterium rubi* for *Rubus*, *Agrobacterium vitis* for grape vine, and *Agrobacterium larrymoorei* on *Ficus* (Willems 2006). It contains 11 validly described species, including the previously described six species.

Allorhizobium

The genus *Allorhizobium* was proposed by de Lajudie et al. (1998) to describe bacteria nodulating the legume *Neptunia natans*. *Allorhizobium undicola* had been isolated from an annual aquatic legume that is indigenous to waterlogged areas of Senegal. This genus is phylogenetically separate from other species of rhizobia. Member of this genus cannot produce 3-ketolactose from lactose. However, in addition to nodule formation, members of this genus can produce hairy roots on host legumes. So far only one nodulating species has been described in this genus.

Sinorhizobium

Based on numerical taxonomy, guanine cytosine content, DNA hybridization, serological data, cellular composition, bacteriophage typing, and protein pattern, Chen et al. (1988) proposed the new genus *Sinorhizobium* with the type species *Sinrhizobium fredi*. Although their initially the proposal did not provide phylogenetic evidence, but later de Lajudie et al. (1994) provided phylogenetic evidence in favor of *Sinorhizobium*. Members of this genus have been isolated from different host like *Melilotus*, *Medicago*, *Acacia*, *Kummerowia*, and *Leucaena* (Willems 2006). In a comparative study, Willems et al. (2003) concluded that *Ensifer* and *Sinorhizobium* were synonyms. Almost at the same time, Young (2003) proposed that *Ensifer* is the correct name of the genus, as it has been proposed earlier. This proposal is now well-accepted, and the genus is generally known as *Ensifer*. At present this genus has 25 valid nodulating species.

Mesorhizobium

Jarvis et al. (1997) proposed *Mesorhizobium* as a new genus from five previously-described *Rhizobium* species such as *Rhizobium loti*, *Rhizobium huakui*, *Rhizobium ciceri*, *Rhizobium mediterraneum*, *Rhizobium tiashanense* following the rules of bacterial taxonomy. '*Mesorhizobium*' means between *Rhizobium* and *Bradyrhizobium*, different species of this genus show growth rates intermediate between *Rhizobium* and *Bradyrhizobium*. Strains of this genus also show high nodulation host specificity, and cross-inoculation between different hosts was not observed. *Mesorhizobium* species have been isolated from *Lotus*, *Lupinus*, *Leucaena*, *Astralagus*, *Cicer*, *Glycyrrhiza*, *Sophora*, *Acacia*, *Prosopis*, *and Amorpha* (Willems 2006). At present, this genus has 25 nodulating species.

3.1.2 Ambiguity in *Rhizobium-Agrobacterium-Allorhizobium*

All described species in the genus *Agrobacterium* were named and included in this genus on the basis of plasmid-borne characteristics like tumourgenesis, pathogenesis, and rhizogenesis. Plasmids and plasmid-borne genes are not stable characteristics of bacteria, but rather exchangeable between closely- and even distantly-related bacterial species. For example, *Agrobacterium tumafaciens* is well-known for its ability to transfer DNA between themselves and plants, and is now an important tool in all fields of molecular biology research.

Nowadays, taxonomists are trying to establish a natural classification of microorganisms by describing stable characteristics to avoid ambiguity. Several studies have found such ambiguity between *Rhizobium* and *Agrobacterium* and therefore it has been proposed that *Agrobacterium* should be merged with *Rhizobium* (Young et al. 2001). Moreover, de Lajudie et al. (1998) found heterogeneity within *Rhizobium* that unsettled the position of *Agrobacterium* in phylogenetic analyses, and therefore proposed the new genus *Allorhizobium*. Nonetheless, phenotypic differences are absent and phylogenetic evidence was not satisfactory to distinguish the three genera such as *Agrobacterium*, *Allorhizobium and Rhizobium*. Thus these genera should be included under the single genus *Rhizobium* (Young et al. 2001).

3.1.3 Some Major Revisions in Rhizobial Taxonomy

A valid list of bacterial species was first published by Skerman et al. (1980), and after that it became mandatory to publish or validate all newly-described bacterial species in the International Journal of Systematic and Evolutionary Microbiology (Rivas et al. 2009a). In this list, four rhizobial species namely *Rhizobium leguminosarum*, *Rhizobium phaseoli*, *Rhizobium trifoli and Rhizobium meliloti*, were included. In 1984, following the proposal of the International Committee on Bacterial Taxonomy, Jordan (1984) proposed the new genus *Bradyrhizobium* and rearranged previously described species. The proposals were published in Bergey's Manual on Systematic Bacteriology but had not been validated in International Journal of Systematic and Evolutionary Microbiology. Therefore, the previously described species names remained valid until 2008. However, Ramirez-Bahena et al. (2008) revised the taxonomic status of *Rhizobium trifoli*, *Rhizobium phaseoli* and *Rhizobium leguminosarum*, merging *R. trifoli* into *R. leguminosarum* but retaining *R. phaseoli* as a separate species.

Since, the 16S rRNA gene of *Agrobacterium tumefaciens* and *Agrobacterium radiobacter* are identical to *Agrobacterium radiobacter* which had been proposed earlier. Thus, Sawada et al. (1993) proposed to merge *Agrobacterium tumifaciens* into *Agrobacterium radiobacter*. However, after several proposals for the revision of the three genera *Rhizobium*, *Allorhizobium* and *Agrobacterium*, the proposal of Young et al. (2001) has been accepted and *Agrobacterium* and *Allorhizobium* have now been merged into the genus *Rhizobium*. Currently, 145 species of rhizobia have described from the genera *Azorhizobium*, *Allorhizobium*, *Agrobacterium*, *Bradyrhizobium*, *Ensifer* and *Rhizobium*. The taxonomic status of some genera and species has been revised and the genus *Agrobacterium* and *Allorhizobium* have now been merged into the genus *Rhizobium*, and *Rhizobium trifolii* has merged into the species *Rhizobium leguminosarum*.

3.2 Taxonomy of Beta-rhizobia

Nodulating beta- and gamma-proteobacteria have only been described relatively recently and are known as beta-rhizobia or non-traditional rhizobia (Gyaneshwar et al. 2011). In total, 18 validly published, beta-rhizobia rhizobial species have been described from the following genera: *Aminobacter* (Urakami et al. 1992), *Devosia* (Nakagawa et al. 1996), *Methylobacterium* (Patt et al. 1976), *Microvigra* (Kanso and Patel 2003), *Ochrobactrum* (Holmes et al. 1988), *Phyllobacterium* (Knosel 1984), Shinella (An et al. 2006), *Burkholderia* (Yabuuchi et al. 1992), *Cupriavidus* (Makkar and Casida 1987), and *Herbaspirillum* (Baldani et al. 1986).

3.2.1 *Burkholderia*

Burkholderia is a diverse genus and different species have been isolated from plant, soil, and clinical samples, and have symbiotic and pathogenic properties. *Burkholderia* symbionts have been isolated from native and introduced *Mimosa* species across South Africa, South, North and Central America, Taiwan, China, and Australia (Gyaneshwar et al. 2011). Among the different beta-proteobacteria associated with legumes, *Burkholderia* is the biggest genus and contains 50 species (Gyaneshwar et al. 2011) and contains eight nodule forming symbiotic and nitrogen-fixing species namely *Burkholderia carebensis*, *Burkholderia cepacia*, *Burkholderia tuberum*, *Burkholderia sabiae*, *Burkholderia phymatum*, *Burkholderia mimosarum*, *Burkholderia diazotrophica*, *and Burkholderia symbiotica*.

3.2.2 *Cupriavidus*

This genus was described by Makkar and Casida (1987). Recently, the genome of one species, *Cupriavidus necator*, has been completely sequenced and shown to possess two large chromosomes and two plasmids (Lykidis et al. 2010). *Cupriavidus necator* can also degrade a number of chloroaromatic compound and chemically related pollutant from polluted soil.

Nodule forming *Cupriavidus taiwanensis* species have isolated from *Mimosa pudica* from Taiwan, India and the other *Mimosa* species has been discovered from other parts of world (Gyaneshwar et al. 2011). *Cupriavidus necator* have been isolated from host such as *Parapiptadenia rigida* from Uruguay and *Leucaena leucocephala* and *Phaseolus vulgaris* from Brazil (da Silva et al. 2012).

3.2.3 *Herbaspirillum*

Different members of this genus have been isolated from plant material, clinical samples, and root nodules of *Phaseolus vulgaris* (Valverde et al. 2003). Different species of the genus *Herbaspirillum* isolated from soil and plant material showed an ability to fix nitrogen (Schmid et al. 2006). Although morphological characteristics such as cell structure, growth behaviour and habitat have similarity with *Azospirillum*, DNA hybridization and 16S rRNA sequence analyses did not find a close relationship to *Azospirillum* (Falk et al. 1986). The most-studied species is *Herbaspirillum seropedicae*, which is also used as a bioinoculant or biofertilizer. This genus has one nodulating species, *Herbaspirillum lusitanum*, which had been isolated from *Phaseolus vulgaris* by Valverde et al. (2003) but it failed to renodulate under laboratory conditions.

3.2.4 *Devosia*

This genus was proposed by Nakagawa et al. (1996). Phylogenetically this genus belongs to the alpha-proteobacteria, the type strain is *Devosia riboflabina* isolated from soil. Later, *Devosia neptuniae*, nodulating the legume *Neptunia natans*, has been described from India by Rivas et al. (2003). Although this genus is in the order Rhizobiales, it belongs to family *Hyphomicrobiaceae*. Currently there is one nodulating species in this genus.

3.2.5 *Shinella*

This genus was proposed by An et al. (2006) with the type species *Shinella granuli*. Recently, Lin et al. (2008) described a nodulating species, *Shinella kummerowiae*. This species can grow at pH 5–11, tolerate 3 % NaCl, and can grow on lysogeny broth medium. The type strain is CCBAU 25048, which was isolated from root nodules of *Kummerowia stipulacea* grown in Shandong province, China. The type strain contains symbiotic *nodC*, *nodD* and *nifH* genes similar to those of *R. tropici* CIAT 899T and CFN 299.

3.2.6 *Microvirga*

Kanso and Patel (2003) proposed the new genus *Microvigra*. Members of this genus showed a close relationship to *Rhizobiacae* in phylogenetic analyses. The three nodulating species have been described by from the legumes *Listia angolensis* from Zambia and *Lupinus texensis* from Texas, USA. These three species are *Microvirga lupini* sp. nov., *Microvirga lotononidis* sp. nov., and *Microvirga zambiensis* sp. nov. These species are root nodulating alpha-proteobacteria that specifically nodulate and fix nitrogen with geographically and taxonomically distinct legume hosts. The *nodA* gene from the type strain to *Bradyrhizobium*, *Burkholderia* and *Methylobacterium*, *Microvirga lupine* Lut6 showed similarity with *Rhizobium*, *Sinorhizobium*, and *Mesorhizobium*, while *nodA* from the type strains of *Microvirga lotononidis* and *Microvirga zambiensis* showed similarity to *Bradyrhizobium*, *Burkholderia* and *Methylobacterium*.

3.2.7 *Ochrobactrum*

This genus was proposed by Holmes et al. (1988) with the novel species *Ochrobactrum anthropi* isolated from human specimens. Two nodulating strains had been described by Trujillo et al. (2005) from nodules of *Lupinus honoratus*. Phylogenetic analysis, based on the 16S and 23S rRNA gene sequences, showed that these belonged to the genus *Ochrobactrum*; their symbiotic *nodA* and *nifH* genes showed similarity to *Rhizobium*. A nodulating species, *Ochrobactrum cytisi*, was also isolated from nodules of *Cytisus scoparius* in Spain by Zurdo-Pineiro et al. (2007).

3.2.8 *Phyllobacterium*

The genus *Phyllobacterium* was proposed by Knosel (1984) with *Phyllobacterium myrsinacearum* as type species but is was validated by Skerman et al. (1980). Members of this genus were isolated from the leaf nodules of plants from the families *Mysinaceae* and *Rubiaceae*. Later, Valverde et al. (2005) described a root-nodulating species, *Phyllobacterium trifolii*, from *Trifolium* and *Lupinus* from Spain.

3.2.9 *Methylobacterium*

The genus *Methylobacterium* was proposed by Patt et al. (1976) with the type species *Methylobacterium organophillum*. Later, Jourand et al. (2004) describe root nodulating species *Methylobacterium nodulans* from the legume species *Crotalaria*. Strains from this species showed well separated group from other rhizobial species in protein profile and 16S-rDNA analyses. Different strains from this species can grow on C_1 compounds such as formate, formaldehyde, methanol and on a wide range of multi-carbon growth substrates (Jourand et al. 2004).

Although nodulating beta-rhizobia from different legumes have only been described recently, molecular evidence showed that they existed as legume symbionts for 50 million years (Gyaneshwar et al. 2011). *Burkholderia* and *Cupriavidus* have symbiotic, pathogenic and endophytic properties and have been isolated from soil, water, plants, rhizosphere, insects, and clinical specimens. Plant nodulating species have been described from different countries, mainly from *Mimosa* spp., suggesting that this genus is an important host for beta-rhizobia.

Different species of beta-rhizobia contain common nodulation genes (*nodABC*, *nodD*, *nifH*), and these genes are very similar to the symbiotic genes of traditional rhizobia (Rivas et al. 2009a). It is now well accepted that lateral transfer of plasmids and plasmid-borne genes are very common in bacteria, and therefore it has been hypothesized that beta-rhizobia have recruited symbiotic genes from unknown sources by lateral gene transfer (Rivas et al. 2009a).

4 Reconstructing Rhizobial Phylogenies

Initially bacterial phylogenies were reconstructed on the basis of morphological characteristics of the cell by Kluyver and van Niel (1936). Following the principles from Zoology, morphological characteristics were used to define larger units like tribes, families and so on. As it became apparent that morphology-based phylogeny was insufficient for bacteria, physiological characteristics like oxygen requirements were included to reconstruct evolutionary relationships in the 1940s (Oren 2010).

Later, sequence analysis and chemical content analysis were introduced to further improve phylogenies. These methods include sequences of the 16S rRNA gene, restriction fragment length polymorphism typing, multi-locus sequencing, whole genome sequence analysis, Fourier-transform infrared spectroscopy, and pyrolysis-mass spectrometry. The sequencing of housekeeping genes, DNA profiling and the application of DNA arrays have become standard methods in bacterial taxonomy (Brenner et al. 2005). There are also powerful PCR-based techniques like REP, BOX, or ERIC-PCR for bacterial taxonomy (Vinuesa et al. 2005). As these PCR-based techniques amplify DNA from the chromosome, plasmids and other genetic elements (Martens et al. 2008) DNA fingerprinting is useful for studying DNA sequence polymorphism or genetic composition of plasmids or chromosomes in an indirect way.

The 16S rRNA and 18S rRNA genes fulfill all the requirements for good phylogenetic marker sequences. For example, they are functionally conserved and universally distributed in all three domain of life. Analyses of 16S rRNA gene sequences revolutionized rhizobial taxonomy like other bacteria and help to eliminate host nodulation capacity as a principle characteristic for taxonomy. However, several limitations like patterns and speed of sequence change mean that 16S rRNA has a low phylogenetic resolving power for separating closely related organism (>97 % similarity), and analyses of this gene are not suitable to distinguish prokaryotes at the species level (Ludwig 2010). Recently, by phylogenetic analyses of housekeeping genes, we found three distinct phylogenetic clades which have almost identical 16S rRNA sequences (Rashid et al. 2012). Considering the limitations of the 16S rRNA gene in bacterial taxonomy, multi-locus sequence analysis and multi locus sequence typing using different protein-coding genes have been preferred for studying closely-related species of bacteria, including rhizobia (Maiden et al. 1998; Mutch and Young 2004; Konstantinidis et al. 2006; Martens et al. 2008). After their introduction, multi-locus approaches have been applied to the classification of a variety of bacteria and fungi, and have helped to develop universally portable and reproducible methods. In brief, multi-locus methods represent a universal language for bacterial characterization (Maiden et al. 1998).

There are few protein coding genes which could be used to evaluate rRNA-based phylogenetic conclusions (Ludwig and Klenk 2005). These are translation elongation factors (IF2, EF-G, EF-TU) and initiation factors, RNA polymerase sub-units, DNA gyrase, *recA*, and ribosomal and heat shock proteins. Among these, *recA* is an important marker for phylogenetic analyses of rhizobia (Fig. 3). However, some others housekeeping genes (*rpoB*, *gyrB*, *groEL*, *atpD*) are also important for bacterial phylogenetics (Adekambi and Drancourt 2004; Glazunova et al. 2009).

4.1 Symbiotic Genes for Phylogenetic Analyses

From the very beginning of rhizobial characterization, classification and identification were dominated by nodulation capacity and morphological characteristics. After the proposal of Woese (Woese et al. 1984), analyses of the 16S rRNA

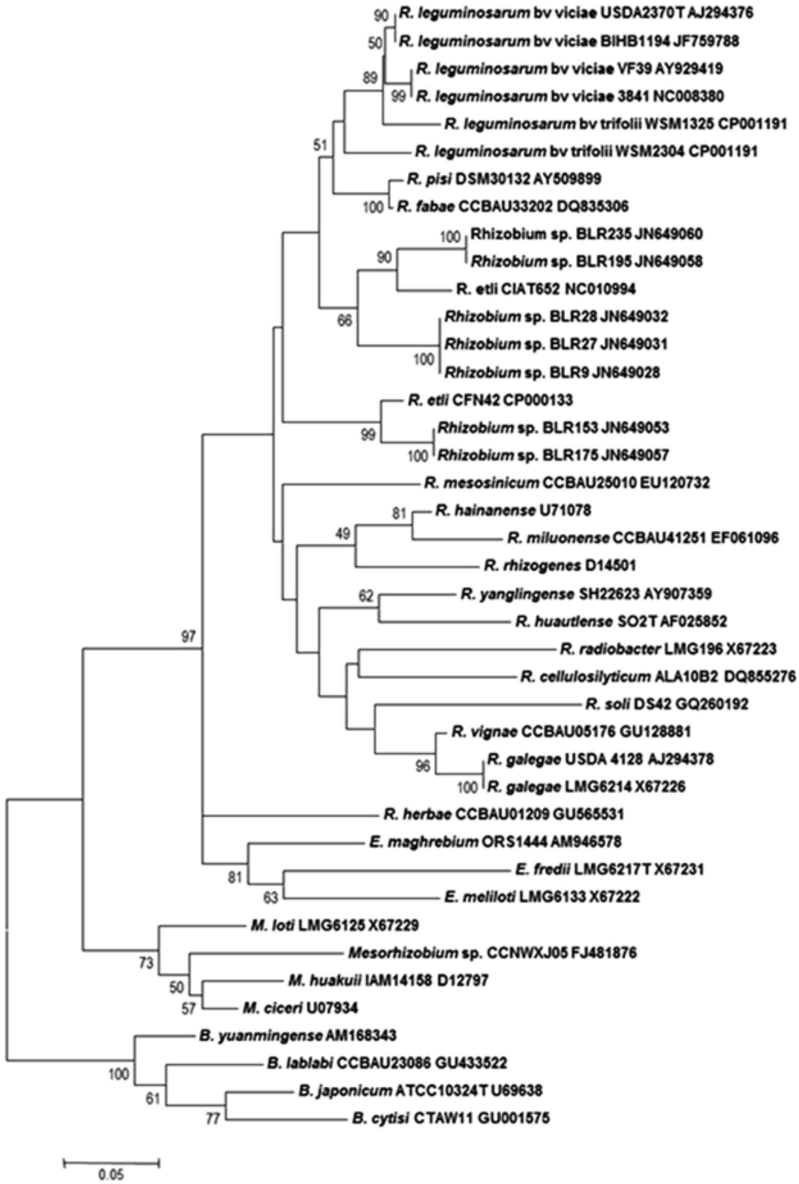

Fig. 3 Maximum likelihood tree based on partial *recA* gene sequences. Bootstrap values ≥50 are indicated for each node (1,000 replicates). *R* = *Rhizobium*, *E* = *Ensifer*, *Brady* = *Bradyrhizobium*, *M* = *Mesorhizobium*. The ML tree shows the reconstructed phylogeny of selected rhizobial species based on partial *recA* gene sequences. The species selected for this tree have more than 97 % sequence identity for their respective 16S rRNA gene sequences with close relatives. Although species are not well separated by their 16S rRNA sequences, the *recA* tree shows their distinctiveness with high bootstrap values and good branch lengths. Importantly, some lineages (three lineages of BLR strains) and most of the species are not resolved by 16S rRNA gene sequence analyses, but are apparent in *recA* gene analysis. This demonstrates the high resolution/resolving power of the *recA* gene for phylogenetic analyses of closely related rhizobial species

gene helped to eliminate plasmid-borne characteristics (nodulation, pathogenicity, hairy root formation) for rhizobial identification and have had significant contributions in the identification of non-traditional rhizobia. However, for the description of root- and stem-nodulating bacteria, a minimum standard has been proposed by Graham et al. (1991) who suggested using traditional morphological and physiological characteristics, symbiotic properties, and DNA fingerprinting methods like restriction fragment length polymorphism, multi locus enzyme electrophoresis, 16S rRNA gene sequencing, and DNA hybridization.

As the evolutionary histories of chromosomal genes and symbiotic genes are often different, different phylogenies can be implied from the phylogenetic analyses of nodulation genes and 16S rRNA gene (Mergaert et al. 1997; Haukka et al. 1998). The genes responsible for nodulation and nitrogen fixation are commonly known as symbiotic genes. With few exceptions, symbiotic genes are located on plasmids in fast growing rhizobia and on chromosomal symbiotic islands in slow growing rhizobia. Whether the symbiotic genes are located on plasmids or chromosomal islands, they are transmissible between different bacteria (Doyle 1998; Sullivan and Ronson 1998).

Although there are a large number of symbiotic genes present in rhizobia, only universally conserved core genes like *nodD*, *nodC*, *nodA* and *nifH* are commonly included for phylogenetic analyses of rhizobial species. Due to the horizontal transfer of symbiotic genes, they do not provide useful information for taxonomy, but provide complementary information for host nodulation and nitrogen fixation (Mergaert et al. 1997; Rashid et al. 2012). The description of symbiotic genes is also useful for the proper identification of non-traditional rhizobia for biogeographic studies and or biovar identification in traditional rhizobia (Rivas et al. 2009b).

The evolutionary history of a single gene or an entire genome can be understood by phylogenetic analysis, and hence is used extensively by microbiologists. Initially, rhizobial phylogeny was reconstructed using morphological and nodulation characteristics. Nowadays sequencing of different housekeeping genes, restriction fragment length polymorphism typing, and DNA fingerprinting are being routinely used for phylogenetic analysis. Sequencing of 16S rRNA genes not only contributes significantly to the elimination of plasmid-borne characteristics from rhizobial taxonomy, but also helps in the identification of beta and gamma rhizobia.

4.1.1 Phylogenetic Analysis Methods and Programs

Phylogenetic analyses help to understand the evolutionary history of individual genes or entire genomes of microorganisms, and hence are used extensively by microbiologists from the fields of systematic, taxonomy and epidemiology. Four steps are necessary for the phylogenetic analysis of molecular sequences. These are the selection of molecular markers, sequencing of the selected molecules, multiple sequence alignment, and tree reconstruction.

4.1.2 Tree Reconstruction Methods

The main objective of phylogenetic analyses is to find the evolutionary relationship of the studied organisms, which is generally represented as a phylogenetic tree. There are four widespread methods for reconstructing phylogenetic trees. These are distance matrix, maximum parsimony, and maximum likelihood and Bayesian analyses.

4.1.3 Reliability of Phylogenetic Trees

Bootstrapping

After the reconstruction of a phylogenetic tree, the reliability and quality of the tree has to be assessed to ensure that the tree accurately represents the actual relationship between strains as closely as possible. Therefore, different methods have been developed to determine the reliability and robustness of tree topology. These are bootstrapping and branch length estimation. The most common method is the bootstrap analysis, which can be implemented in different tree reconstruction methods. This method was introduced by Felsenstein (1985) and is a widely and successfully applied procedure. Bootstrapping programs resample the columns of a multiple sequence alignment group and create many new alignment groups by random sampling, replacing the original data set. New sets are similar to each other or the original alignment, but are rarely absolutely identical. Multiple bootstrap trees are then generated from the new sets of alignment and the statistical confidence of each branch is calculated. This value is known as the bootstrap value. Generally 200–2,000 resamplings should be used for bootstrapping (Chun and Hong 2010), but 1,000 resample are frequently being used. A bootstrap support of 70 % or higher is often considered as indicative of a reliable grouping or clustering for a phylogenetic tree. Bootstrapping method can be used in available tree building methods (van de Peer 2003; Chun and Hong 2010). In Bayesian analyses, similar concept is being used and it is known as posterior probability.

Tree Rooting

The purpose of phylogenetic studies is to find the evolutionary relationships between organisms. In the absence of directly observable relationships between ancestor and descendent, the direction of the changes must be inferred by rooting the tree (Nei and Kumar 2000). From an un-rooted tree, it is impossible to determine the directions of changes in traits of organisms. Although rooting is not essential for some cases, such as for knowing whether a group of genes is orthologous or dispersed among others genes on the tree, in most of the cases knowledge of the direction of changes is essential to reconstruct the evolutionary process (Graur and Li 2000). There are two methods for molecular tree rooting: out-group rooting and duplicate gene rooting. Out-group rooting compares character states of the group of interest i.e., in-group with a closely-related but sufficiently distant group i.e., out-group. The differences between in-group and out-group help to understand the direction of changes in resultant tree (Maddison et al. 1984). Prior knowledge is required to select out-group or it can be appeared during alignment.

In duplicated gene rooting, sequences from one gene clade are used to root another gene clade (Simmons et al. 2000). Duplicated gene rooting helps to unveil unexpected relationships among species or genes in the main clade (Brown and Doolittle 1995; Mathews and Donoghue 1999). However, a tree can also be rooted using evidence from ontogeny or the fossil record, which is known as midpoint rooting (Smith 1994).

4.2 Non-rhizobial Nitrogen-Fixing Symbiosis

Although the vast majority of nitrogen-fixing symbioses involve legumes and rhizobia, other plants and microbes also form nitrogen-fixing associations of various degrees of intimacy. These are ranges from very loose associations of essentially independent organisms to complex symbiosis that rival the rhizobia-legume symbiosis in complexity (Fig. 4).

4.2.1 Nitrogen Fixing Cyanobacteria

Cyanobacteria are a large and diverse group of oxygenic photoautotrophic bacteria. The photosynthetic apparatus of these organisms shares many similarities with plant plastids, and indeed it is now almost universally accepted that primordial cyanobacteria were recruited as endosymbionts by the ancestors of green plants, where they eventually evolved into plastids. Aside from their photosynthetic ability, a large number of cyanobacterial strains are also capable of nitrogen fixation, and plants have taken advantage of this by forming various associations with these organisms. The simplest of these is the mere co-occurrence of plants and cyanobacteria in the same environment. Although in this situation there is not directed transfer of nutrients between cyanobacteria and plants, the transfer of fixed nitrogen from cyanobacteria to plants can nevertheless be quite significant (Mishra and Pabbi 2004), probably through the release of nitrogenous compounds upon the death and lysis of the bacterial cells. As cyanobacteria are usually dependent on abundant water, this form of nitrogen transfer is particularly important in aqueous environments, and thus can play a significant role in agricultural contexts such as rice paddy fields (Vaishampayan et al. 2001; Mishra and Pabbi 2004).

A more intimate symbiosis takes place between the aquatic fern genus *Azolla* and strains of the cyanobacterium *Anabaena azollae. Azolla* spp., are small floating plants that inhabit standing or slowly flowing waters throughout the tropics and temperate zones. Although a single plant is only of a few centimeters in diameter, the presence of a nitrogen-fixing symbiont means that *Azolla* can rapidly cover vast areas

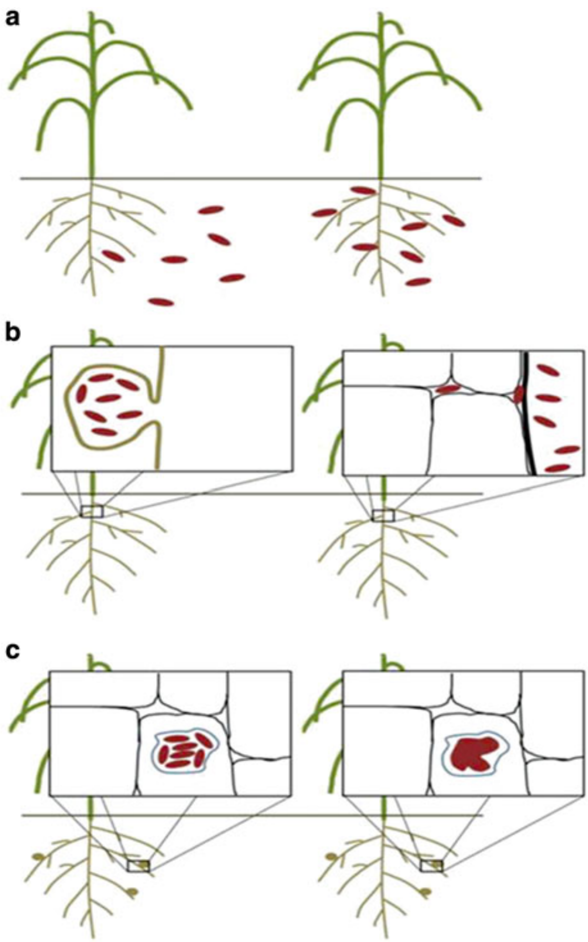

Fig. 4 Nitrogen-fixing plant-microbe associations exhibit different degrees of intimacy. (**a**) Soil associations. Plants may simply grow in soils also harbouring saprophytic diazotrophs (*left*). No specific exchange of nutrients, but bacteria may benefit from decaying plant matter and the plant may benefit from fixed nitrogen released by the bacteria. Diazotrophs may also be enriched within the rhizosphere and benefit more directly from plant root exudates (*right*). The plant may benefit from released nitrogen, but has little control over the exchange. (**b**) Specific diazotrophs may be allowed to enter the plant body within specialized cavities (*left*, e.g. Azolla-Anabaena symbiosis) or within the plant tissue itself (*right*, e.g. Kallar grass-Azoarcus symbiosis). Nutrient exchange is specific and directed; plant can select symbionts to some degree. (**c**) True intracellular symbioses. Symbionts are completely enclosed by a host membrane and reside within the host cytoplasm. Symbiotic form may either be similar to the free-living organism (*left*, e.g. Gunnera-cyanobacteria symbiosis and non-swollen bacteroids) or terminally differentiated into an organelle-like state (*right*, swollen bacteroids). The plant has a high degree of control and symbiosis is highly specific

through fast growth and vegetative reproduction. Due to its fast growth and ability to clog waterways it is considered a noxious weed in many areas of the world. A thick carpet of *Azolla* on the surface of the water also prevents sunlight from reaching other aquatic vegetation, which in turn leads to an accumulation of dead rotting plant matter and subsequent death of aquatic animals due to oxygen depletion (Hussner 2010). In other situations, such as rice paddies, the growth of *Azolla* may however be actively encouraged as the nitrogen-fixing ability of the symbiotic cyanobacterium ultimately fertilizes the paddy water and soil, increasing rice crop yield. Unlike the rhizobia-legume symbiosis, which is an intracellular association, the cyanobacterial symbiont in the *Anabaena-Azolla* symbiosis is instead housed in modified leaf cavities. *Anabaena azolla* is highly adapted to this environment and in nature is only found within *Azolla* leaf cavities. The *Azolla* symbiont can be cured of the diazothrophic partner, but such plants are unable to grow in low nitrogen environments and are rarely found outside of the laboratory (Peters and Meeks 1989). Fixed nitrogen is most likely released into the leaf cavity in the form of ammonia, which is then absorbed by specialized plant leaf hairs (Fig. 5a) and used in the GOGAT-dependent biosynthesis of glutamine (Vaishampayan et al. 2001).

Cyanobacteria are also able to form nitrogen-fixing symbioses with other plants, notable cycads and the South American *Gunnera* genus. In these symbioses the bacteria partner is either housed within modified coralloid root structures (Thajuddin et al. 2010) or intracellularly within modified mucilage glands (Khamar et al. 2010). In the case of the *Gunnera* symbiosis, the symbiont is attracted towards the glands by the secretion of sugars, which also serve as carbon source for the symbiont (Khamar et al. 2010).

4.2.2 Endophytic Diazotrophs

The intercellular spaces of all species of plant examined so far are inhabited by various benign or even beneficial species of fungi and bacteria collectively known as endophytes (Reinhold-Hurek and Hurek 2011). Although nitrogen fixation by endophytic bacteria is relatively rare and usually does not contribute much to the nitrogen supply of the host plant, some grasses such as sugar cane or kallar grass (*Leptochloa fusca*) obtain a substantial part of their nitrogen from nitrogen-fixing symbionts (Boddey et al. 1995; Reinhold-Hurek and Hurek 2011). Among the best studied of these symbionts are *Gluconacetobacter diazotrophicus* from sugar cane and *Azoarcus* spp., from Kallar grass, which is also able to infect the roots of rice (Hurek and Reinhold-Hurek 2003). Although no plant is known to be able to rely entirely on endophytic nitrogen fixation for its nitrogen supply, *nif* gene expression in *Azoarcus* is highly up regulated in the symbiotic state, and at least some fixed nitrogen is transferred to the plant host (Hurek et al. 2002). In its simplest form, infection of the host plant by diazotrophs takes place via crack entry, where breaches in the root epidermis are used by the bacteria as points of entry to the intracellular space. The bacteria are then able to spread throughout the continuum of the apoplast (Hurek et al. 2002). In addition to this simple mode of infection, the spread of bacterial endophytes through association with fungal spores has also been observed in various grasses (Boddey et al. 1995; Hurek et al. 1997).

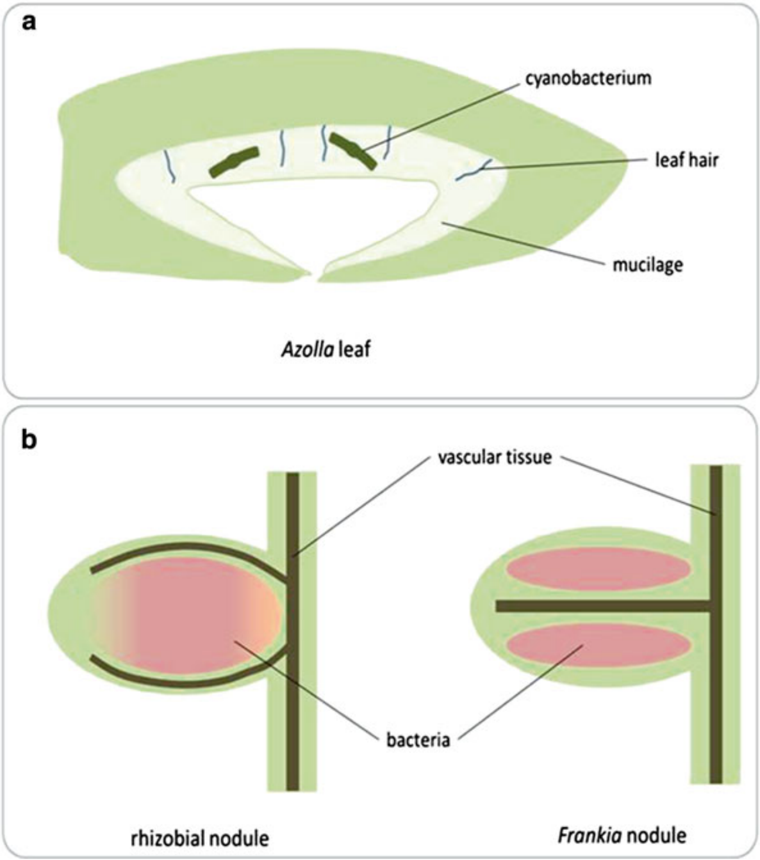

Fig. 5 Non-rhizobial nitrogen-fixing symbiosis. (**a**) Longitudinal cross-section of an *Azolla* leaf showing the cyanobacterial symbiont. The cavity contains a central air space and a layer of mucilage containing the *Anabaena* symbiont. Fixed nitrogen is released by the diazotroph as ammonia, which is absorbed by specialized leaf hairs. (**b**) Longitudinal cross-sections of rhizobial and *Frankia* nodules showing morphological differences. Rhizobial nodules are vascularized peripherally, *Frankia* nodules have a central vascular bundle

4.2.3 Frankia Symbiosis

A number of mostly woody non-legume species in the plant families *Betulaceae*, *Casuarinaceae*, *Coriariaceae*, *Datiscaceae*, *Eleagnaceae*, *Myricaceae*, *Rhamnaceae*, and *Rosaceae* are able to produce nitrogen-fixing root symbiotic nodules that superficially resemble rhizobia-legume root nodules. Unlike legume root nodules, which possess a peripheral vascular system and a central infected tissue, the root nodules of these non-legumes are modified lateral roots with a central vascular system and a peripheral infected expanded cortex (Fig. 5b) (Pawlowski and Demchenko 2012).

Although the symbiont is housed intracellularly within membrane-bounded vesicles as in the rhizobia-legume symbiosis (Kneip et al. 2007), the symbiont is not a gram-negative bacterium but gram-positive actinomycetes of the genus *Frankia*. In the free-living state, *Frankia* grows in the form of hypha that can also form multilocular sporangia in a manner similar to streptomycetes. Unlike most rhizobia, most *Frankia* spp. are able to fix nitrogen under nitrogen-limiting conditions in the free-living state (Pawlowski and Demchenko 2012). In both the free-living and the symbiotic state nitrogen fixation takes place within the swollen tips of hyphae termed vesicles that are encased in multilayered hopanoid-rich membrane envelopes (Parsons et al. 1987; Berry et al. 1993). Depending on the plant species, infection can either take place via infection threads or by crack entry, again similar to the situation observed in the rhizobia-legume symbiosis (Pawlowski and Demchenko 2012). Although *Frankia* spp., do not appear to produce Nod factors, it was shown that similar plant signaling pathways as those involved in rhizobial symbiosis or arbuscular mycorrhiza are also involved in the recognition of *Frankia* by the host plant (Gherbi et al. 2008; Markmann et al. 2008).

The legume-rhizobia association contributes significantly to the symbiotic biological nitrogen-fixing process, but other microbes such as cyanobacteria, endophytic bacteria and fungi, or *Frankia* sp. also form nitrogen-fixing associations of various degrees of intimacy for nitrogen fixation with plants (Fig. 4).

5 Agronomic Benefit of Legume-Rhizobium Symbiosis

The benefit of legume cultivation has been known since ancient times. The remediation of poor soils by the addition of soil from fields in which legumes had been grown was suggested by Theophrastus in the third century BCE (Vessey 2004). As legumes fix nitrogen biologically, the cultivation of legumes reduces greenhouse gasses by reducing the need for artificial nitrogenous fertilizer. Alternating other crops with legumes also decreases diseases and pest infestation by breaking pathogen life cycles and positively impact soil biota from sowing until after harvest (Lupwayi and Kennedy 2007). Of course, the main benefit of legume cultivation comes from their ability to fix nitrogen fixation in symbiosis with rhizobia. For instance, the introduction of legume-based pasture to Australia significantly increased and stabilized crop yields, mainly due to the benefit from legume nitrogen fixation (Herridge et al. 1998). Aside from its biological benefit, the legume-rhizobium symbiosis is also critical for agriculture due to its impact on farming cost, sustainable land use, soil quality, and mitigation of greenhouse gas emissions (Black et al. 2012).

The role of root nodulating bacteria on legume growth was observed in various experiments as soil inoculants (Akhtar and Siddiqui 2008; Akhtar et al. 2010). However, Nautiyal et al. (2008) have observed the effect of rhizobia on the growth of legumes, produced the first commercial bacterial inoculum containing rhizobia ("nitragin") for agricultural practices.

A large number of studies have been performed around the world to examine and enhance the beneficial effect of the legume-rhizobium symbiosis on plant growth. During the 1930s, a massive research effort was undertaken in Australia to enhance the pasture and grazing industries. A large collection of strains had been isolated and significant contributions to the study of strain-host specificity were made. It was concluded that infectivity i.e., nodule formation capacity and affectivity i.e., nitrogen fixation capacity of rhizobia depend on the relationship between specific strains and their hosts (Deaker et al. 2004). It is now well-established that inoculation with rhizobia is necessary to introduce legumes to new areas, to improve soil health, and to enhance legume production. Before 1970, only a few research groups in Europe, the US, and Australia was conducting research on biological nitrogen fixation. Because of the energy crisis of 1973–74 and its subsequent effects on fertilizer price, interest in biological nitrogen fixation research increased rapidly in developed and developing countries, and more attention was paid to biological nitrogen fixation with a view to replace chemical fertilizers with organic alternatives and reduce the cost of production (IAEA 1998). Rhizobial inoculation has the potential to increase crop productivity, nitrogen yields, and residual nitrogen levels of forage and grain legumes.

5.1 Nitrogen Fixation

Depending on rhizobial strains and legume species, nitrogen fixation differs greatly. In 2008, the total annual nitrogen fixation by leguminous pulse and oil seed crops were 2.95 Mt and 18.5 Mt, respectively. The cultivation of crops other than legumes also fixes a large amount of nitrogen per year from atmosphere. For instance, rice cultivation fixes 5Mt and sugar cane fixes 0.5 Mt. Non-legume crop lands (<4 Mt/year) and extensive savannas (<14 Mt/year) also contribute to biological nitrogen fixation. Therefore for it has been estimated that total biological nitrogen fixation is around 50–70 Mt/year (Herridge et al. 2008; Morel et al. 2012).

Unkovich and Pate (2000) reviewed the capacity of nitrogen fixation by legume-rhizobium symbiosis and estimate a global fixation rate of 450 kg nitrogen/ha/year. In Canada, 171 million kg of nitrogen were fixed by pea, lentil, dry bean and chickpea. This value corresponds to 40 million kg nitrogen for lentil and dry bean in the USA in 2004 (Lupwayi and Kennedy 2007). However, the amount of nitrogen fixation by different legumes is mentioned in the following Table 1.

Legume has residual effects on next cultivated crops. Nitrogen balances in crop field, the differences between fixed nitrogen and the nitrogen contained in the harvested crop, can be positive or negative for legumes crops. For example, mentioned that pea, lentil and dry bean showed nitrogen balances of 18 kg/ha, 9 kg/ha, and 0 kg/ha, respectively (Lupwayi and Kennedy 2007).

Table 1 The amount of nitrogen fixation by different field legumes

Crop	Nitrogen fixation (kg/hectare/year)
Soybean	0–450
Lupine	19–327
Field pea	8–200
Faba bean	12–330
Common bean	0–165
Lentil	5–191
Chickpea	0–144

Cited from Unkovich and Pate (2000) and Lupwayi and Kennedy (2007)

5.2 *Dry Matter and Seed Yield*

Rhizobial inoculation showed a positive effect on dry matter and grain yield of different crop in different countries. A clear and significant response to inoculation was always found in “virgin” soils, meaning soils that had never been inoculated with rhizobia specific for the planted legume species (Vessey 2004). A good correlation between dry matter yield and nitrogen fixation in different legume species was observed by (Peoples et al. 2002). Rhizobial inoculation was performed with different legume crops in Bangladesh using single or mixed strains in liquid and/or peat-based rhizobial inoculants. In most of the cases, the yield was significantly increased (Saha et al. 2008; Kabir et al. 2008) under field conditions. Rhizobial inoculation also significantly increased dry matter yield of different legumes in Canada (Vessey 2004).

In the Middle East, the geographical origin of many crop legumes, significant yield increases have been observed after rhizobium inoculation of different legumes, e.g. in Syria and Turkey (Albayrak et al. 2006; Yadegari et al. 2008). A coordinated research project conducted by the IAEA in Australia, Bangladesh, China, India, Malaysia, Pakistan, the Philippines, Sri Lanka, Thailand, and Vietnam to increase N_2 fixation, grain yields and observe the possible benefits on the nitrogen balance of selected legumes like chickpea, ground nut, soybean and lentil showed significant yield and nitrogen fixation responses after rhizobial inoculation in most field trials (IAEA 1998).

5.3 *Effects of Inoculation in the Presence of Indigenous Rhizobial Populations*

Legume inoculation with rhizobia greatly influenced by the presence of indigenous and/or naturalized rhizobila population in soil. For example, in the presence of endemic rhizobia in soil, different legumes generally showed variable yield responses to rhizobial inoculation. This might be due to the presence of an insufficient number of suitable rhizobia or the presence of an ineffective/less effective competing endemic population. Although field soil may already contain appropriate rhizobia, inoculation may still have a significant effect on the grain yield of different legumes (Vessey 2004). For instance, even in the presence of native rhizobial strains that form nodules with *Lathyrus* and *Vicia*, Cheminingwa (2002) found an excellent response (six fold increase) of pea crops following inoculation in Canada. However, such significant responses to inoculation are to be expected if legumes are grown in virgin soils. However, Moawad et al. (1998) investigated the competitive ability and yield response of inoculant strains for lentil in Egypt and found that 76–88 % of nodules were occupied by native rhizobia, and there was no yield response from inoculation. However, a good response of other legumes like clover and common bean were observed, where 52–79 % of nodules were occupied by native rhizobia. In the presence of indigenous rhizobial populations in soil, a potential benefit from inoculation could be obtained by using more infective and effective strains of rhizobia.

5.4 Different Factors for Nitrogen Fixation by Rhizobia

The potential benefit of the legume-rhizobium symbiosis depends on many factors, including inoculants quality like strain effectivity, viable cell content, moisture content, inocula aeration, legume species like varieties, seed quality, production of seed exudates, inoculation techniques such as seed or soil inoculation, and soil conditions such as acidity, texture, moisture contents, etc. (Brockwell et al. 1995; Brahmaprakash and Sahu 2012). Moreover, there are several reasons for the failure of inoculation such as soil nitrogen content, drought stress, phosphorus limitation, and disease infestation. Among these, high mineral nitrogen content dominates over any other factor (Vessey 2004). In the presence of abundant nitrogen, legumes tend to exclude nitrogen fixing microorganisms because biological nitrogen fixation is bio-energetically costly (Houlton et al. 2008). In fact nitrogen fixation depends on a feedback control mechanism between legume demand and nitrogen availability in a particular ecosystem (Soussana and Tallec 2010). Moreover, rhizobial infection process can be hampered by a number of factors, like the absence of appropriate rhizobia or too few rhizobia in the soil, the presence of competing rhizobia, a late response of the plant due to a lack of nutrients, or through altered soil metabolism, for example nitrate reduction in soil high in available nitrogen. In any of these situations fixation may be reduced, and subsequently grain yield and/or rotational value of the legume will be reduced (McNeil and Materne 2007). However, plant breeders can enhance biological nitrogen fixation by selecting more effective varieties. Selection of crop varieties based on plant growth under stress conditions produces higher yields and higher nitrogen fixation rates. For instance, boron-tolerant lentil varieties exhibit a significantly increased photosynthetic rate, green matter accumulation, and subsequently good nitrogen fixation in high-boron soils (Hobson et al. 2004). The groundnut genotype G-7 produced better yields and higher nitrogen fixation with inoculated strains than other genotypes in Bangladesh (Sattar et al. 1998). From the different studies on agronomic benefit of rhizoa it is clear that for any interpretation of legume inoculation different factors like endemic rhizobial population in the field, the percentage of nodules by the inoculant, and the survival of the inoculant in the soil need to be considered.

6 Conclusion

The association of plants with nitrogen-fixing bacteria range from casual coexistence in the same environment to complex intracellular symbioses and allow plants to flourish in situations in which plant-assimilable nitrogen is limiting or even absent. Diazotrophic bacteria may be coincidentally present and grow saprophytically in the soil, preferentially occur in the rhizosphere and benefit from carbon sources exuded by the plant root, be housed within specialized cavities on the plant where they are provisioned with carbon and energy by the plant e.g. *Azolla*, grow intracellularly within the plant tissue e.g. endophytes, or exist as true intracellular symbionts e.g. rhizobia and *Frankia* that may be terminally differentiated, in effect turning the bacterial symbiont into a nitrogen-fixing organelle. The rhizobia-legume symbiosis is the most thoroughly researched form of symbiotic nitrogen fixation and is often considered the agriculturally important. Rhizobial and *Frankia* symbioses depend on a complex exchange of information between the two partners and use similar plant signaling pathways to fungal mycorrhizal symbiosis, which may be ancestral to the nitrogen-fixing symbioses. The plant appears to be in control of the symbiosis and in some cases able to force the bacterial partner into a terminally differentiated state that is unable to reproduce. Nevertheless, symbiosis must nevertheless be advantageous to the bacterial partner as well; bacterial symbiosis genes should rapidly be lost if they were a reproductive disadvantage for the bacterium. The means by which the rhizobia-legume symbiosis is controlled and stabilized against defector and free-loader bacteria, ineffective symbionts, and potential pathogens is currently being actively researched.

A detailed understanding of nodulation is also important for sustainable agriculture. Inoculation with highly infective and effective strains of rhizobia with high survival capacity under field conditions rhizobia can achieve great yield improvements. Therefore, whether in virgin soils or soils already containing endemic rhizobial populations, legume producers should inoculate with rhizobia for better yields and soil nitrogen balances. To find effective and infective rhizobial strains, rhizobia need to be isolated from diverse plant genotypes and species, and from different ecological conditions. Subsequently their performance needs to be evaluated under laboratory and field conditions using different crop genotypes. The inoculation of legume crops with effective rhizobial strains is necessary to counterbalance and reduce the environmental pollution associated with the production and use of artificial nitrogenous fertilizers. If the current human population growth rate is sustained, the world population will double every 54 years and will stand at about 12 billion by 2054. The proper use of soil and the development of new technologies based on ecologically friendly microorganisms to control pests and improve plant growth could help to feed this huge population.

References

Adekambi T, Drancourt M (2004) Dissection of phylogenetic relationships among 19 rapidly growing Mycobacterium species by 16S rRNA, hsp65, sodA, recA and fpoB gene sequencing. Int J Syst Evol Microbiol 54:2095–2105. doi:10.1099/ijs.0.63094-0

Akhtar MS, Siddiqui ZA (2008) Biocontrol of a root-rot disease complex of chickpea by *Glomus intraradices*, *Rhizobium* sp. and *Pseudomonas straita*. Crop Prot 27:410–417. doi:10.1016/j.cropro.2007.07.009

Akhtar MS, Shakeel U, Siddiqui ZA (2010) Biocontrol of *Fusarium* wilt by *Bacillus pumilus*, *Pseudomonas alcaligenes*, and *Rhizobium* sp. on lentil. Turk J Biol 34:1–7. doi:10.3906/biy-0809-12

Albayrak S, Sevimay CS, Tongel O (2006) Effect of inoculation of *Rhizobium* on seed yield and yield components of common vetch (*Vicia sativa* L.). Turk J Agric For 30:31–37

Alunni B, Kevei Z, Redondo-Nieto M, Kondorosi A, Mergaert P, Kondorosi E (2007) Genomic organization and evolutionary insights on GRP and NCR genes, two large nodule-specific gene families in *Medicago truncatula*. Mol Plant Microbe Interact 20:1138–1148. doi:10.1094/MPMI-20-9-1138

An DS, Im WT, Yang HC, Lee ST (2006) *Shinella granuli* gen. nov., sp. nov., and proposal of the reclassification of *Zoogloea ramigera* ATCC 19623 as *Shinella zoogloeoides* sp. nov. Int J Syst Evol Microbiol 56:443–448. doi:10.1099/ijs.0.63942-0

Appleby CA (1984) Leghemoglobin and *Rhizobium* respiration. Annu Rev Plant Physiol 35: 443–478. doi:10.1146/annurev.pp.35.060184.002303

Atkinson EM, Long SR (1992) Homology of *Rhizobium meliloti* NodC to polysaccharide polymerizing enzymes. Mol Plant Microbe Interact 5:439–442

Atkinson EM, Palcic MM, Hindsgaul O, Long SR (1994) Biosynthesis of *Rhizobium meliloti* lipooligosaccharide Nod factors: NodA is required for an N-acyltransferase activity. Proc Natl Acad Sci U S A 91:8418–8422

Ausmees N, Jonsson H, Hoglund S, Ljunggren H, Lindberg M (1999) Structural and putative regulatory genes involved in cellulose synthesis in *Rhizobium leguminosarum* bv. *trifolii*. Microbiology 145:1253–1262

Baldani JI, Baldani VLD, Seldin L, Dobereiner J (1986) Characterization of *Herbaspirillum seropedicae* gen. nov., sp. nov., a root-associated nitrogen-fixing bacterium. Int J Syst Evol Microbiol 36:86–93. doi:10.1099/00207713-36-1-86

Bergey DH, Harrison FC, Breed RS, Hammer BW, Huntoon FM (1923) Bergey's manual of determinative bacteriology, 1st edn. Williams and Wilkins, Baltimore

Berry AM, Harriott OT, Moreau RA, Osman SF, Benson DR, Jones AD (1993) Hopanoid lipids compose the *Frankia* vesicle envelope, presumptive barrier of oxygen diffusion to nitrogenase. Proc Natl Acad Sci U S A 90:6091–6094

Black M, Moolhuijzen P, Chapman B, Barrero R, Howieson J, Hungria M, Bellgard M (2012) The genetics of symbiotic nitrogen fixation: comparative genomics of 14 rhizobia strains by resolution of protein clusters. Genes 3:138–166. doi:10.3390/genes3010138

Boddey RM, de Oliveira OC, Urquiaga S, Reis VM, de Olivares FL, Baldani VLD, Dobereiner J (1995) Biological nitrogen fixation associated with sugar cane and rice: contributions and prospects for improvement. Plant Soil 174:195–209. doi:10.1007/BF00032247

Brahmaprakash GP, Sahu PK (2012) Biofertilizers for sustainability. J Indian Inst Sci 92:37–62

Brenner DJ, Staley JT, Krieg NR (2005) Classification of prokaryotic organism and concept of bacterial speciation. In: Brenner DJ, Krieg NR, Staley JT (eds) Bergey's manual of systematic bacteriology. Springer, New York, pp 27–32

Brockwell J, Bottomley PJ, Thies JE (1995) Manipulation of rhizobia microflora for improving legume productivity and soil fertility: a critical assessment. Plant Soil 174:143–180. doi:10.1007/BF00032245

Brown JR, Doolittle WF (1995) Root of the universal tree of life based on ancient aminoacyl-tRNA synthesize gene duplications. Proc Natl Acad Sci U S A 92:2441–2445. doi:10.1073/pnas.92.7.2441

Calvert HE, Lalonde M, Bhuvaneswari TV, Bauer WD (1978) Role of lectins in plant-microorganism interactions. IV. Ultrastructural localization of soybean lectin binding sites of *Rhizobium japonicum*. Can J Microbiol 24:785–793

Cheminingwa GN (2002) The abundance, efficacy, and diversity of *Rhizobium leguminosarum* bv. *viciae* populations in southern Manitoba soils. Ph.D. thesis, University of Manitoba, Winnipeg, Canada

Chen WX, Yan GH, Li JL (1988) Numerical taxonomic study of fast-growing soybean rhizobia and a proposal that *Rhizobium fredii* be assigned to *Sinorhizobium* gen. nov. Int J Syst Evol Microbiol 38:392–397. doi:10.1099/00207713-38-4-392

Chun J, Hong SG (2010) Methods and programs for calculation of phylogenetic relationships from molecular sequences. In: Oren A, Papke RT (eds) Molecular phylogeny of microorganisms. Caister Academic Press, Norfolk, pp 23–39. ISBN 978-1-904455-67-7

Conn HJ (1942) Validity of the genus *Alcaligenes*. J Bacteriol 44:353–360

Cullimore JV, Ranjeva R, Bono JJ (2001) Perception of lipo-chitooligosaccharidic Nod factors in legumes. Trends Plant Sci 6:24–30. doi:10.1016/S1360-1385(00)01810-0

da Silva K, Florentino LA, da Silva KB, de Brandt E, Vandamme P, de Souza Moreira FM (2012) Cupriavidus necator isolates are able to fix nitrogen in symbiosis with different legume species. Syst Appl Microbiol 35:175–182. doi:10.1016/j.syapm.2011.10.005

de Lajudie P, Willems A, Pot B, Dewettinck D, Maestrojuan G, Neyra M, Collins MD, Dreyfus B, Kersters K, Gillis M (1994) Polyphasic taxonomy of rhizobia: emendation of the genus *Sinorhizobium* and description of *Sinorhizobium meliloti* comb. nov., *Sinorhizobium saheli* sp. nov., and *Sinorhizobium teranga* sp. nov. Int J Syst Evol Microbiol 44:715–733. doi:10.1099/00207713-44-4-715

de Lajudie P, Laurent-Fulele E, Willems A, Torck U, Coopman R, Collins MD, Kersters K, Dreyfus B, Gillis M (1998) *Allorhizobium undicola* gen. nov., sp. nov., nitrogen-fixing bacteria that efficiently nodulate *Neptunia natans* in Senegal. Int J Syst Bacteriol 48:1277–1290. doi:10.1099/00207713-48-4-1277

de Velde WV, Zehirov G, Szatmari A, Debreczeny M, Ishihara H, Kevei Z, Farkas A, Mikulass K, Nagy A, Tiricz H, Satiat-Jeunemaitre B, Alunni B, Bourge M, Kucho K, Abe M, Kereszt A, Maroti G, Uchiumi T, Kondorosi E, Mergaert P (2010) Plant peptides govern terminal differentiation of bacteria in symbiosis. Science 327:1122–1126. doi:10.1126/science.1184057

Deaker R, Roughley RJ, Kennedy IR (2004) Legume seed inoculation technology – a review. Soil Biol Biochem 36:1275–1288. doi:10.1016/j.soilbio.2004.04.009

Djordjevic MA, Redmond JW, Batley M, Rolfe BG (1987) Clovers secrete specific phenolic compounds which either stimulate or repress nod gene expression in *Rhizobium trifolii*. EMBO J 6:1173–1179

Downie JA (2005) Legume haemoglobins: symbiotic nitrogen fixation needs bloody nodules. Curr Biol 15:R196–R198. doi:10.1016/j.cub.2005.03.007

Downie JA, Knight CA, Johnston AWB, Rossen L (1985) Identification of genes and gene products involved in the nodulation of peas by *Rhizobium leguminosarum*. Mol Gen Genet 198:255–262. doi:10.1007/BF00383003

Doyle JJ (1998) Phylogenetic perspectives on nodulation: an evolving view of plants and symbiotic bacteria. Trends Plant Sci 3:473–478. doi:10.1016/S1360-1385(98)01340-5

Doyle JJ, Luckow MA (2003) The rest of the Iceberg. Legume diversity and evolution in a phylogenetic context. Plant Physiol 131:900–910. doi:10.1104/pp.102.018150

Dreyfus B, Garcia JL, Gillis M (1988) Characterization of *Azorhizobium caulinodans* gen. nov., sp. nov., a stem-nodulating nitrogen-fixing bacterium isolated from *Sesbania rostrata*. Int J Syst Evol Microbiol 38:89–98. doi:10.1099/00207713-38-1-89

Esseling JJ, Lhuissier FGP, Emons AMC (2003) Nod factor-induced root hair curling: continuous polar growth towards the point of nod factor application. Plant Physiol 132:1982–1988. doi:10.1104/pp.103.021634

Falk EC, Johnson JL, Baldani VLD, Dobereiner J, Krieg NR (1986) Deoxyribonucleic and ribonucleic acid homology studies of the genera *Azospirillum* and conglomeromonas. Int J Syst Evol Microbiol 36:80–85. doi:10.1099/00207713-36-1-80

Fellay R, Perret X, Viprey V, Broughton WJ, Brenner S (1995) Organization of host-inducible transcripts on the symbiotic plasmid of *Rhizobium* sp. NGR234. Mol Microbiol 16:657–667. doi:10.1111/j.1365-2958.1995.tb02428.x

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Ferguson GP, Jansen A, Marlow VL, Walker GC (2006) BacA-mediated bleomycin sensitivity in *Sinorhizobium meliloti* is independent of the unusual lipid A modification. J Bacteriol 188:3143–3148. doi:10.1128/JB.188.8.3143-3148.2006

Finnie C, Hartley NM, Findlay KC, Downie JA (1997) The *Rhizobium leguminosarum* prsDE genes are required for secretion of several proteins, some of which influence nodulation, symbiotic nitrogen fixation and exopolysaccharide modification. Mol Microbiol 25:135–146. doi:10.1046/j.1365-2958.1997.4471803.x

Finnie C, Zorreguieta A, Hartley NM, Downie JA (1998) Characterization of *Rhizobium leguminosarum* exopolysaccharide glycanases that are secreted via a type I exporter and have a novel heptapeptide repeat motif. J Bacteriol 180:1691–1699

Frank B (1889) Uber die pilzsymbiose der leguminosen. Berichte Deutschen Botanischen Gesellschaft 7:332–346

Gage DJ (2002) Analysis of infection thread development using Gfp- and DsRed-expressing *Sinorhizobium meliloti*. J Bacteriol 184:7042–7046. doi:10.1128/JB.184.24.7042-7046.2002

Gherbi H, Markmann K, Svistoonoff S, Estevan J, Autran D, Giczey G, Auguy F, Peret B, Laplaze L, Franche C, Parniske M, Bogusz D (2008) SymRK defines a common genetic basis for plant root endosymbioses with arbuscular mycorrhiza fungi, rhizobia, and Frankia bacteria. Proc Natl Acad Sci U S A 105:4928–4932. doi:10.1073/pnas.0710618105

Glazebrook J, Ichige A, Walker GC (1993) A *Rhizobium meliloti* homolog of the *Escherichia coli* peptide-antibiotic transport protein SbmA is essential for bacteroid development. Genes Dev 7:1485–1497. doi:10.1101/gad.7.8.1485

Glazunova OO, Raoult D, Roux V (2009) Partial sequence comparison of the rpoB, sodA, groEL and gyrB genes within the genus *Streptococcus*. Int J Syst Evol Microbiol 59:2317–2322. doi:10.1099/ijs.0.005488-0

Graham PH, Sadowsky MJ, Keyser HH, Barnet YM, Bardley RS, Cooper JE, De Lay DJ, Jarvis BDW, Roslycky EB, Strijdom BW, Young JPW (1991) Proposed minimal standards for the description of new genera and species of root- and stem-nodulating bacteria. Int J Syst Evol Microbiol 41:582–587. doi:10.1099/00207713-41-4-582

Graur D, Li WH (2000) Fundamentals of molecular evolution, 2nd edn. Sinauer Associates, Sunderland

Gyaneshwar P, Hirsch AM, Moulin L, Chen WM, Elliott GN, Bontemps C, Estrada-de Los Santos P, Gross E, Dos Reis FB, Sprent JI, Young JP, James EK (2011) Legume-nodulating betaproteobacteria: diversity, host range, and future prospects. Mol Plant Microbe Interact 24:1276–1288. doi:10.1094/MPMI-06-11-0172

Haag AF, Baloban M, Sani M, Kerscher B, Pierre O, Farkas A, Longhi R, Boncompagni E, Herouart D, Dall'Angelo S, Kondorosi E, Zanda M, Mergaert P, Ferguson GP (2011) Protection of *Sinorhizobium* against host cysteine-rich antimicrobial peptides is critical for symbiosis. PLoS Biol 9:e1001169. doi:10.1371/journal.pbio.1001169

Hakoyama T, Niimi K, Watanabe H, Tabata R, Matsubara J, Sato S, Nakamura Y, Tabata S, Jichun L, Matsumoto T, Tatsumi K, Nomura M, Tajima S, Ishizaka M, Yano K, Imaizumi-Anraku H, Kawaguchi M, Kouchi H, Suganuma N (2009) Host plant genome overcomes the lack of a bacterial gene for symbiotic nitrogen fixation. Nature 462:514–517. doi:10.1038/nature08594

Haukka K, Lindstrom K, Young JPW (1998) Three phylogenetic groups of nodA and nifH genes in *Sinorhizobium* and *Mesorhizobium* isolated from leguminous trees growing in Africa and Latin America. Appl Environ Microbiol 64:419–426

Heidstra R, Geurts R, Franssen H, Spaink HP, Kammen AV, Bisseling T (1994) Root hair deformation activity of nodulation factors and their fate on *Vicia sativa*. Plant Physiol 105:787–797

Herridge DF, Holland JF, Rose LA, Redden RJ (1998) Selection and breeding of grain legumes in Australia for enhanced nodulation and N_2 fixation. In: Improving yield and nitrogen fixation of grain legumes in the tropics and sub-tropics of Asia. Soil and Water Management and Crop Nutrition Section Joint FAO/IAEA Division International Atomic Energy Agency, Vienna, Austria

Herridge DF, Peoples MB, Boddey RM (2008) Global inputs of biological nitrogen fixation in agricultural systems. Plant Soil 311:1–18. doi:10.1007/s11104-008-9668-3

Ho SC, Wang JL, Schindler M, Loh JT (1994) Carbohydrate binding activities of *Bradyrhizobium japonicum*. III. Lectin expression, bacterial binding, and nodulation efficiency. Plant J 5:873–884. doi:10.1046/j.1365-313X.1994.5060873.x

Hobson KB, Armstrong RD, Nicolas M, Connor D, Marterne MA (2004) Boron tolerance of lentil-highlights of a research program. In: Fischer RA (ed) New directions for a diverse planet. Proceedings of the 4th international crop science congress, Brisbane, Australia, 26th September–1st October 2004. http://www.regional.org.au/au/asa/2004/symposia/6/2/1028_hobsonkb.htm. Assessed on 24 Nov 2013

Holmes B, Popoff M, Kiredjian M, Kersters K (1988) *Ochrobactrum anthropi* gen. nov., sp. nov. from human clinical specimens and previously known as group Vd. Int J Syst Bacteriol 38:406–416

Houlton BZ, Wang YP, Vitousek PM, Field CB (2008) A unifying framework for dinitrogen fixation in the terrestrial biosphere. Nature 454:327–331. doi:10.1038/nature07028

Hurek T, Reinhold-Hurek B (2003) *Azoarcus* sp. strain BH72 as a model for nitrogen-fixing grass endophytes. J Biotechnol 106:169–178. doi:10.1016/j.jbiotec.2003.07.010

Hurek T, Wagner B, Reinhold-Hurek B (1997) Identification of N_2-fixing plant- and fungus-associated Azoarcus species by PCR-based genomic fingerprints. Appl Environ Microbiol 63:4331–4339

Hurek T, Handley LL, Reinhold-Hurek B, Piche Y (2002) *Azoarcus* grass endophytes contribute fixed nitrogen to the plant in an unculturable state. Mol Plant Microbe Interact 15:233–242. doi:10.1094/MPMI.2002.15.3.233

Hussner A (2010) Nobanis–Invasive alien species fact sheet–*Azolla filiculoides*. www.nobanis.org. Assessed on 24 Nov 2013

IAEA (1998) Improving yield and nitrogen fixation of grain legumes in the tropics and sub-tropics of Asia, International Atomic Energy Agency, (IAEA-TECDOC-1027), Vienna, Austria

Jarvis BDW, van Berkum P, Chen WX, Nour SM, Fernandez MP, Cleyet-Marel JC, Gillis M (1997) Transfer of *Rhizobium loti*, *Rhizobium huakuii*, *Rhizobium ciceri*, *Rhizobium mediterraneum*, and *Rhizobium tianshanense* to *Mesorhizobium* gen. nov. Int J Syst Bacteriol 47:895–898

John M, Rohrig H, Schmidt J, Wieneke U, Schell J (1993) *Rhizobium* NodB protein involved in nodulation signal synthesis is a chitooligosaccharide deacetylase. Proc Natl Acad Sci U S A 90:625–629. doi:10.1073/pnas.90.2.625

Jones KM, Sharopova N, Lohar DP, Zhang JQ, VandenBosch KA, Walker GC (2008) Differential response of the plant *Medicago truncatula* to its symbiont *Sinorhizobium meliloti* or an exopolysaccharide-deficient mutant. Proc Natl Acad Sci U S A 105:704–709. doi:10.1073/pnas.0709338105

Jordan DC (1982) Transfer of *Rhizobium japonicum* Buchanan 1980 to *Bradyrhizobium* gen. nov., a genus of slow-growing, root nodule bacteria from leguminous plants. Int J Syst Bacteriol 32:136–139

Jordan DC (1984) Family III. Rhizobiaceae. In: Krieg NR, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 1. Williams and Wilkins, Baltimore, pp 234–242

Jordan DC, Allen ON (1974) GenusII, *Rhizobium*. In: Buchanan RE, Gibbons NE (eds) Bergey's manual of determinative bacteriology, 8th edn. Williams and Wilkins, Baltimore, pp 262–264

Jourand P, Giraud E, Bena G, Sy A, Willems A, Gillis M, Dreyfus B, de Lajudie P (2004) Methylobacterium nodulans sp. nov., for a group of aerobic, facultatively methylotrophic, legume root-nodule-forming and nitrogen-fixing bacteria. Int J Syst Evol Microbiol 54:2269–2273. doi:10.1099/ijs.0.02902-0

Kabir MH, Chowdhury AKM, Rashid MH, Malaker JC, Hassan SMM (2008) Effect of rhizobial inoculation on nodulation and growth of summer mungbean. Bangladesh J Environ Sci 14:129–133

Kamst E, Pilling J, Raamsdonk LM, Lugtenberg BJ, Spaink HP (1997) *Rhizobium* nodulation protein NodC is an important determinant of chitin oligosaccharide chain length in Nod factor biosynthesis. J Bacteriol 179:2103–2108

Kanso S, Patel BKC (2003) Microvirga subterranea gen. nov., sp. nov., a moderate thermophile from a deep subsurface Australian thermal aquifer. Int J Syst Evol Microbiol 53:401–406. doi:10.1099/ijs.0.02348-0

Karunakaran R, Haag AF, East AK, Ramachandran VK, Prell J, James EK, Scocchi M, Ferguson GP, Poole PS (2010) BacA is essential for bacteroid development in nodules of galegoid, but not phaseoloid, legumes. J Bacteriol 192:2920–2928. doi:10.1128/JB.00020-10

Kereszt A, Mergaert P, Kondorosi E (2011) Bacteroid development in legume nodules: evolution of mutual benefit or of sacrificial victims. Mol Plant Microbe Interact 24:1300–1309. doi:10.1094/MPMI-06-11-0152

Khamar HJ, Breathwaite EK, Prasse CE, Fraley ER, Secor CR, Chibane FL, Elhai J, Chiu WL (2010) Multiple roles of soluble sugars in the establishment of Gunnera-Nostoc endosymbiosis. Plant Physiol 154:1381–1389. doi:10.1104/pp. 110.162529

Kluyver AJ, van Neil CB (1936) Prospects for natural system of classification of bacteria. Zentralblatt fur Bakteriologie, Parasitenkunde und Infektionskrankheiten. II. 94:396–403

Kneip C, Lockhart P, Voß C, Maier UG (2007) Nitrogen fixation in eukaryotes – new models for symbiosis. BMC Evol Biol 7:55. doi:10.1186/1471-2148-7-55

Knosel DH (1984) Genus: *Phyllobacterium*. In: Krieg NR, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 1. Williams and Wilkins, Baltimore, pp 254–256

Konstantinidis KT, Ramette A, Tiedje JM (2006) Toward a more robust assessment of intraspecies diversity, using fewer genetic markers. Appl Environ Microbiol 72:7286–7293. doi:10.1128/AEM.01398-06

Kouchi H, Imaizumi-Anraku H, Hayashi M, Hakoyama T, Nakagawa T, Umehara Y, Suganuma N, Kawaguchi M (2010) How many peas in a pod. Legume genes responsible for mutualistic symbioses underground. Plant Cell Physiol 51:1381–1397. doi:10.1093/pcp/pcq107

Krehenbrink M, Downie JA (2008) Identification of protein secretion systems and novel secreted proteins in *Rhizobium leguminosarum* bv. *viciae*. BMC Genomics 9:55

Krehenbrink M, Edwards A, Downie JA (2011) The superoxide dismutase SodA is targeted to the periplasm in a SecA-dependent manner by a novel mechanism. Mol Microbiol 82:164–179. doi:10.1111/j.1365-2958.2011.07803.x

Lin DX, Wang ET, Tang H, Han TX, He YR, Guan SH, Chen WX (2008) *Shinella kummerowiae* sp. nov., a symbiotic bacterium isolated from root nodules of the herbal legume *Kummerowia stipulacea*. Int J Syst Evol Microbiol 58:1409–1413. doi:10.1099/ijs.0.65723-0

Loh JT, Ho SC, de Feijter AW, Wang JL, Schindler M (1993) Carbohydrate binding activities of *Bradyrhizobium japonicum*: unipolar localization of the lectin BJ38 on the bacterial cell surface. Proc Natl Acad Sci U S A 90:3033–3037

Ludwig W (2010) Molecular phylogeny of microorganisms: is rRNA still a useful marker. In: Oren A, Papke RT (eds) Molecular phylogeny of microroganisms. Caister Academic Press, Norfolk, pp 65–83. ISBN 978-1-904455-67-7

Ludwig W, Klenk HP (2005) Overview: a phylogenetic backbone and taxonomic framework for prokaryotic systematic. In: Brenner DJ, Krieg NR, Staley JT (eds) Bergey's manual of systematic bacteriology. Springer, New York, pp 49–65

Lupwayi NZ, Kennedy CA (2007) Grain legumes in northern great plains: impacts on selected biological soil processes. Agron J 99:1700–1709

Lykidis A, Perez-Pantoja D, Ledger T, Mavromatis K, Anderson IJ, Ivanova NN, Hooper SD, Lapidus A, Lucas S, Gonzalez B, Kyrpides NC (2010) The complete multipartite genome sequence of *Cupriavidus necator* JMP134, a versatile pollutant degrader. PLoS One 5:e9729. doi:10.1371/journal.pone.0009729

Maddison WP, Donoghue MJ, Maddison DR (1984) Outgroup analyses and parsimony. Syst Zool 33:83–103

Maiden MCJ, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, Zhang Q, Zhou J, Zurth K, Caugant DA, Feavers IM, Achtman M, Spratt BG (1998) Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc Natl Acad Sci U S A 95:3140–3145

Makkar NS, Casida LE Jr (1987) *Cupriavidus necator* gen. nov., sp. nov.: a nonobligate bacterial predator of bacteria in soil. Int J Syst Evol Microbiol 37:323–326. doi:10.1099/00207713-37-4-323

Markmann K, Giczey G, Parniske M (2008) Functional adaptation of a plant receptor-kinase paved the way for the evolution of intracellular root symbioses with bacteria. PLoS Biol 6:e68. doi:10.1371/journal.pbio.0060068

Marlow VL, Haag AF, Kobayashi H, Fletcher V, Scocchi M, Walker GC, Ferguson GP (2009) Essential role for the *BacA* protein in the uptake of a truncated eukaryotic peptide in *Sinorhizobium meliloti*. J Bacteriol 191:1519–1527. doi:10.1128/JB.01661-08

Martens M, Dawyndt P, Coopman R, Gillis M, De Vos P, Willems A (2008) Advantages of multilocus sequence analysis for taxonomic studies: a case study using housekeeping genes in the genus *Ensifer* (including former *Sinorhizobium*). Int J Syst Evol Microbiol 58:200–214. doi:10.1099/ijs.0.65392-0

Mateos PF, Baker DL, Petersen M, Velazquez E, Jimenez-Zurdo JI, Martínez-Molina E, Squartini A, Orgambide G, Hubbell DH, Dazzo FB (2001) Erosion of root epidermal cell walls by *Rhizobium* polysaccharide-degrading enzymes as related to primary host infection in the *Rhizobium*-legume symbiosis. Can J Microbiol 47:475–487

Mathews S, Donoghue MJ (1999) The root of angiosperm phylogeny inferred from duplicate phytochrome genes. Science 286:947–950. doi:10.1126/science.286.5441.947

McNeil DL, Materne M (2007) *Rhizobium* management and nitrogen fixation. In: Yadav SS, McNeil DL, Stevenson PC (eds) Lentil: an ancient crop for modern times. Springer, Dordrecht, pp 127–143

Mergaert P, Van Montagu M, Holsters M (1997) Molecular mechanisms of Nod factor diversity. Mol Microbiol 25:811–817. doi:10.1111/j.1365-2958.1997.mmi526.x

Mergaert P, Nikovics K, Kelemen Z, Maunoury N, Vaubert D, Kondorosi A, Kondorosi E (2003) A novel family in *Medicago truncatula* consisting of more than 300 nodule-specific genes coding for small, secreted polypeptides with conserved cysteine motifs. Plant Physiol 132:161–173, http://dx.doi.org/10.1104/pp.102.018192

Mergaert P, Uchiumi T, Alunni B, Evanno G, Cheron A, Catrice O, Mausset AE, Barloy-Hubler F, Galibert F, Kondorosi A, Kondorosi E (2006) Eukaryotic control on bacterial cell cycle and differentiation in the *Rhizobium*-legume symbiosis. Proc Natl Acad Sci U S A 103:5230–5235. doi:10.1073/pnas.0600912103

Mishra U, Pabbi S (2004) Cyanobacteria: a potential biofertilizer for rice. Resonance 9:6–10. doi:10.1007/BF02839213

Moawad H, Badr El-Din SMS, Abdel-Aziz RA (1998) Improvement of biological nitrogen fixation in Egyptian winter legumes through better management of *Rhizobium*. Plant Soil 204:95–106. doi:10.1023/A:1004335112402

Morel MA, Brana V, Castro-Sowinski S (2012) Legume crops, importance and use of bacterial inoculation to increase production. In: Akash G (ed) Crop plant, In Tech publisher, Croatia. http://www.intechopen.com/books/crop-plant/legume-crops-importance-and-use-ofbacterial-inoculation-to-increaseproduction

Mulley G, White JP, Karunakaran R, Prell J, Bourdes A, Bunnewell S, Hill L, Poole PS (2011) Mutation of GOGAT prevents pea bacteroid formation and N_2 fixation by globally down-regulating transport of organic nitrogen sources. Mol Microbiol 80:149–167. doi:10.1111/j.1365-2958.2011.07565.x

Mutch LA, Young JPW (2004) Diversity and specificity of *Rhizobium leguminosarum* biovar *viciae* on wild and cultivated legumes. Mol Ecol 13:2435–2444. doi:10.1111/j.1365-294X.2004.02259.x

Nakagawa Y, Sakane T, Yokota A (1996) Transfer of "*Pseudomonas riboflavina*" (Foster 1944), a gram-negative, motile rod with long-chain 3-hydroxy fatty acids, to *Devosia riboflavina* gen. nov., sp. nov., nom. rev. Int J Syst Bacteriol 46:16–22. doi:10.1099/00207713-46-1-16

Nautiyal CS, Srivastava S, Chauhan PS (2008) Rhizosphere colonization: molecular determinant from plant-microbe coexistence perspective. In: Nautiyal CS, Dion P (eds) Molecular mechanisms of plant and microbe coexistence. Springer, Berlin/Heidelberg

Nei M, Kumar S (2000) Molecular evolution and phylogenetics, 1st edn. Oxford University Press, New York

O'Brian MR, Vance CP, VandenBosch KA (2009) Legume focus: model species sequenced, mutagenesis approaches extended, and debut of a new model. Plant Physiol 151:969. doi:10.1104/pp. 109.900305

Oldroyd GE, Murray JD, Poole PS, Downie JA (2011) The rules of engagement in the legume-rhizobial symbiosis. Annu Rev Genet 45:119–144. doi:10.1146/annurev-genet-110410-132549

Oono R, Denison RF (2010) Comparing symbiotic efficiency between swollen versus nonswollen rhizobial bacteroids. Plant Physiol 154:1541–1548. doi:10.1104/pp.110.163436

Oren A (2010) Concepts about phylogeny of microorganisms – an historical overview. In: Oren A, Papke RT (eds) Molecular phylogeny of microorganisms. Caister Academic Press, Norfolk, pp 1–21. ISBN 978-1-904455-67-7

Palomares A, Montoya E, Olivares J (1978) Quality and rate of extracellular polysaccharides produced by *Rhizobium meliloti* and their inducing effect on polygalacturonase production in legume roots as derived from the presence of extrachromosomal DNA. Microbios 22:7–13

Parsons R, Silvester WB, Harris S, Gruijters WTM, Bullivant S (1987) Frankia vesicles provide inducible and absolute oxygen protection for nitrogenase. Plant Physiol 83:728–731

Patriarca EJ, Tate R, Iaccarino M (2002) Key role of bacterial NH_4^+ metabolism in *Rhizobium-plant* symbiosis. Microbiol Mol Biol Rev 66:203–222. doi:10.1128/MMBR.66.2.203-222.2002

Patt TE, Cole GC, Hanson RS (1976) Methylobacterium, a new genus of facultatively methylotrophic bacteria. Int J Syst Evol Microbiol 26:226–229. doi:10.1099/00207713-26-2-226

Pawlowski K, Demchenko KN (2012) The diversity of actinorhizal symbiosis. Protoplasma 249:967–979. doi:10.1007/s00709-012-0388-4

Peoples MB, Giller KE, Herridge DF, Vessey JK (2002) Limitations to biological nitrogen fixation as a renewable source of nitrogen for agriculture. In: Finan TM, O'Brian MR, Layzell DB, Vessey JK, Newton W (eds) Nitrogen fixation-global perspectives. CABI Publishing, New York, pp 356–360

Perret X, Staehelin C, Broughton WJ (2000) Molecular basis of symbiotic promiscuity. Microbiol Mol Biol Rev 64:180–201

Peters GA, Meeks JC (1989) The Azolla-anabaena symbiosis: basic biology. Annu Rev Plant Physiol Plant Mol Biol 40:193–210. doi:10.1146/annurev.pp. 40.060189.001205

Peters NK, Frost JW, Long SR (1986) A plant flavone, luteolin, induces expression of *Rhizobium meliloti* nodulation genes. Science 233:977–980. doi:10.1126/science.373 8520

Preisig O, Zufferey R, Thony-Meyer L, Appleby CA, Hennecke H (1996) A high-affinity cbb3-type cytochrome oxidase terminates the symbiosis-specific respiratory chain of *Bradyrhizobium japonicum*. J Bacteriol 178:1532–1538

Ramirez-Bahena MH, Garcia-Fraile P, Peix A, Valverde A, Rivas R, Igual JM, Mateos PF, Martinez-Molina E, Velazquez E (2008) Revision of the taxonomic status of the species *Rhizobium leguminosarum* (Frank 1879) Frank 1889^{AL}, *Rhizobium phaseoli* Dangeard 1926^{AL} and *Rhizobium trifolii* Dangeard 1926^{AL}. *R. trifolii* is a later synonym of *R. leguminosarum*. Reclassification of the strain *R. leguminosarum* DSM 30132 (=NCIMB 11478) as *Rhizobium pisi* sp. nov. Int J Syst Evol Microbiol 58:2484–2490. doi:10.1099/ijs.0.65621-0

Rashid MH, Schafer H, Gonzalez J, Wink M (2012) Genetic diversity of rhizobia nodulating lentil (*Lens culinaris*) in Bangladesh. Syst Appl Microbiol 35:98–109. doi:10.1016/j.syapm.2011.11.008

Rashid MH, Gonzalez J, Young JPW, Wink M (2014) *Rhizobium leguminosarum* is symbiont of lentils in the Middle East and Europe but not in Bangladesh. FEMS Microbiol Ecol 87(1):64–77. doi:10.1111/1574-6941.12190

Reinhold-Hurek B, Hurek T (2011) Living inside plants: bacterial endophytes. Curr Opin Plant Biol 14:435–443. doi:10.1016/j.pbi.2011.04.004

Rivas R, Willems A, Subbarao NS, Mateos PF, Dazzo FB, Kroppenstedt RM, Martinez-Molina E, Gillis M, Velazquez E (2003) Description of *Devosia neptuniae* sp. nov. that nodulates and fixes nitrogen in symbiosis with *Neptunia natans,* an aquatic legume from India. Syst Appl Microbiol 26:47–53. doi:10.1078/072320203322337308

Rivas R, Garcia-Fraile P, Velazquez E (2009a) Taxonomy of bacteria nodulating legumes. Microbiol Insight 2:51–69

Rivas R, Martens M, de Lajudie P, Willemes A (2009b) Multilocus sequence analysis of the genus *Bradyrhizobium*. Syst Appl Microbiol 32:101–110. doi:10.1016/j.syapm.2008.12.005

Roche P, Maillet F, Plazanet C, Debelle F, Ferro M, Georges Truchet G, Prome JC, Denarie J (1996) The common *nodABC* genes of *Rhizobium meliloti* are host-range determinants. Proc Natl Acad Sci U S A 93:15305–15310

Rohrig H, Schmidt J, Wieneke U, Kondorosi E, Barlier I, Schell J, John M (1994) Biosynthesis of lipooligosaccharide nodulation factors: *Rhizobium* NodA protein is involved in N-acylation of the chitooligosaccharide backbone. Proc Natl Acad Sci U S A 91:3122–3126

Rossen L, Shearman CA, Johnston AW, Downie JA (1985) The nodD gene of *Rhizobium leguminosarum* is autoregulatory and in the presence of plant exudate induces the nodA, B, C genes. EMBO J 4:3369–3373

Saha BK, Chowdhury MAH, Rashid MH (2008) Effect of rhizobia and host cultivar on some biochemical constituents and yield of lentil. Int J Biol Res 4:100–107

Santos R, Herouart D, Sigaud S, Touati D, Puppo A (2001) Oxidative burst in alfalfa-*Sinorhizobium meliloti* symbiotic interaction. Mol Plant Microbe Interact 14:86–89. doi:10.1094/MPMI.2001.14.1.86

Sattar MA, Podder AK, Das ML, Shaikh MAQ, Danso SKA (1998) Evaluation of chickpea and groundnut for N_2 fixation and yield in Bangladesh. In: Improving yield and nitrogen fixation of grain legumes in the tropics and sub-tropics of Asia (IAEA-TECDOC-1027), International Atomic Energy Agency, Vienna, Austria, pp 131–146

Sawada H, Ieki H, Oyaizu H, Matsumoto S (1993) Proposal for rejection of *Agrobacterium tumefaciens* and revised descriptions for the genus *Agrobacterium* and for *Agrobacterium radiobacter* and *Agrobacterium rhizogenes*. Int J Syst Bacteriol 43:694–702. doi:10.1099/00207713-43-4-694

Schlaman HR, Okker RJ, Lugtenberg BJ (1992) Regulation of nodulation gene expression by *NodD* in rhizobia. J Bacteriol 174:5177–5182

Schloter M, Wiehe W, Assmus B, Steindl H, Becke H, Hoflich G, Hartmann A (1997) Root colonization of different plants by plant-growth-promoting *Rhizobium leguminosarum* bv. *trifolii* R39 studied with monospecific polyclonal antisera. Appl Environ Microbiol 63:2038–2046

Schmid M, Baldani JI, Hartmann A (2006) The genus *Herbaspirillum*. Prokaryotes 5:141–150. doi:10.1007/0-387-30745-1_7

Scholla MH, Elkan GH (1984) *Rhizobium fredii* sp. nov., a fast-growing species that effectively nodulates soybeans. Int J Syst Bacteriol 34:484–486

Sieberer BJ, Timmers ACJ, Emons AMC (2005) Nod factors alter the microtubule cytoskeleton in *Medicago truncatula* root hairs to allow root hair reorientation. Mol Plant Microbe Interact 18:1195–1204. doi:10.1094/MPMI-18-1195

Simmons MP, Bailey CD, Nixon KC (2000) Phylogeny reconstruction using duplicate genes. Mol Biol Evol 17:469–473

Skerman VBD, Mcgowan V, Sneath PHA (1980) Approved lists of bacterial names (Amemded). Int J Syst Bacteriol 30:225–420. doi:10.1099/00207713-30-1-225

Smit G, Swart S, Lugtenberg BJ, Kijne JW (1992) Molecular mechanisms of attachment of *Rhizobium* bacteria to plant roots. Mol Microbiol 6:2897–2903. doi:10.1111/j.1365-2958.1992.tb01748.x

Smith AB (1994) Rooting molecular trees: problem and strategies. Biol J Linn Soc 51:279–292. doi:10.1006/bijl.1994.1024

Soltis DE, Soltis PS, Morgan DR, Swensen SM, Mullin BC, Dowd JM, Martin PG (1995) Chloroplast gene sequence data suggest a single origin of the predisposition for symbiotic nitrogen fixation in angiosperms. Proc Natl Acad Sci U S A 92:2647–2651

Soussana JF, Tallec T (2010) Can we understand and predict the regulation of biological N_2 fixation in grassland ecosystems. Nutr Cycl Agroecosyst 88:197–213. doi:10.1007/s10705-009-9335-y
Spaink HP, Okker RJ, Wijffelman CA, Tak T, Goosen-de Roo L, Pees E, van Brussel AA, Lugtenberg BJ (1989) Symbiotic properties of rhizobia containing a flavonoid-independent hybrid *nodD* product. J Bacteriol 171:4045–4053
Spaink HP, Sheeley DM, van Brussel AA, Glushka J, York WS, Tak T, Geiger O, Kennedy EP, Reinhold VN, Lugtenberg BJ (1991) A novel highly unsaturated fatty acid moiety of lipo-oligosaccharide signals determines host specificity of *Rhizobium*. Nature 354:125–130. doi:10.1038/354125a0
Sprent JI (2001) Nodulation in legumes. Royal Botanic Gardens, London
Sullivan JT, Ronson CW (1998) Evolution of rhizobia by acquisition of a 500-kb symbiosis island that integrates into a phe-tRNA gene. Proc Natl Acad Sci U S A 95:5145–5149
Tan XJ, Cheng Y, Li YX, Li YG, Zhou JC (2009) BacA is indispensable for successful *Mesorhizobium-Astragalus* symbiosis. Appl Microbiol Biotechnol 84:519–526. doi:10.1016/B978-0-12-398264-3.00005-X
Terpolilli JJ, Hood GA, Poole PS (2012) What determines the efficiency of N(2)-fixing *Rhizobium-legume* symbioses. Adv Microb Physiol 60:325–389. doi:10.1016/B978-0-12-398264-3.00005-X
Thajuddin N, Muralitharan G, Sundaramoorthy M, Ramamoorthy R, Ramachandran S, Akbarsha MA, Gunasekaran M (2010) Morphological and genetic diversity of symbiotic cyanobacteria from cycads. J Basic Microbiol 50:254–265. doi:10.1002/jobm.200900343
Tian CF, Young JPW, Wang ET, Tamimi SM, Chen WX (2010) Population mixing of *Rhizobium leguminosarum* bv. *viciae* nodulating *Vicia faba*: the role of recombination and lateral gene transfer. FEMS Microbiol Ecol 73:563–576. doi:10.1111/j.1574-6941.2010.00909.x
Timmers AC, Soupene E, Auriac MC, de Billy F, Vasse J, Boistard P, Truchet G (2000) Saprophytic intracellular rhizobia in alfalfa nodules. Mol Plant Microbe Interact 13:1204–1213
Trujillo ME, Willems A, Abril A, Planchuelo A, Rivas R, Ludena D, Mateos PF, Martinez-Molina E, Velazquez E (2005) Nodulation of *Lupinus albus* by Strains of *Ochrobactrum lupini* sp. nov. Appl Environ Microbiol 71:1318–1327. doi:10.1128/AEM.71.3.1318-1327.2005
Unkovich MJ, Pate JS (2000) An appraisal of recent field measurements of symbiotic N_2 fixation by annual legumes. Field Crop Res 65:211–228. doi:10.1016/S0378-4290(99)00088-X
Urakami T, Araki H, Oyanagi H, Suzuki KI, Komagata K (1992) Transfer of *Pseudomonas aminovorans* (den Dooren de Jong 1926) to *Aminobacter* gen. nov. as *Aminobacter aminovorans* comb. nov. and description of *Aminobacter aganoensis* sp. nov. and *Aminobacter niigataensis* sp. nov. Int J Syst Evol Microbiol 42:84–92. doi:10.1099/00207713-42-1-84
Vaishampayan A, Sinha RP, Hader DP, Dey T, Gupta AK, Bhan U, Rao AL (2001) Cyanobacterial biofertilizers in rice agriculture. Bot Rev 67:453–516. doi:10.1007/BF02857893
Valverde A, Velazquez E, Gutierrez C, Cervantes E, Ventosa A, Igual JM (2003) *Herbaspirillum lusitanum* sp. nov., a novel nitrogen-fixing bacterium associated with root nodules of *Phaseolus vulgaris*. Int J Syst Evol Microbiol 53:1979–1983. doi:10.1099/ijs.0.02677-0
Valverde A, Velazquez E, Fernandez-Santos F, Vizcaino N, Rivas R, Mateos PF, Martinez-Molina E, Igual JM, Willems A (2005) *Phyllobacterium trifolii* sp. nov., nodulating *Trifolium* and *Lupinus* in Spanish soils. Int J Syst Evol Microbiol 55:1985–1989. doi:10.1099/ijs.0.63551-0
van de Peer Y (2003) Phylogeny inference based on distance methods. In: Salemi M, Vandamme AM (eds) The phylogenetic handbook: a practical approach to DNA and protein phylogeny. Cambridge University Press, Cambridge, pp 101–119
Vasse J, de Billy F, Camut S, Truchet G (1990) Correlation between ultrastructural differentiation of bacteroids and nitrogen fixation in alfalfa nodules. J Bacteriol 172:4295–4305
Vessey JK (2004) Benefits of inoculating legume crops with Rhizobia in the Northern Great Plains. Crop Manage. Online: http://www.plantmanagementnetwork.org/pub/cm/review/2004/legume
Vinuesa P, Silvaa C, Wernerb D, Martinez-Romero E (2005) Population genetics and phylogenetic inference in bacterial molecular systematics: the roles of migration and recombination in *Bradyrhizobium* species cohesion and delineation. Mol Phylogenet Evol 34:29–54. doi:10.1016/j.ympev.2004.08.020

Wang D, Griffitts J, Starker C, Fedorova E, Limpens E, Ivanov S, Bisseling T, Long S (2010) A nodule-specific protein secretory pathway required for nitrogen-fixing symbiosis. Science 26:1126–1129. doi:10.1126/science.1184096

Willems A (2006) The taxonomy of rhizobia: an overview. Plant Soil 287:3–14. doi:10.1007/s11104-006-9058-7

Willems A, Fernandez Lopez M, Munoz-Adelantado E, Goris J, De Vos P, Martinez-Romero E, Toro N, Gillis M (2003) Description of New Ensifer strains from nodules and proposal to transfer Ensifer adhaerens Cassida 1982 to *Sinorhizobium* as *Sinorhizobium adhaerens* comb. nov. Request for an Opinion. Int J Syst Evol Microbiol 53:1207–1217. doi:10.1099/ijs.0.02264-0

Woese CR, Stackebrandt E, Weisburg WG, Paster BJ, Madigan MT, Fowler VJ, Hahn CM, Blanz P, Gupta R, Nealson KH, Fox GE (1984) The phylogeny of purple bacteria: the alpha subdivision. Syst Appl Microbiol 5:315–326

Wolfe DW (2001) Tales from the underground: a natural history of subterranean life. Perseus Publishing, Cambridge, MA

Yabuuchi E, Kosako Y, Oyaizu H, Yano I, Hotta H, Hashimoto Y, Ezaki T, Arakawa M (1992) Proposal of *Burkholderia* gen. nov. and transfer of seven species of the genus *Pseudomonas* homology group II to the new genus, with the type species *Burkholderia cepacia* (Palleroni and Holmes 1981) comb. nov. Microbiol Immunol 36:1251–1275

Yadegari M, Rahmani HA, Noormohammadi G, Ayneband A (2008) Evaluation of bean (*Phaseolus vulgaris*) seeds inoculation with *Rhizobium phaseoli* and plant growth promoting rhizobacteria on yield and yield components. Pak J Biol Sci 11:1935–1939

Young JPW (1996) Phylogeny and taxonomy of rhizobia. Plant Soil 186:45–52. doi:10.1007/BF00035054

Young JM (2003) The genus name *Ensifer* Casida 1982 takes priority over *Sinorhizobium* Chen et al. 1988, and S*inorhizobium* morelense Wang et al. 2002 is a later synonym of *Ensifer adhaerens* Casida 1982. Is the combination '*Sinorhizobium adhaerens*' (Casida 1982) Willems et al. 2003 legitimate? Request for an Opinion. Int J Syst Evol Microbiol 53:2107–2110. doi:10.1099/ijs.0.02665-0

Young JM, Kuykendall LD, Martinenez-Romero E, Kerr A, Sawada H (2001) A revision of *Rhizobium* Frank 1889, with an emended description of the genus, and the inclusion of all species of *Agrobacterium* Conn 1942 and *Allorhizobium undicola* de Lajudie et al. 1998 as new combinations: *Rhizobium radiobacter*, *R. rhizogenes, R. rubi, R. undicola* and *R. vitis*. Int J Syst Evol Microbiol 51:89–103. doi:10.1099/00207713-51-1-89

Zheng L, White RH, Dean DR (1997) Purification of the *Azotobacter vinelandii* nifV-encoded homocitrate synthase. J Bacteriol 179:5963–5966

Zurdo-Pineiro JL, Rivas R, Trujillo ME, Vizcaıno N, Carrasco JA, Chamber M, Palomares A, Mateos PF, Martinez-Molina E, Velázquez E (2007) *Ochrobactrum cytisi* sp. nov., isolated from nodules of *Cytisus scoparius* in Spain. Int J Syst Evol Microbiol 57:784–788. doi:10.1099/ijs.0.64613-0

Zurkowski W, Lorkiewicz Z (1976) Plasmid deoxyribonucleic acid in *Rhizobium trifolii*. J Bacteriol 128:481–484

Factors Influencing Farm Profitability

Yeong Sheng Tey and Mark Brindal

Abstract Improving economic, environmental, and social sustainability is a major goal for agriculture. In particular economic sustainability is a key concern for most farmers. Many farmers have indeed left agriculture due to low or no profit. Many studies have attempted to understand why some farms are more profitable than others. This paper reviews factors that regularly influence farm profitability, and thereby generate policy implications to improve farm economic sustainability. A vote count method was employed to review past findings of 16 studies which were published between 1988 and 2013. All studies were conducted in the United States, covering the dairy, soybean, corn, and general farms. Many variables have been found to be significant in explaining farm profitability. They are related to management and financial capacity, farm resource quality and operation, farm and financial management, and skills. The primary finding of this synthesis is that operational scale, operational efficiency, and output prices regularly have a positive impact on earnings across past studies.

In general, the findings imply that sustaining in farming and rural business is not an easy task. While it is challenging, the common factors clearly indicate that farm profit can be achieved by reducing cost through economies of scale and input-output improvement, and by increasing revenue through production capacity expansion and better crop prices. These intertwined features should be included in any design or promotion of sustainable agricultural systems and agricultural technologies. Nevertheless, more research is needed to establish a knowledge base reflecting the particular conditions relevant to individual locales and sectors.

Keywords Economic sustainability • Farm profitability • Systematic literature review • Vote count

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1 Introduction

Ensuring sustainable development is a primary goal for agriculture (UNCED 1992; FAO 2002; WSSD 2002). Under the sustainability umbrella, sustainable development is achieved when economic, environmental, and social sustainability coexists. Seeking to achieve these goals, a number of alternative agricultural systems (e.g., organic agriculture, low-inputs agriculture, precision agriculture, etc.) have been promoted with the aim of providing greater sustainability than is achieved by prevailing systems.

Sustainable agricultural systems are commonly considered to efficiently use locally available resources and less non-renewable inputs through improved management techniques and practices (Lee 2005). Under this notion, the emphasis is to prescribe and optimize input application according to agronomic needs. When implemented properly, alternative agricultural systems are evident in the ecological principles said to characterize sustainable agriculture: saving input costs, improving soil conditions, reducing input run-off and leaching, enhancing biological interactions, minimizing negative environmental externalities, promoting crop quality, and ensuring worker safety. In the long-run, they improve the sustainability of the environment, society, and economy as a whole.

However, economic sustainability is the over-riding priority of many farmers and farm enterprises (Pannell et al. 2006; Tey et al. 2012). This is axiomatic since agriculture is a business: continuity of any commercial activity depends upon the capacity to remain financially viable over time and this implies a positive return (i.e., profitability) on all inputs. To farmers, agriculture is an important income source. It might be their only economic means of meeting their financial needs or, sometimes, an additional financial means of improving their quality of life. Therefore, farm profitability has a critical implication for farm survival, food security, and farmer welfare.

Failure to sustain farm profitability and growth has driven some farmers and farm enterprises out of the industry. In the United States, the last three decades recorded an average annual gross farm exit rate of about 10 % per year (Mishra et al. 2014). The number of New York dairy farms declined from 19,000 to 7,900 between 1980 and 2000 (USDA 2002). Within the Australian agriculture industry, farmers exit rate is described stable, standing at around 5 % per annum between 1976 and 2001 (Barr et al. 2005). Similarly, the Malaysian agricultural labor force shrunk from 1.52 million people to 1.39 million people between 1994 and 2010 (Ministry of Finance 1995, 2013). While these are just some examples, other developed and developing countries exhibit similar patterns of change. This phenomenon is worrisome (Fig. 1).

In order to understand and perhaps capture the capacity to reverse this shrinkage, both researchers and policymakers have expressed a desire to understand why some farms are more profitable than others. Past studies of those factors thought to determine farm profitability have been reviewed, specifically for American agriculture, by Fox et al. (1993). They conclude that personal attributes and goals are the

Fig. 1 Inability to sustain farm profitability and growth has driven farmers and farm enterprises out of the industry (Source: sevenacres.wordpress.com (2014) – "'Farm for Sale' signs are not uncommon sight up here… Most properties go up for sale and their fates can vary")

primary factors determining differences in profitability across farms. Their work, however, represents a selective review and both the recent and wider body of literature concerning the determinants of farm profitability remains un-synthesized. Therefore, this issue can be better understood by a systematic analysis of the knowledge bank that is formed by the body of recent literature.

Responding to the above mentioned gap, the objective of this paper is to review the factors influencing farm profitability in the recent research. This paper is the first in this area to conduct systematic literature review using a vote count method. Without limiting our scope to any particular agricultural system, our findings will indicate certain factors as more or less important determinants for farm earnings. The general findings will serve as a knowledge base for policymakers to understand how to better steer farm enterprises into profitability and for researchers to develop avenues for future investigation. Such understanding will also form the basis in designing the promotion of sustainable agricultural systems and agricultural technologies.

2 Methodology

A vote count analysis is a common method for conducting systematic literature review in agricultural studies. For example, it has recently been applied to review the literature on the adoption of sustainable agriculture (Tey et al. 2014a), precision farming technologies (Tey and Brindal 2012), agricultural best management practices (Prokopy et al. 2008), and agricultural conservation practices (Knowler and Bradshaw 2007). As demonstrated by these studies, using vote count analysis, a score is given to individual statistically insignificant factors as well as statistically significant positive and negative factors. Votes are then tallied. The results can be used to draw conclusions about the literature as a whole.

2.1 Dataset

As the interest of this review has been in summarizing past findings on factors influencing farm profitability, the dataset of this paper is formed by the relevant literature. To obtain the input, a comprehensive search was conducted through appropriate databases (Scopus® and Google Scholar®) and also the reference section of relevant articles. The central criteria for assessing past studies relevance were dependent variables which represented or measured farm profitability. Studies computing inputs-outputs and the resultant economic performance were excluded because they did neither include statistical significance in their empirical analysis nor did they match the scope of this paper.

As a result, the dataset comprises 16 studies (see Table 1) conducted over the past 25 years (1988–2013). According to these studies, there were four measures of profitability:

- Accounting profit: total revenue minus total expenses. It does not place an arbitrary monetary value on the unpaid expenses (labor, management, and financing interests) and helps equalize different standard of farms (Dartt et al. 1999).
- Net farm income: total accrual revenue minus total accrual expenses (including labor, management, and financing interests) when cash income is adjusted for inventory changes (El-Osta and Johnson 1998). This measure is appropriate to investigate the extent to which farm profits is generated from the use of labor, management, and capital (Lins et al. 1987).
- Net farm income per farm or herd size: net farm income divides by number of hectares or head of livestock. As a result, an average profit, which corrects for the operation scale in order to compare the financial performance across different farm sizes can be calculated (Gloy et al. 2002a).
- Rate of return on assets: interest expense is added, and both unpaid labor and management expenses are subtracted from net farm income; the capital return is then divided by the total assets. This measure eliminates differences in farm size and expenses such as labor, management, and financing (Dartt et al. 1999).

Throughout the literature, two groups of statistical methods, according to the individual research interests of the authors, were applied. Firstly, two-stage estimation methods were deployed to examine the relationship between the adoption of certain practices and farm profitability. In the stage one of this process, probit and multivariate probit models aimed to analyze adoptive behavior. In the stage two, the

Table 1 The past studies used in this review

No.	Study	Countries	Sectors	Research questions	Dependent variable	Statistical methods
1	Haden and Johnson (1989)	USA	Dairy farms	Profitability	Accounting profit	OLS
					Net farm income	OLS
					Return on assets	OLS
2	Ford and Shonkwiler (1994)	USA	Dairy farms	Profitability	Net farm income	SEM
3	Stefanides and Tauer (1999)	USA	Dairy farms	Adoption – Profitability	Net farm income/cow	Probit – OLS
4	Dartt et al. (1999)	USA	Dairy farms	Adoption – Profitability	Net farm income	OLS
5	Mishra et al. (1999a)	USA	Limited resource farms	Profitability	Net farm income	WLS
6	Winsten et al. (2000)	USA	Dairy farms	Adoption – Profitability	Net farm income	OLS
7	Gloy et al. (2002a)	USA	Dairy farms	Profitability	Return on assets	2-stage LS
			Limited resource farms	Profitability	Return on assets	WLS
			Small farms	Profitability	Net farm income	WLS
			Small farms	Profitability	Return on assets	WLS
8	Gloy et al. (2002a)	USA	Dairy farms	Adoption – Profitability	Return on assets	Probit – OLS
					Return on assets	OLS
9	McBride and El-Osta (2002)	USA	Corn farms	Adoption – Profitability	Net farm income	Probit – OLS
			Soybean farms	Adoption – Profitability	Net farm income	Probit – OLS
10	Foltz and Chang (2002)	USA	Dairy farms	Adoption – Profitability	Net farm income/cow	Probit – OLS
11	Jackson-Smith et al. (2004)	USA	Dairy farms	Profitability	Return on assets	OLS
12	Gloy and LaDue (2003)	USA	Dairy farms	Profitability	Return on assets	OLS
13	Foltz and Lang (2003)	USA	Dairy farms	Adoption – Profitability	Net farm income/cow	Probit – OLS
14	McBride et al. (2004)	USA	Dairy farms	Adoption – Profitability	Accounting profit/production	Probit – OLS
15	Fernandez-Cornejo et al. (2005)	USA	Soybean farms	Adoption – Profitability	Accounting profit	Multivariate probit – OLS
16	Gillespie et al. (2010)	USA	Dairy farms	Adoption – Profitability	Net farm income/cow	Probit – OLS
				Adoption – Profitability	Net farm income/production	Probit – OLS

Notes: We reviewed the best model when authors presented a number of models. *OLS* denotes ordinary least squares, *SEM* denotes structural equation model, *WLS* denotes weighted least squares

All these studies were conducted in the United States. The studies largely covered the dairy production sector; soybean, corn, and general farms to a small extent. The majority of studies attempted to answer the question as to whether adoption of a certain production practices, among other factors, resulted in higher profitability resultant predicted values were incorporated into an ordinary least squares model to examine its impact, among other factors, on farm profitability. Secondly, ordinary least squares, weighted least squares, and structural equation models were used to identify the underlying determinants of farm profitability in general.

2.2 Vote Count Procedures

A vote count method was utilized following the direction proposed by Tey et al. (2014a, b), Tey and Brindal (2012), Prokopy et al. (2008), and Knowler and Bradshaw (2007). Careful attention was paid to the statistical significance of variables covered by the 16 reviewed studies. In a progressive manner, a vote was assigned to each statistically significant variable either as displaying positive or negative association with farm profitability at a 0.10, 0.05, and 0.01 significance levels; a vote was also allocated for every statistically insignificant variable. Finally, votes for statistically significant positive variables, statistically significant negative variables, and statistically insignificant variables were inventoried respectively.

Guided by the relevant literature, the summarized variables, which shared similarities, were divided into four categories: (1) farmer and farm household characteristics, (2) farm and biophysical characteristics, (3) farm management and financial characteristics, and (4) exogenous factors. The placement of each variable into a category was determined based upon careful reading and understanding of each study.

3 Findings

In the 24 analyses reviewed (see Table 2), many variables have been investigated to explain why some farms generate higher profits than others. These variables are presented in a coherent manner under the four category headings mentioned earlier.

3.1 Farmer and Farm Household Characteristics

Farmer and farm household characteristics are often used to reflect management capacity (Prokopy et al. 2008). Good management requires farmers to possess an ability to maintain the function of a system as it undergoes changes (Carpenter et al. 2001). For example, horticultural farms require broad and varied expertise in managing cropping programs, farm finances, and human resources. Similar challenges are faced by livestock farms, but they also have to manage a herd. These examples demonstrate that each agricultural sector is managerially demanding and has specific management requirements. Additionally, differences in farmer and farm

Table 2 Significant and insignificant factors (at α = 0.10) influencing farm profitability

Factors	Sig. (+)	Sig.(−)	Insig.	Total	Status
Farmer and farm household characteristics					
Education	1	1	9	11	*
Age	2	3	6	11	*
Years of farming experience	0	0	2	2	
Household size	0	0	1	1	
Assets	0	0	3	3	^
Asset-related ratios	2	0	1	3	**
Sole proprietor	1	0	2	3	**
Off-farm employment	1	0	1	2	
Farmer risk taking behavior	0	1	1	2	
Farm and biophysical characteristics					
Farm typology	0	0	1	1	
Soil fertility	1	1	3	5	*
Region	1	3	4	8	*
Farm size	1	1	3	5	*
Livestock holdings or ratio	7	0	5	12	**
Farm management and financial characteristics					
Land tenure	0	0	1	1	
Input costs	0	2	1	3	**
Hired laborers working hours	0	1	3	4	**
Output prices and value	6	1	4	11	*
Farm diversification	1	1	1	3	*
% of revenue from core production	2	0	6	8	**
% of revenue from non-core production	0	1	3	4	**
Use of computer	0	0	2	2	
Hire or create record keeping	1	0	5	6	**
Hire or create financial analysis	3	0	8	11	**
Debt-related ratios	1	4	1	6	*
Cost efficiency ratios	2	4	6	12	*
Financial health ratios	2	0	0	2	
Operational efficiency ratios	9	0	6	15	**
Adoption of innovation	2	0	12	14	**
Technology index	0	1	1	2	
Experience with the innovation	0	0	2	2	
Breeding system	0	0	3	3	^
Veterinary service	0	1	1	2	
Parlor milking system	0	2	5	7	*
Milking frequency	0	0	4	4	^
Exogenous factors					
Covered by crop insurance	2	0	0	2	
Participate in training program	2	0	5	7	**

Notes: *Sig.* is significant; *Insig.* is insignificant; (**) a factor that has a mix of insignificance and significance and has different sign when significant; (*) a factor that is always insignificant; factors that have frequency below three instances are not rated; (^) a factor that has a mix of insignificance and significance and has the same sign when significant

In total, 28 variables have been found to be significant in explaining farm profitability. These findings imply that farm economic sustainability is influenced by many factors. Therefore, sustaining farming and rural business is not an easy task household characteristics often result in management variations which affect the financial outcomes of farms, notwithstanding that they share a similar resource base and production systems (Ford and Shonkwiler 1994).

One common factor that is assessed within 11 of the 24 reviewed analyses is the educational attainment level achieved by farm operators. This factor is thought to yield insights into the influence of training (Mishra et al. 2009). It is based on a premise that better educated farmers have a tendency to be more knowledgeable and possess greater management capacity. As a result, they are likely to manage their farm enterprises more effectively and efficiently. Therefore, a higher education level is hypothesized to have a positive impact on economic performance. Support for this hypothesis was given by Stefanides and Tauer (1999). However, in other studies, education was largely found to be an insignificant factor in relation to farm profitability (e.g., Foltz and Chang 2002; Fernandez-Cornejo et al. 2005). The studies offer no explanation for that counter intuitive finding. Since agriculture is a complicated economic activity, which involves farming techniques and business skills that are both specific and experiential, formal education may play a less significant role than might be expected in agricultural management. However, since education is generally considered to include the ability to more easily acquire knowledge which might be translated into practical skills, this area is in need of more detailed and extensive research.

Age, as a common factor, and experience are used as two proxies for on-farm human capital. These two factors are often interrelated. Given that experience is accumulated over time, older farmers have a greater farming experience. They are thought to be more likely to manage a farm better and to achieve healthy financial results. Contrary to this expectation, age and farming experience were found to be largely insignificant (e.g., Gloy et al. 2002a; McBride and El-Osta 2002); a negative impact on farm profitability in some studies (e.g., Haden and Johnson 1989; Foltz and Lang 2003). The "insignificant" findings must be considered counter intuitive since they imply that a young farmer with no experience could run a farm and achieve the same profitability as an old farmer with years of experience. The negative findings could, if we can assume that the sample was skewed by a predominance of older farmers, be explained in terms of the shorter career horizon of ageing farmers. They have less motivation to strive for efficiency as they may have reached a point at which they consider their own experience as such that they cannot further improve their practices and tend to value other life aspects more than monetary reward. Notwithstanding these explanations, it is surprising that only two analyses in Mishra et al. (1999a) found a positive association between age and farm earnings. We believe that a more logical explanation might lie in an assumption that age/experience show either a linear or exponential increase; in fact, it is more likely that these factors might more rightly be expressed as a classic bell curve. Such an assumption could explain some of the counter intuitive findings of earlier works. We offer this hypothecation to explain an obvious conundrum: proving or disproving it is a matter for future research.

Agricultural activities remain labor-intensive, and the human resources who are employed on the farm are often household members. A larger household is assumed to overcome labor constraints and provide economical labor input. Thus, household size is expected to have a positive correlation with a greater profitability. However, this factor was proved insignificant in Fernandez-Cornejo et al. (2005). Logically, this is due to opportunity cost considerations: non-agricultural jobs are more economically attractive and available to household members while foreign farm workers are readily available at a lesser cost, and therefore more economically profitable.

Financial capital plays an important role in generating return on investment. A central measure in this regard is farmer or household's asset. These are considered to reflect financial status. This measure is sometimes replaced using asset-related ratios (e.g., farm asset per cow). Farmers who are endowed with a greater asset base are considered to have the ability to invest more and experience the benefit of higher profits even when the rate of return is fixed. Such impact was evidenced for asset-related ratios (Ford and Shonkwiler 1994), but was found to be insignificant for asset value (e.g., Dartt et al. 1999; Gloy et al. 2002b). Such apparently contradictory findings demand further investigation.

Another proxy for financial capital concerns a farmer's ability to raise financial capital. This is interrelated with the farmer's asset base but contains additional dimensions. Business partnerships and corporate ownership structures tend to possess and have the ability to acquire more capital than sole proprietorships. The financial constraint of the latter might therefore restrain expansion in farm enterprises. Such reasoning implies that a farm which is owned and run by an individual is less likely to be associated with higher profits than one which involves a partnership or corporate structure. Contrary to this hypothesis, a positive relationship was found to exist between sole proprietors and farm financial performance (Mishra et al. 1999a). According to the authors, the single owner manages farm resources efficiently, has solely responsibility in decision making, and does not have to share profits like those in business partnerships.

An alternative source of financial capital is off-farm employment, which generates additional income for potential farm investment. This hypothesis was reinforced by an analysis, but was found to be insignificant in another analysis of the work of McBride and El-Osta (2002). The study suggests that involvement in off-farm economic activities may potentially compete with on-farm involvement.

The degree to which farmers are prepared to assume risk correlates with their degree of investment to achieve capital growth. It is specified using a risk index that is built from a series of questions exploring how farmers react toward risk. A high index score indicates that farmers are likely to take a risk and make a farm investment. Given that investment is made with an expectation of profit, the risk taking behavior is expected to be positively related to farm net incomes. Contrary to that expectation, empirical findings suggest an inverse relationship between the risk index and the dependent variable (McBride and El-Osta 2002). Though no explanation was offered, it might be attributed to the fact that an investment may or may not be productive. When the investment is not fruitful, risk taking farmers suffer losses; risk-adverse farmers, however, remain unaffected and with their stable incomes.

3.2 Farm and Biophysical Characteristics

Farm and biophysical characteristics directly correlate with endowment quality at any farmer enterprise. A favorable farm and biophysical environment helps to increase productive farm outputs. To achieve a yield, for instance, vegetable cultivation in resource-rich areas does not need the same quantities of inputs as those which are required to achieve similar production in resource-poor areas. This variation demonstrates that a different effort is needed in accordance with both scale and the asymmetric resource distribution across regions and farm enterprises. Consequently, variable farm and biophysical characteristics have an impact on a farm's economic performance.

Natural endowments vary across farm locations. The United States has developed a farm typology. Farms are divided into three groups: rural residential farms, intermediate farms, and commercial farms. Amongst these, commercial farms are highly profit-driven and invest in efficient technologies. Despite this, Winsten et al. (2000) quantified no significant difference between these categories of farm typology in relation to financial performance.

Soil fertility and soil color are measured to reflect farm productivity. Dark and fertile soils generally need less nutrient input and, thus, are cost saving. The studies, however, produced mixed results: some did not find a relationship between soil fertility and farm profitability (e.g., Mishra et al. 1999a, b) revealed a positive correlation; Ford and Shonkwiler (1994) found a negative correlation.

Moving away from site-specific conditions, resources differ across farm locations and regions. With respect to the regional difference, positive, negative, and insignificant association with farm returns were all claimed. We must therefore conclude that studies with respect to the correlation of farm and biophysical characteristics are confused, inconclusive, and even contradictory. Clearly a greatly increased endeavor must be devoted to this area before clarity can be achieved.

The scale of farm operation is commonly categorized using number of livestock for livestock farms and/or acreage for other farms. In fact, this factor was utilized by 17 of 24 reviewed analyses. The rationale behind the importance assigned to this is related to two economic benefits that are enjoyed by large-scale farms. Firstly, cost per unit of output generally decreases as farm size increases since the fixed costs are spread over more output units. Thus economies of scale are increased. Secondly, increased asset base (greater equity in tangible assets) facilitates a capacity to access increased levels of financial assistance (e.g., credit or loans). Given that large-scale farms are more likely to be cost efficient and are able to raise capital for expansion and investment purposes, it can be hypothecated to enjoy greater profits. This preposition was supported by most studies (Winsten et al. 2000; Jackson-Smith et al. 2004). A single exception was found by Dartt et al. (1999), who suggested that increased farm size may depress labor efficiency. This is plausible when workforce employed must husband a farm which is beyond their capacity or when the size of the enterprise necessitates inefficient communication and/or the need for second tier management. Notwithstanding this, there is a general consensus that operational scale is a critical factor affecting farm profitability.

3.3 Farm Management and Financial Characteristics

Farm management and financial characteristics are proxy descriptors for the use of resources and their function in optimizing whole farm systems (McConnell and Dillon 1997). Optimal farm enterprise operation often depends on production practices that emphasize efficiency. Farm operation may include financial analysis which assists the understanding farm practices, managing uncertainty, and helps towards making guided decisions. Clearly, farms involve complex management. Similar farming enterprises of similar size in similar locations with similar resources are operated and managed dissimilarly. However, differences in farm management techniques and decision making influence business profit.

Tenure to farm land is either freehold or lease. Freehold farms are often better maintained than leased land (Knowler and Bradshaw 2007). It should be noted, however, that this generalization is in part at least dependent on the period of tenure of the lease. In Australia, for instance, some publically owned land is leased to cattle and sheep farmers on a “perpetual lease”. The rents charged are insignificant. This type of lease guarantees occupancy of the land for 99 years with an automatic right or renewal for a further 99 years in perpetuity. Obviously, this type of lease should almost be regarded as an equivalent to a freehold entitlement. Where there freehold property, the owners do not have to pay rent and they can therefore enjoy greater profit margins since their fixed costs are diminished. On the other hand, leased farms (which in Asia might only be a relatively short period) face a pressure to sustain in business profitability the tenure of the lease. They seem therefore more likely to exploit on-farm resources or to increase efficiency exclusively in exchange for economic benefit. Based on these arguments, it is reasonable to assume that there will be little significant relationship between land tenure and farm profitability. Indeed, this was confirmed by the work of Gloy et al. (2002a).

Input costs, be they in general or specifically related to workforce employment, have a direct impact on farm profit margin (e.g., Dartt et al. 1999). With respect to workforce expenditure, more farm revenue has to be spent on the wages when they work for longer hours. Consequently, farm profitability is diminished. Nevertheless, other analysis within the same study indicated that working hour had no impact on the financial output. Because longer working hours represent a greater farming effort, the increased productivity may compensate for the increased labor costs. This, once again, finds a locus in farm enterprise management skills: the skillful manager provides for additional labor cost only to the extent that those costs are justifiable in terms of enterprise profitability.

Similarly, farm revenue is a closely related factor in farm enterprise financial performance. The entire amount of income comes from the sale of farm produce. Being common factors, higher output prices and revenue saw a significant uptrend in farm profit in most studies (e.g., Haden and Johnson 1989; McBride et al. 2004). Different from this finding, their relationship was insignificant in some literature (e.g., Fernandez-Cornejo et al. 2005), and even inversely significant in Dartt et al. (1999). Such case arises because revenue is subject to deductions (e.g., input cost, taxation) in order to get the net incomes. Therefore, it is not impossible that farms make loss when they have a high sale.

Some farms tend to spread their risk by undertaking diversification. Such diversification may lead to economies of scope, which lowers the average cost of producing two or more commodities. However, empirical correlation for this hypothesis was mixed: Mishra et al. (1999b) found positive significance; McBride at al. (2004) found negative significance, while Mishra et al. (1999a) found diversification to be insignificant. In view of this inconclusiveness, further attempts were made to look at the role of revenue from core (e.g., dairy) and non-core produce (e.g., non-dairy). When a greater share of revenue derives from the core product, there exists an inherent implication that productivity is derived from specialization. Though such concentration of effort resulted in higher net incomes in certain studies (e.g., Foltz and Chang 2002), it was not an influential factor in most cases (e.g., Haden and Johnson 1989; Fernandez-Cornejo et al. 2005). On the other hand, non-core production diverts effort from the primary agricultural activity. If the financial contribution of the non-core activity is positive (after factoring in the potentially weaker profits from the core activity), its contribution to enterprise revenue means that non-core product is economically rewarding. However, Dartt et al. (1999) found diversification be significantly associated with low farm profits whereas Fernandez-Cornejo et al. (2005) did not find any relationship between the two. We again point to the locus of these factors with enlightened efficient management practice. If any diversification undertaken diminishes enterprise efficiency and lowers profit, the results found by Darrt et al. (1999) are axiomatic. Where such diversification is managed in a way which does not diminish profit, results as those found by Fernandez-Cornejo et al. (2005) are to be expected. However, where an efficient manager diversifies in a manner which increases efficiency lowers input costs, or offsets lower returns on one product by enhanced profitability of the other, financial returns will be enhanced. That means intelligent diversification enhances profitability (Rumley et al. 2007).

Record keeping and the use of computers are also used as a proxy for managerial ability. Farmers who record or hire accounting services to record inflows and outflows monitor their incomes and expenses closely. Alternatively, this task can also be completed efficiently using a computer. The notion suggests that both bookkeeping and the user of computational recording assist farmers to make sound decisions (Mishra et al. 1999a). According to Gloy et al. (2002a), those farm managers who utilized accounting services exhibited a significantly positive correlation with the enterprise's financial performance. Conversely, according to Foltz and Chang (2002) and McBride and El-Osta (2002), the same association was insignificant overall. This could be because, in some instances, ledgers are largely kept exclusively for recording and taxation purposes. In such cases, their effect of farm profitability might prove insignificant.

Nevertheless, the accounting information remain important inputs for financial analysis. Such analysis helps farmers to better understand their business. Based on its outputs, farmers are able to make guided decisions. Notwithstanding this, research results remain contradictory. While Gloy and LaDue (2003) linked the practice of financial analysis to business viability, most studies did not identify it as being a significant factor (e.g., Haden and Johnson 1989). That leads researchers to explore a better understanding through the relationship between specific subsets of financial analysis and farm profitability.

Debt-related analyses which are explored in the literature include debt-equity ratio, debt per head of cattle, and the share of loan interest as a percentage of the total expenditure. They indicate the proportion of debt a farm enterprise uses to finance its assets and operation. More debt is hypothecated to be carried by those more profitable farms which possess the ability to meet repayment schedules. However, such a hypothesis was only supported by Gloy et al. (2002a). In contradiction, since debt is repaid at the expense of farm revenue, debt-related ratios were found to have had a significant inverse relationship with farm financial outputs (e.g., Haden and Johnson 1989).

Ratios like input expenses per acre, feed expenses per head of livestock, veterinary expenses per animal, and labor cost per animal, capture the average production costs and are commonly examined. A high ratio shows that a substantial portion of revenue needs to be subtracted since it constitutes production expenditure. While this accounting rationale was evidenced in some studies (e.g., Mishra et al. 1999a), it is not found by other researchers (e.g., Winsten et al. 2000) and perversely, is posited in the opposite direction (e.g., Ford and Shonkwiler 1994). This latter study refers particularly to the cost of veterinary expenses, which help to maintain cattle health. This input cost is relatively small when compared to the loss of cattle and diminishing dairy production. As such, different production costs should have a varied impact on farm net incomes.

Financial health is diagnosed using equity-asset ratio and farm equity ratio; operational efficiency is calculated through operating margins, production per labor unit/head of cattle/or acreage, farm size per sales per labor/cattle, and livestock holdings per acre. Both of these ratios paint a contemporary picture of any farm enterprise. For example, a low efficiency ratio encapsulates a possibility for productivity growth. Farmers can then consider a course of action either for business expansion or to improve resource allocation. Driven by both financial health and operational efficiency ratios, farmers were generally able to achieve substantial net earnings (e.g., Ford and Shonkwiler 1994; Gloy and LaDue 2003) Despite some research findings of “insignificance” in this area (e.g., Mishra et al. 1999a), as these ratios have a direct implications for profitability, they are likely the most important explanatory factors to farm economic performance.

Agricultural innovations are developed to enhance farm productivity. Innovations that are encompassed by this review include livestock hormones, livestock feeding systems, and genetically modified crops. A growth hormone like recombinant bovine somatotropin aims to increase milk output (Dohoo et al. 2003a, b); management intensive rotational grazing (MIRG) reduces production cost (Foltz and Lang 2003); Roundup Ready soybeans boost crop yield by suppressing weed growth (Carpenter and Gianessi 1999). Given these production benefits, adoption of these innovations is commonly investigated and is anticipated to have a positive impact on farm financial outputs. While this anticipation was confirmed for Roundup Ready soybeans

(Dartt et al. 1999) and MIRG (Fernandez-Cornejo et al. 2005), it was insignificant in most cases (e.g., Winsten et al. 2000; Gillespie et al. 2010). These findings suggest that the overall impact of innovations is uncertain as they may decrease input cost while producing consequences which result in lower production or increase production only to an extent. In that case, the production does not compensate for greater investment costs. Such an inverse relationship was indeed evidenced through the technology index developed by Foltz and Lang (2003). Explanation for their findings might relate to the spatial factor inherent in innovation. Greater experience in using an innovation implies familiarity and efficiency. It should be noted that, as has previously been mentioned, there was no significant correlation between the years of experience and farm profitability (Stefanides and Tauer 1999). However, spatial factors also affect the relationship between innovation and farm income returns in complex ways. Innovation often involves outlay costs, but its benefits might be realized in a different time frame to the annualized method usually reckoned for farm profitability. Sometimes this will deter farmers from adopting the innovation. Alternatively, if they do adopt it, the result may be skewed if accounted annually. If an innovation with expenditure outlays in year one increases profit from year two, with annual accounting, such an innovation will show diminished profits in the first year and an inflated return in year two since the out-goings and incomings are realized in different accounting periods.

While all the farm management and financial characteristics above apply to general farms, livestock farms have additional requirements in respect to breeding practices, and animal husbandry. For the dairy industry, milking systems and milking frequency must also be added. Heifers (female calves) are kept to breed replacement cows for the dairy herd. To lower reproduction cost, artificial insemination and embryo transfer are used by increasing herd fertility without needing to invest in one or more bulls. As the reproduction cost is a marginal expenditure, these cost-saving practices did not have a significant contribution to net returns (e.g., McBride et al. 2004). However, it should be noted that this highlights one of the conundrums which plague many of the studies: while the reproduction costs are marginal for the current paradigm, the alternative is to keep a number of bulls on the property. Their fertility may be variable and certainly cannot be guaranteed. They also require grazing space on the property, fodder and animal husbandry. In such a regime, production costs are increased and profits are lowered. If it viewed from that perspective, not of the reproductive costs as they currently apply by from the costs saving achieved from the alternative regime, the current practices might be found to make a significant contribution to net returns. In current regimes, greater cash flow is allocated to maintain cattle health since herd wellbeing and freedom from disease directly influence dairy production. This cost, represented by veterinary service expenditure, significantly diminishes farm profits (Foltz and Chang 2002). In respect, specifically to dairy production, parlor milking systems have eased the strenuous activity of milking and provided greater efficiency, but these systems come at a substantial investment cost. In weighing benefit against monetary cost, the parlor was not a significant factor in some studies (e.g., Gloy et al. 2002a; Gillespie et al. 2010); in others, it caused a significant slide in farm earnings (Stefanides and Tauer 1999; Gloy et al. 2002b).

Such contradictory findings point to the need for more research in the area. When there is no difference in the milking systems, researchers posit that farmers who milk their cow more regularly have higher dairy production. In fact, milking frequency depends on the speed of the beast's lactation. Therefore, it is unsurprising that past studies have failed to establish this factor as a significant predictor of farm profitability (e.g., Foltz and Chang 2002).

3.4 Exogenous Factors

There are additional factors within agricultural systems that are exogenous to a farm and farmer. Crop insurance is purchased to protect farms against either yield loss due to natural disasters or revenue loss due to price depreciation. They are therefore used as a risk management tool. Given that the premium demanded for such insurance is high, it is more affordable as a tool for more profitable farms. Consequently, crop insurance has a positive association with farm profitability (Mishra et al. 1999b).

To enhance farmer management skills, training is provided by extension organizations. Capacity building programs are conducted in order to keep farmers updated on developments in agricultural innovations and operation. This is especially important as the competition for efficiency and sustainability makes agriculture increasingly complex. For example, the Dairy Herd Improvement Association has advocated input-output data and sample analyses to guide farm decision-making in the United States. Participants of such programs are shown to achieve higher financial returns (Foltz and Chang 2002; Jackson-Smith et al. 2004). However, the same relationship does not exist in other studies (e.g., McBride et al. 2004; Gillespie et al. 2010). These latter findings might be explained in terms of the problem associated with the assumption behind this factor, which accepts that participants of a program learn from it and implement the methods recommended. In fact, as noted by one strand of innovation-adoption research, farmers are generally reluctant to change their routines (e.g., Knowler and Bradshaw 2007; Tey and Brindal 2012). Consequently, a better proxy should be found to represent the effect of training.

4 Discussions

Retaining farmers in agriculture is no easy task and, especially given the intertwined problems of population growth and food security, it has become a priority for many governments. Policymakers should be mindful when attempting to utilize research findings that attempt to explain farm profitability. This is because our analysis has shown that research in the area is very much a work in progress. Past findings must be understood in their context and their limitations must also be considered. Having made that observation, it currently appears that certain significant factors (e.g., age and farm location) do associate with the dependent variable, they might not cause changes to farm financial performance. Other factors (e.g., input costs and output prices) which determine production cost and farm revenue and, in turn, affect farm profitability have more certain efficacy. Consequently, those common determinants which have been generally confirmed as having a significant and consistent causal relationship with the dependent variable, at this time, deserve greater attention in the policy development of this area.

Operational scale should be a key policy initiative in facilitating farm returns. Before prescribing any measures, both policymakers and farmers should understand the inputs used for each unit of yield and look at maximizing output in order to increase revenue and earnings. Policy which affects the expansion of production capacity and the agricultural enterprise scale is important. This helps to achieve economies of scale by reducing its average production cost. Consequently, profit margins are improved. Such an improvement in production capacity might also create an opportunity for strategic advantage: supply continuity (i.e., allowing customers to deal continuously with the same enterprise). By doing so, customers need not turn to competitors for unfulfilled demand and farmers do not risk losing their customers permanently. Notwithstanding this, relevant policy facilitating operational expansion should be inclusive and must take into account the scale of farming in developing countries like Malaysia (which are often characterized by smallholdings). Failure to do so could result in social dislocation and produce political consequences.

Another key concern is operational efficiency. Relevant policy efforts should empower farmers to better understand their farm enterprises, to see where the business will be in the future, who the direct or indirect competitors and the likelihood of the business is succeeding. In regard to such operational efficiency, emphasis should be placed on understanding the significance of the ratio formed between production input (e.g., labor and fertilizers) to run farm business and economic output (e.g., margin and profit) gained from the business. This ratio has been demonstrated to provide critical information to guide farm decisions, particularly with respect to the optimal use of resources. Common alternatives for improving operational efficiency include using less input for the same output, the same input for more output, and more input for much more output. The first two options aim to improve profit margin: farmers reap premium dividends when they produce at the lowest or the same cost while, at the same time, maintaining and increasing the output respectively. The last choice requires investment in technologies (e.g., modern varieties) which will increase yield. Such products, when sold, are either in their quantity and/or quality as to compensate for the additional investment cost. Any of these improvements in operational efficiency provides farmers with an edge over their competitors and assists in sustaining them in the industry.

Agricultural commodity prices affect farm earnings directly and demand special policy focus. Typical measures in this regard include guaranteed minimum price and crop insurance. The guaranteed minimum price scheme assures that the prices stay above certain level in depressed markets. As such, its use is activated on an ad-hoc basis and should be valid only for a short-term. Abuse of such policy instruments will distort the market, discourage competitiveness, burden taxpayers, and run contrary to various free trade agreements to which many nations have committed. Similarly, crop insurance indemnifies farm enterprises when revenue falls below certain guaranteed levels. Effectively it is a private arrangement which guarantees a minimum price independent of government. Given that insurance claim depends on the result of a claim and subsequent justification, crop insurance is seen as a more efficient instrument to deter fraud. On top of these two typical measures, new policy intervention is possibly needed to balance the interest of farmers and the emergence of large supermarkets. Supermarket chains have increasingly slashed produce prices at consumer-level and pressed farmers to cope financially with lower farm gate receipts (Tey and Brindal 2014). Such prolonged price cut will eventually force farmers out of business.

In addition, it is apparent that more research is needed to establish an empirical base to foster sustainable economic development in agriculture. This is because past findings have largely been mixed and are often counter intuitive and contradictory. Such inconclusiveness neither produces clear implications for policymaking purposes nor gives policymakers confidence in usefulness of the research. This is more critical since policy prescription is increasingly required to target at the most effective areas, especially as there is a trend to increasingly limit funds for agricultural development (Tey et al. 2014b). Such pressure calls for future research to clearly prioritize the factors determining farm profits in order to order to assist policymaking in the agricultural industry.

Future research should aim to produce empirical knowledge that is meaningful especially for the local management and sector. Most past studies are based on U.S. context: their findings and their variables are developed in a western and developed society. With a significantly different cultural setting, their research implications must be used with care. As one example, major farms in developed economies are large scale operations and are highly mechanized. Most farms in developing economies are owned by smallholders and remain labor intensive. Such difference points to the need to gain local information on factors facilitating farm success and those forcing farmers to quit agriculture, as they apply in a more regionalized context. Moreover, different agricultural sectors involve specific operational and management skills. Their particular complexity and peculiarities demand special knowledge and skills. Taking dairy farming as an example, we have shown their specific requirements (breeding practices, livestock healthcare, milking system, and milking frequency), which are not applicable to crop cultivation. Therefore, sector-based investigation is also essential to identify such factors and tailor promotional efforts according to their importance within these individual sectoral contexts.

5 Conclusions

The economic sustainability of long-term profits is the key for farmers continuing their business enterprises. Failure to achieve economic sustainability has forced an increasing number of farmers and farm enterprises exit the industry.

This phenomenon can be understood by summarizing the findings offered by the literature. For this reason, it has been the objective of this paper to systematically review the determinants of farm profitability as found in the recent research.

In this systematic literature review, a vote count method has been used to synthesize evidence from 24 reviewed analyses. It has inventoried the number of statistically insignificant as well as statistically significant positive and negative results for each investigated variable. In total, 28 out of the many possible variables proved significant in influencing farm economic performance. The summarized variables were grouped into four categories according to their similarity and their description in the literature: farmer and farm household characteristics, farm and biophysical characteristics, farm management and financial characteristics, and exogenous factors.

Farmer and farm household characteristics are used as the proxy of management capacity. Among them, education level and farmer age were investigated by most studies. As the number of investigations increases, these factors appear to have a greater likelihood of producing a mixed relationship with farm net earnings. Unless some explanation can be found in factors such as regional of cultural differences, such contradictory findings are counter intuitive and an explanation must be sought in the presumptions made or the methodology used. We have offered a tentative theory that such apparently contradictory results might be achieved if researchers are assuming that the relationship is linear, where, in fact, it is most likely to resemble a bell curve. Similar contradictions were also revealed in the research in other reviewed areas (e.g., the adoption of conservation tillage by Knowler and Bradshaw (2007)). This again indicates the need for continuing a more thorough research to provide some logic to the contradictory findings. Notwithstanding this, considering the current available studies, we can conclude that education level and farmer age together with other management proxies (farming experience and household size) skewed towards insignificance; financial-related variables (assets and off-farm employment) proved significant; and efficiency-based sole proprietorship variable proved significant. These significant factors suggest the importance of capital for expanding production capacity and efficiency for optimizing operation in springing farm profits.

Farm and biophysical characteristics denote the asymmetric distribution of resources across farms. Most of them did not produce a consistent result. A single exception was livestock holdings or ratio, which represents the operational scale of livestock farms. Its convergence towards positivity highlights the contribution of cost efficiency and capital enlargement to farm economic performance.

Farm management and financial characteristics describe how effectively a farm business is run. In other words, business growth is dependent on its management. Therefore, this category was seen as the most important explanatory dimension (based on its underlying number of variables and their frequency of investigation in the literature). The impact of this factor on farm profits was relatively more consistent than in any of the preceding categories. Input costs, debts, expenses on technology investment, and marginalized effort for cultivating core crops reduced the net earnings significantly. Increased output prices, financial recording and analysis, operational analysis, technology, and attention on core crops increased farm economic performance significantly. Among these positive factors, it is axiomatic that higher output prices always result in greater profitability and that operational analysis facilitate a stellar improvement in operation efficiency. These findings imply that not only produce prices matter, understanding the business is also critical if farmers want to outperform their competitors.

It is obvious that sustaining farming and rural business is not an easy task. Farm profitability is even influenced by many complex factors. Nevertheless, our synthesis concludes that farm profit is mostly determined by operational scale, operational efficiency, and output prices. Earnings can be improved by reducing cost (through economies of scale and input-output improvement) and increasing revenue (through production capacity expansion and better crop prices). Given by this empirical knowledge, these intertwined features should be included in any design or promotion of sustainable agricultural systems and agricultural technologies. For better local management, more research is needed to establish a knowledge base reflecting the particular conditions relevant to individual locales and sectors.

References

Barr N, Karunaratne K, Wilkinson R (2005) Australia's farmers: past, present and future. Land & Water Australia, Melbourne

Carpenter J, Gianessi L (1999) Herbicide tolerant soybeans: why growers are adopting Roundup Ready varieties. AgBioforum 2(2):65–72

Carpenter S, Walker B, Anderies JM, Abel N (2001) From metaphor to measurement: resilience of what to what? Ecosystems 4(8):765–781. doi:10.1007/s10021-001-0045-9

Dartt BA, Lloyd JW, Radke BR, Black JR, Kaneene JB (1999) A comparison of profitability and economic efficiencies between management-intensive grazing and conventionally managed dairies in Michigan. J Dairy Sci 82(11):2412–2420. doi:10.3168/jds.S0022-0302(99)75492-5

Dohoo IR, Descôteaux L, Leslie K, Fredeen A, Shewfelt W, Preston A, Dowling P (2003a) A meta-analysis review of the effects of recombinant bovine somatotropin: 2. Effects on animal health, reproductive performance, and culling. Can J Vet Res 67(4):252–264

Dohoo IR, Leslie K, Descôteaux L, Fredeen A, Dowling P, Preston A, Shewfelt W (2003b) A meta-analysis review of the effects of recombinant bovine somatotropin: 1. Methodology and effects on production. Can J Vet Res 67(4):241–251

El-Osta HS, Johnson JD (1998) Determinants of financial performance of commercial dairy farms, Technical bulletin. U.S. Department of Agriculture, Washington, DC

FAO (2002) Foreword. In: Bruinsma J (ed) World agriculture: towards 2015/2030. An FAO perspective. Earthscan, London, pp iii–iv

Fernandez-Cornejo J, Hendricks C, Mishra A (2005) Technology adoption and off-farm household income: the case of herbicide-tolerant soybeans. J Agric Appl Econ 37(3):549–563

Foltz J, Chang HH (2002) The adoption and profitability of rbST on Connecticut dairy farms. Am J Agric Econ 84(4):1021–1032. doi:10.1111/1467-8276.00364

Foltz J, Lang G (2003) The adoption and impact of management intensive rotational grazing (MIRG) on Connecticut dairy farms. Renew Agric Food Syst 20(4):261–266. doi:10.1079/RAF2005127

Ford SA, Shonkwiler JS (1994) The effect of managerial ability on farm financial success. Agric Resour Econ Rev 23(2):150–157. doi:10.1108/00021460910978661

Fox G, Bergen PA, Dickson E (1993) Why are some farms more successful than others? A review. In: Hallam A (ed) Size, structure, and the changing face of American agriculture. Westview Press, Boulder, pp 232–250

Gillespie J, Nehring R, Hallahan C, Sandretto C, Tauer L (2010) Adoption of recombinant bovine somatotropin and farm profitability: does farm size matter? AgBioforum 13(3):251–262

Gloy BA, LaDue EL (2003) Financial management practices and farm profitability. Agric Financ Rev 63(2):157–174. doi:10.1108/00215060380001147

Gloy BA, Hyde J, LaDue EL (2002a) Dairy farm management and long-term farm financial performance. Agric Resour Econ Rev 31(2):233–247

Gloy BA, Tauer LW, Knoblauch W (2002b) Profitability of grazing versus mechanical forage harvesting on New York dairy farms. J Dairy Sci 85(9):2215–2222. doi:10.3168/jds.S0022-0302(02)74301-4

Haden KL, Johnson LA (1989) Factors which contribute to the financial performance of selected Tennessee dairies. South J Agric Econ 21(1):105–112

Jackson-Smith D, Trechter D, Splett N (2004) The contribution of financial management training and knowledge to dairy farm financial performance. Rev Agric Econ 26(1):132–147. doi:10.1111/j.1467-9353.2003.00166.x

Knowler D, Bradshaw B (2007) Farmers' adoption of conservation agriculture: a review and synthesis of recent research. Food Policy 32(1):25–48. doi:10.1016/j.foodpol.2006.01.003

Lee DR (2005) Agricultural sustainability and technology adoption: issues and policies for developing countries. Am J Agric Econ 87(5):1325–1334. doi:10.1111/j.1467-8276.2005.00826.x

Lins DA, Ellinger PA, Lattz DH (1987) Measurement of financial stress in agriculture. Agric Financ Rev 47:53–61

McBride WD, El-Osta HS (2002) Impacts of the adoption of genetically engineered crops on farm financial performance. J Agric Appl Econ 34(1):175–192

McBride WD, Short S, El-Osta H (2004) The adoption and impact of bovine somatotropin on US dairy farms. Rev Agric Econ 26(4):472–488. doi:10.1111/j.1467-9353.2004.00194.x

McConnell DJ, Dillon JL (1997) Farm management and farm types. In: Farm management for Asia: a systems approach, FAO farm systems management series, 13. Food and Agriculture Organization of the United States, Rome

Ministry of Finance (1995) Economic report 1994/95. Ministry of Finance, Kuala Lumpur

Ministry of Finance (2013) Economic report 2012/13. Ministry of Finance, Putrajaya

Mishra AK, El-Osta HS, Johnson JD (1999a) Factors contributing to earnings success of cash grain farms. J Agric Appl Econ 31:627–637

Mishra AK, El-Osta HS, Steele CJ (1999b) Factors affecting the profitability of limited resource and other small farms. Agric Financ Rev 59:123–140

Mishra A, Wilson C, Williams R (2009) Factors affecting financial performance of new and beginning farmers. Agric Financ Rev 69(2):160–179. doi:10.1108/00021460910978661

Mishra AK, Fannin JM, Joo H (2014) Off-farm work, intensity of government payments, and farm exits: evidence from a national survey in the United States. Can J Agric Econ 62:283–306. doi:10.1111/cjag.12027

Pannell DJ, Marshall GR, Barr N, Curtis A, Vanclay F, Wilkinson R (2006) Understanding and promoting adoption of conservation practices by rural landholders. Aust J Exp Agric 46(11):1407–1424. doi:10.1071/EA05037

Prokopy LS, Floress K, Klotthor-Weinkauf D, Baumgart-Getz A (2008) Determinants of agricultural best management practice adoption: evidence from the literature. J Soil Water Conserv 63(3):300–311. doi:10.2489/jswc.63.5.300

Rumley R, Muthuri C, Ong C (2007) More trees with less water. The role of deciduous trees. Policy Brief. World Water Week, Stockholm

Sevenacres.wordpress.com (2014) Small scale farming and a simpler life. Sevenacres. http://sevenacres.wordpress.com/

Stefanides Z, Tauer LW (1999) The empirical impact of bovine somatotropin on a group of New York dairy farms. Am J Agric Econ 81(1):95–102

Tey YS, Brindal M (2012) Factors influencing the adoption of precision agricultural technologies: a review for policy implications. Precis Agric 13(6):713–730. doi:10.1007/s11119-012-9273-6

Tey YS, Brindal M (2014) Adapting importation policy to global commodity markets: implications of rice import allocation in Singapore. Mitig Adapt Strateg Glob Chang. doi:10.1007/s11027-013-9474-y

Tey YS, Li E, Bruwer J, Amin Mahir A, Cummins J, Alias R, Mohd Mansor I, Suryani D (2012) Refining the definition of sustainable agriculture: an inclusive perspective from the Malaysian vegetable sector. Maejo Int J Sci Technol 6(3):379–396

Tey YS, Li E, Bruwer J, Amin Mahir A, Brindal M, Alias R, Mohd Mansor I, Suryani D (2014a) Factors influencing the adoption of sustainable agricultural practices in developing countries: a review. Environ Eng Manag J (in Press)

Tey YS, Li E, Bruwer J, Amin Mahir A, Brindal M, Alias R, Mohd Mansor I, Suryani D (2014b) The relative importance of factors influencing the adoption of sustainable agricultural practices: a factor approach for Malaysian vegetable farmers. Sustain Sci 9(1):17–29. doi:10.1007/s11625-013-0219-3

UNCED (United Nation Conference on Environment and Development) (1992) Agenda 21 – an action plan for the next century. The United Nations, New York

USDA (U. S. Department of Agriculture) (2002) Published estimates data base, U.S. and state-level data for dairy. USDA/NASS. http://www.nass.usda.gov:81/ipedb/

Winsten JR, Parsons RL, Hanson GD (2000) A profitability analysis of dairy feeding systems in the Northeast. Agric Resour Econ Rev 29(2):220–228

WSSD (World Summit on Sustainable Development) (2002) Report of the World Summit on Sustainable Development. The United Nations, Johannesburg

Soil Fertility and Crop Productivity in African Sustainable Agriculture

Alfred Oghode Misaiti Okorogbona and Patrick Olusanmi Adebola

Abstract Agriculture currently feeds over six billion people as compared to an estimated population of four million people fed during the period of hunting and gathering practiced by man in the primeval days. While agriculture is considered to have the capability of taking care of the food needs of 8–10 billion people, concern is raised on how this thoughtful ideology could continually be achieved, using measures inclined with sustainable approach. Sub-Saharan Africa (SSA) remains the poorest when compared to other regions of the world, as the average real per capita income in 2010 was \$688, in constant 2000 US\$, compared to \$1717 in the rest of the developing world. While agricultural productivity continues to improve in industrialised nations, the reverse is the case in Africa. In 1983, 1990 and 1993, Africa had a declining grain production and food legume yields from 87 % ha^{-1}, during 1979–1981 to 71 % for 1991–1993, compared to a change of 78–74 % in South America and 151–308 % in Europe. African farmer obtains over 2.47 t of cereal per hectare as compared to the 5 t; 12.5 t; and 17.5 t cereal per hectare obtained by the Indian, Chinese and American farmers, respectively. There is a need for the agrarian community to have more information on soil fertility and crop productivity.

This review reveals that soil texture, soil structure and soil constituents include the critical factors for crop productivity. N and organic C are higher in clayey soils than in loam and sandy soils. Best management of irrigated soils includes increasing organic content by incorporating pasture phases into cropping rotations; reducing tillage or avoiding the tilling of the soil; covering the soil to avoid raindrop impact; and the addition of gypsum for displacement of sodium cations. Soil salinity remain the most serious problem of soils in many parts of Africa. Plant species reach an optimum growth at salinities of 5–25 % of standard salt water level. Tree planting, use of halophytic plants and the application of plant physiology, genetics and biochemical approaches involving the modification and breeding of salt stress tolerant varieties or cultivars are means of managing soil salinity problems. Biological process of nitrogen (N_2) fixation, precision nutrient management and the use of organic

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fertilisers are effective tools for maintaining sustainable yields. It was suggested that chemical fertilisers could be safely applied to soils used for leafy vegetable growth, in the neighbourhood of 20 kg N ha^{-1}, 30 kg P ha^{-1}, 40 kg K ha^{-1} in form of (2:3:4) fertiliser in planting furrow. Depending on the type of crop, composted, dried and pulverised chicken manure may be applied from 12 to 24 t ha^{-1} for the growth of leafy vegetables in soils with low nutrient availability. Also, soils of low biological activities. Cattle manure can be applied to soils between 180 and 210 t ha^{-1} while goat manure can be applied from 60 to 90 t ha^{-1}. Farmers should always give consideration to the sources from which manures are obtained as storage conditions of these organic materials affect the quality.

Keywords Soil • Soil texture • Soil structure • Nitrogen fixation • Chemical fertiliser • Animal manure

1 Introduction

The provision of food to meet up with the increasing human population throughout the world requires adequate preservation and upgrading of agricultural land, as this is vital for both crop cultivation and animal husbandry. There is a general consensus that food sustainability for future generations could only be guaranteed if crucial research strategies are earmarked for the maintenance and improvement of soil and environmental qualities, considering the enhancement of agricultural productivity. This ideology was supported and fine-tuned by Foster and Lufafa (2002) who opined that future agricultural productivity gains in terms of food security should not be achieved at the expense of environmental conservation. Worldwide, there is continuous campaign on the improvement of agricultural productivity in recent times, as economists, scientists and agriculturists have realised that this phenomenon alleviates poverty in poor and developing countries, where agriculture often employs the greatest portion of the population and in essence tremendously lead to the growth of these countries' economies. Increase in agricultural productivity leads to agricultural growth which in turn contributes immensely to the economic growth of most agrarian societies, especially in countries with low level of industrialisation. Throughout the globe, agricultural productivity is often inclined with sustainability and sustainable development.

As explained by Fulginiti and Perrin (1998) together with Liverpool-Tasie et al. (2011), agricultural productivity is the agricultural output produced by a given level of agricultural input(s) in the agricultural sector of a particular economy. A concise definition of sustainable agriculture given by Tilman et al. (2002) described the concept as practices that meet current and future societal needs for food and fibre, for ecosystem services, and for healthy lives, which do so by maximising the net benefit to society when all costs and benefits of the practices are considered. Ukpore (2009) defined sustainable development as the mode of human development in which the use of resources are aimed at meeting human needs while preserving the environment so that these needs can be met not only in the present, but also for future generations. The concept of sustainable development is often classified in three ways and these include environmental sustainability, economic sustainability and sociopolitical sustainability. For the purpose of this chapter, emphasis will only be laid on environmental sustainability.

The hunter–gatherer lifestyle supported about four million people globally before the dawn of agriculture but today over six billion people are fed from modern Agriculture (Cohen 1995; Tilman et al. 2002). Scientists postulated that agriculture has the capability to meet the food needs of 8–10 billion people while substantially decreasing the proportion of the population who go hungry (Sen 1981; Plucknett 1993; Waggoner 1995; Kates 1996) but according to Tilman et al. (2002), there is little consensus on how this can be achieved by sustainable means. Sustainability implies both high yields that can be maintained, even in the face of major shocks, and agricultural practices that have acceptable environmental impacts (Conway 1997; Tilman et al. 2002). According to Tilman et al. (2002), the main environmental impacts of agriculture come from the conversion of natural ecosystems to agriculture; from agricultural nutrients that pollute aquatic and terrestrial habitats and groundwater. Also, from pesticides, especially bioaccumulating or persistent organic agricultural pollutants. The mining of soil with off take greater than input which results in nutrient imbalances also has negative impact on many agricultural lands (Stoorvogel and Smiling 1990). This situation has resulted to decline in the yield of crops over time. Humans are considered to be the largest factors causing this havoc due to unprofessional use of agricultural inputs on farmlands which results in a situation where loss of the land's nutrients/organic matters occur with a consequential loss of soil fertility (Katsunori 2003).

The supply of agricultural products and ecosystem services are both essential to human existence and quality of life. However, recent agricultural practices that have greatly increased global food supply have had inadvertent detrimental impacts on the environment and on ecosystem services, highlighting the need for more sustainable agricultural methods (Tilman et al. 2002). A few strategies that have been used by various scientists to address the issue of adverse effect of inefficient utilisation of nutrient sources on agricultural land need to be uncovered to the agricultural and scientific communities in order to avoid some draw-backs affecting agricultural productivity. In line with this, review on the positive and negative effects of nutrient sources on plant growth and soil environment is crucial to provide a guideline for crop production and soil fertility management to farmers and emerging researchers. This chapter aligns itself to this agricultural growth course.

According to Björklund et al. (1999), the primary goal of the twentieth century agriculture has been to meet the food demands of the rapidly increasing human population and the development has been highly successful in the industrialised countries with increase in agricultural productivity and the benefit of agricultural mechanisation providing manpower for other economic sectors such as industry and service at the same time. However, the reverse is the case in non- and low-industrialised nations, particular in African countries. Evidence of this was pointed out by Dakora and Keya (1997) in their review work. According to Dakora and Keya (1997), report provided by Food and Agriculture Organisation (FAO) on crop productivity in 1983, 1990 and 1993 indicated a declining grain production and food legume yields from 87 % ha^{-1}, during 1979–1981 to 71 % for 1991–1993, compared to a change of 78–74 % in South America and 151–308 % in Europe. Same depiction of stagnation appears in the structures of African economies as in almost all African countries (excluding South Africa), production is dominated by the primary sector in agriculture. In the primary sector, agriculture is marked by low productivity with little application of science and technology. Research work carried out on food production and consumption trends by Chauvin et al. (2012) pointed out sub-Saharan Africa (SSA) as the poorest region in the world, as the average real per capita income in 2010 was $688 (in constant 2000 US$) compared to $1717 in the rest of the developing world. According to Chauvin et al. (2012), over the past 30 years, GDP growth per capita in SSA has averaged 0.16 % per year and this failure of growth over the long term has resulted in high levels of poverty in the region. The low crop productivity in Africa as compared to other parts of the world highly reflects on cereal product. For example, report provided on agricultural development in Africa by Bill and Melinda Gates foundation (2011) indicated that an average farmer in sub-Saharan Africa obtains over 2.47 t of cereal per hectare, while the average Indian farmer harvests about 5 t of cereal per hectare, the average Chinese farmer obtains 12.5 t cereal per hectare and the average American farmer- about 17.5 t cereal per hectare. Similar trend was observed in the areas of the world relying on genetically modified (GM) crops including soyabean, cotton, maize and canola which all dominates the world agricultural market. For example, James (2009) reported that out of the 134 million hectares of agricultural land cultivated with GM crop globally in the year 2009, the United States of America had 64 million hectares, Brazil- 21.4 million hectares, Argentina (21.3), India (8.4), Canada (8.2), China (3.7), Paraguay (2.2), and South Africa (2.1 million hectares). A situation, that also indicates the low crop productivity level in Africa as compared to other parts of the world.

Generally, most farming systems in sub-Saharan Africa (excluding South Africa) are faced with low crop productivity. This low crop productivity is more pronounced in grain production and food legumes (Dakora and Keya 1997; Jonas et al. 2011). According to Dakora and Keya (1997), these are often associated with declining soil fertility and reduced N_2-fixation, which was pointed out to be due to biological and environmental factors by Jonas et al. (2011). As postulated by Dakora and Keya (1997), the factors responsible for the low crop productivity continue to remain obscured. It was also opined that soil constraints play a major role in limiting crop yields in Africa. Land use, crop selection and nutrient availability is limited by the fundamentally low fertility of African soils and their highly heterogeneous nature, which consists of oxisols, ultisols, alfisols, vertisols, aridisols and inceptisols, (Munns and Franco 1981; Dakora and Keya 1997). One of the crucial ways of overcoming this challenge is to thoroughly espouse efficient strategies of combining land use management technique with crop selection and plant nutrient management measures. Plates 1 and 2 provides indication of the aforementioned soil management techniques

Plate 1 A highly clayey (*reddish*) and nutrient deficient soil considered to be inappropriate for the cultivation of tuber crops due to its muddy and sticky nature when wet. This shows a poor tuber crop vegetative growth where weeds even competes with the plants for nutrients and water

Plate 2 Same clayey-nutrient deficient soil indicated in Plate 1 formerly observed to be unsuitable for tuber crop growth and only after undergoing intensive soil fertility management measure, then used for the cultivation of Lucerne, resulted in high yield of the fodder crop. This soil management technique has basically provided an indication that soil not suitable for the growth of a particle species of crop could be entirely appropriate for the growth of other species of crops

Plate 3 Sandy soil used for the cultivation of maize after incorporating high level of nitrogen, phosphorus and potassium fertilisers

where soils unsuitable for growing particular species of crops are managed and used for the cultivation of other crops for bumper crop harvest.

Similar occurrence was observed in the case of a sandy soil having high nutrient leaching effect but the factor ignored and the soil was supplied with high rate of nitrogen fertiliser, in addition to considerable level of superphosphate and potassium chloride, before being used for the cultivation of maize, which showed poor growth response despite the application of these nutrient sources. But cowpea thrived well when planted in same soil without the addition of nitrogen fertiliser. These developments are articulated in Plates 3 and 4.

As efforts to increase food production continues to be considered to take place within environments characterised by a scarcity of natural resources coupled with the fact that there is little room for expansion of arable land, with virtually no additional land available in many regions including South Asia, the Near East; North Africa inclusive, as indicated by FAO (2011). Emphasis will be laid on soil fertility in this chapter. Considering the low soil fertility condition associated with African soils, improving productivity in agriculture with emphasis on sustainable approach in recent times should be a realistic goal and this chapter intends to offer proven techniques from past research endeavours used in the past and even still in use, to improve crop productivity while conserving valuable resources needed for today and for the future.

Plate 4 Cowpea thriving tremendously without the incorporation of nitrogen fertiliser to same sandy soil indicated in Plate 3, due to the symbiotic association between some soil bacteria and the root noodles of this leguminous crop

Where land is available, in sub-Saharan Africa and Latin America, more than 70 % suffers from soil and terrain constraints (FAO 2011). Unsustainable land use practices, such as overuse, poor land management and nutrient mining, result in global net losses of land productivity of an average 0.2 % per year (Nellemann et al. 2009). The need to assemble research information encompassing soil nutrient resources, unavailability of these nutrient sources to certain group of agriculturists, scientists and farmers-particularly the marginal low input African farmers; the inefficient use of these soil nutrient sources; the low and excessive application of these nutrient materials and ways of increasing crop productivity becomes imperative and this chapter also tends to pursue the course.

The objective of this review is to bare past research works addressing the benefits; technicalities and shortcomings in the use of nutrient sources-to provide information for the facilitation of innovation—capacity building; farmers' empowerment; and for the improvement of efficiency in agricultural research and extension services. In essence, this chapter emphasises on a few phenomena that are neglected or not properly understood and overlooked. The chapter also includes reports on findings from recent research works.

In synopsis, it could be deduced from this section that modern agriculture feeds about six billion people worldwide as compared to the four million people fed by the hunting and gathering life-style practiced before the commencement of land tillage and animal husbandry for crop and animal production respectively. While agriculture is considered to have the capability to care for the food needs of 8–10 billion people, concern continues to be raised on how this can be achieved by sustainable means. However, recent agricultural practices that have greatly increased global food supply have had inadvertent detrimental impacts on the environment and on ecosystem services, highlighting the need for more sustainable agricultural methods. Sub-Saharan Africa (SSA) remains the poorest when compared to other regions in the world. While agricultural productivity improves in industrialised nations, the reverse is the case in Africa. Low crop productivity in Africa as compared to other parts of the world, highly reflects on cereal product. Agricultural research and production activities in the contemporary world of technological dynamism, involves high cost and efforts both from the view point of inputs and human resources. In line with this, the need to provide the agricultural community with beneficial information relating to agronomic management practices that could be considered in crop production activities, in order to avoid escapable losses becomes germane. Hence, it is essential for the agrarian community to have an insight on information relating soil fertility to crop productivity and this review work is focused towards such course.

2 Soil in Agriculture

Soil is a natural body consisting of layers primarily composed of minerals which differ from their parent materials in their texture, structure, consistency, colour, chemical, biological and other characteristics. An intricate explanation of the concept provided by Powlson et al. (2000) described the term 'soil' as an environment for seed germination, root growth; a reservoir for nutrients within organic matter and mineral components released into plant available forms at different rates; a pathway through which water and nutrients move to roots either from its reserve or from external inputs; a matrix where transformations of nutrients occur through biological, chemical and physical processes, with major implications for crop uptake and losses; an environment for beneficial, harmful or neutral microorganisms and fauna, which might have positive or negative impact to plant growth and development and a platform for machinery, humans or animals involved in agricultural operations.

2.1 Soil as a Plant Nutrient Pool

Soil is a heterogeneous material consisting of three major components: namely solid, liquid and gaseous phases which specifically influence the supply of nutrients to plant roots. The solid phase is the main nutrient reservoir. The inorganic particle of the solid phase contain nutrients such as potassium (K), sodium (Na), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) whilst the organic particle form the main reserve of nitrogen (N) and to a lesser extent, also of phosphorus (P) and sulphur (S). The liquid phase of the soil (soil solution) facilitates nutrient transport in the soil. For example, for the transport of nutrients from various parts of the bulk soil to plant roots. Nutrient transported in the liquid phase are mainly present in ionic form but oxygen (O_2) and carbon dioxide (CO_2) are also dissolved in the soil and mediates the gaseous exchange which occurs between the numerous living organisms of the soil (plant roots, bacteria, fungi, animal) together with the atmosphere. Mineral nutrient behaviour in the soil is closely dependent on nutrient interactions between the solid, liquid and gaseous phases (Bassiri 2005).

2.2 Soil as a Milieu for Crop Productivity

It is important to understand the soil environment in which plants grow, to recognise the limitations of that environment and to ameliorate where possible without damaging the soil quality for efficient crop production (FAO 1999). Soil is considered to be one of the most important natural resources for crop production. According to Friend (1992), estimate of the rate of soil formation indicated that about 2.5 cm of soil is formed in every 150 years, which shows that soil is non-renewable within the human-life-span. Henceforth, it is in the interests of the farmer, and the population as a whole, to ensure that good soil management is practiced so that this resource is preserved for continued use by the current and future generations (FAO 1999).

As a result of the growing interest of agriculturists in determining the consequences of management practices on the quality of soil relative to sustainability of crop ecosystem functions in addition to plant productivity, the need to assess soil properties is expanding in recent times. Farmers have always relied on a knowledge of chemical and physical properties of soils to assess the capacity of agricultural land in a particular place in order to support crop productivity (Schoenholtz et al. 2000). Improving crop productivity by farmers requires knowledge of soil characteristics, quality and productivity. These include soil texture, structure and constituents which all determine the quality and productivity of soil.

This section pointed out soil as an environment for plant nutrient and plant roots growth. The role soil plays to plants and its relation to sources of energy such as air, water and sunlight has made soil as a concept in agriculture to be described as one of the most important natural resources for crop production. This review revealed that soil texture, soil structure and soil constituents are among the critical factors that need to be considered in order to improve crop productivity.

2.3 Soil Texture and Crop Growth

Soil texture is the most influential characteristic used in determining the behaviour of any given soil. Soil texture refers to the relative proportion of particles or it is the relative percentage by weight of the three major classes of soil separates, which are

Table 1 Soil textural class, nitrogen and organic carbon content (From Devkota and Jha 2009)

Textural class	Percentage (%) of sand	Soil N (%)	Organic carbon (%)
Clay	0	0.250	4.500
Silt	20	0.220	4.200
Loam soil	40	0.190	3.800
Sandy loam	60	0.120	3.580
Sandy soil	80	0.090	1.310
Sand	100	0.008	0.025

[a]Highest N and OC occurred in clay and the lowest levels of these two constituents are observed in the textural class-sand. The nitrogen and organic carbon percentages in loam and sandy loam soils appeared to be intermediate, although their levels seemed to be more in loam soil than in sandy loam soil

sand, silt and clay or simply refers to the size of soil particles. It is a key variable in the coupled relationship between climate, soil and vegetation (Fernandez-Illescas et al. 2001). Nutrients availability varies with different textural classes of soil. For example, analysis carried out on different classes of soil by Devkota and Jha (2009) indicated that nitrogen (N) and organic carbon (OC) availability in different soil textures decreases with increasing level of the percentage of sand available in the soil content. Evidence of this is shown in Table 1, which indicates that among the six textural classes of soil that featured in the soil N and OC analyses, clay soil with 0 % soil had the highest percentages of N and OC which were 0.25 and 4.5 % respectively while the textural class with 100 % sand had the lowest percentages of N and OC, which were 0.08 and 0.25 % respectively.

Soil texture also influences the growth and yield of crops. In a greenhouse experiment carried out to determine variation in the growth of a medicinal plant (*Centella asiatica*), Devkota and Jha (2009) recorded the highest number of leaves and rosette diameter from the plant grown with sandy loam soil. They indicated the textural class (sandy loam) to probably be the most fertile among all. Hence, suggested that the amount of plant-available N is lower in clay-rich soils, despite the high organic C and total N in the textural class and low fertility level of sand due to less amount of total N. Consequently, it was recommended that medicinal plants of similar agronomic characteristics can maximise growth and yield in habitats with sandy loam than clayey soil (Table 2).

Soils with different textural characteristics also exhibits different productivity levels when nutrients are incorporated in the plant growth material for root absorption. In a greenhouse experiment carried out to determine the effect of different textural classes of soil on the yield of tomato, using five levels of N application rate in the form of 0, 30, 60, 90, 120 kg N ha^{-1}, Oyinlola and Jinadu (2012) reported the highest plant height, fruit weight, fruit yield and dry matter yield at 90 kg N ha^{-1} in the treatment with loam soil at 12 weeks, after planting. In line with this, it was suggested that loam soil could be more suitable for the growth of fruit vegetables such as tomato, despite the fact that analysis carried out on the nutrient content of the three different textural classes indicated that clay soil contain more nutrients than sand and loam as shown in the concentrations of total N and available phosphorus in Table 3.

Table 2 Growth traits and yield of *Centella asiatica* in different textural soil group (From Devkota and Jha 2009)

Textural class	Percentage (%) of sand	No of leaves	Rosette diameter (cm)
Clay	0	3.66	12.4
Silt	20	4.31	15.3
Loam soil	40	3.25	18.2
Sandy loam	60	5.29	19.5
Sandy soil	80	4.65	13.3
Sand	100	3.65	10.5

[a]Highest number of leaves and widest rosette diameter were obtained from *Centella asiatica* grown in sandy loam soil. Crop grown in sandy soil produced higher number of leaves than same medicinal crop grown in loam soil but had lower rosette diameter

Table 3 Soil properties and crop height at different application rates of nitrogen fertiliser on different textural classes of soil (From Oyinlola and Jinadu 2012)

Soil type	Total nitrogen (g kg^{-1})	Available phosphorus (mg kg^{-1})	Organic carbon (g kg^{-1})	Application rate of N (kg ha^{-1})				
				0	30	60	90	120
Sand	0.14	3.24	2.20	16.9 cm	24.1 cm	30.2 cm	30.5 cm	31.0 cm
Loam	0.42	4.50	7.20	18.2 cm	33.7 cm	34.6 cm	41.3 cm	34.2 cm
Clay	0.56	5.40	7.90	17.6 cm	32.6 cm	33.4 cm	33.6 cm	31.4 cm

Tallest tomato plants with mean height of 41.3 cm was reported in crop grown with loam soil which was followed by the height of tomato grown in clay while the crop grown in sandy soil had the shortest height at the application rate of 90 kg N ha^{-1} by the period of 12 weeks

2.4 Soil Structure

Soil structure has seconded soil texture in terms of the important characteristics used in determining the behaviour of a given soil. Soil structure refers to the grouping or arrangement of major soil particles which include sand, silt, clay and organic matter into lesser particles referred to as aggregate which may also be called peds. Lal (1991) defined soil structure as the size, shape and arrangement of solids and voids, continuity of pores and voids, their capacity to retain and transmit fluids and organic and inorganic substances, and ability to support vigorous root growth and development. According to Bronick and Lal (2005), soil structure is a key factor in the functioning of soil due to its ability to support plant and animal life, and moderate environmental quality with particular emphasis on soil carbon (C) sequestration and water quality. Bronick and Lal (2005) postulated that favourable soil structure and high aggregate stability are important in improving soil fertility, increasing agronomic productivity, enhancing porosity and decreasing erodibility. The aggregates are normally described in terms of the shape, size and grades of soil. Soils having similar characteristics such as texture, depth or similar vegetable and climate but different structure will react differently under similar conditions. Soil structure has

Plate 5 Potato plants showing remarkable growth performance in a highly nutrient retention capacity clay loam soil

a strong impact on a range of processes influencing crop yield (Dickson et al. 1990; Dinel et al. 1992; Alami et al. 2000) as it is considered to influence soil water movement and retention, erosion, crusting, nutrient recycling, root penetration (Bronick and Lal 2005). Evidence of this is shown in Plate 3 indicating a condition where the growth of maize planted in a sandy soil with high porosity level (low nutrient retaining capacity) was retarded despite applying nitrogen, phosphorus and potassium fertilisers at almost double the application rates of 175 kg N ha^{-1}; 73 kg P ha^{-1}; 17 kg K ha^{-1} generally recommended for South African soils by Fertiliser Society of South Africa (2004). Also, in Plate 5, where the growth of potato was considered to be significant when cultivated in a clay loam soil with high nutrient holding capacity.

Processes including runoff, surface- and ground-water pollution and CO_2 emissions are also influenced by soil structure.

The structure of the soil in terms of hardness or softness affects both the shoot and root system of plants. The hardness of soil affects root growth which in turn influence the growth and development of the shoot system as this condition hinders plant root system to supply the shoot with water or nutrients (Boone 1986; Brereton et al. 1986; Masle and Passioura 1987; Andrade et al. 1993; Wolfe et al. 1995; Mulholland et al. 1996; Stirzaker et al. 1996; Masle 1998; Passioura 2002). The hardness is usually expressed in terms of penetrometer resistance–the pressure required to push into the soil a cylindrical probe with a conical tip (Passioura 2002). According to Passioura (2002), report provided by Bengough and Mullins and Materechera et al., indicated that root growth slows markedly once the resistance exceeds about 1 MPa and falls away, roughly linearly, to almost nothing at a resistance of about 5 MPa. This hardness is strongly affected by soil compaction.

In an experiment conducted to determine plant response to a range of soil densities (1.12, 1.45, 1.64 and 1.78 Mg m^{-3}) under wet condition and 0.96, 1.32, 1.50, and 1.79 Mg m^{-3} for alternating wet/dry conditions; the effect of artificial and naturally formed biopores, using barley as test crop, Stirzaker et al. (1996) reported that the crop had its highest growth at 1.45 and 1.32 Mg m^{-3} for wet and wet/dry conditions respectively. Consequently, Stirzaker et al. (1996) pointed out that barley developed best at an intermediate bulk density which represented a compromise between soil that was soft enough to allow good root development but sufficiently compact to give good root contact.

However, when considering the use of a given soil for crop production in a particular environment, practices that preserve and improve soil structure becomes necessary. A few agronomic practices that have been undertaken by soil scientists include the increase of soil organic content which is carried out by incorporating pasture phases into cropping rotations; reducing tillage or avoiding the tilling of the soil or the practice of zero tillage, most especially during periods of excessive dryness or wetness when soils have the tendency to shatter or smear and ensuring sufficient ground cover to protect the soil from raindrop impact. *The addition of calcium sulphate, which is also referred to as gypsum for displacement of sodium cations, calcium and the reduction of exchangeable sodium percentage or sodicity were also pointed out as very useful soil structure preservative measures used in irrigated farming schemes*. In a review work on soil management relating to sustainable agriculture and ecosystem services, Powlson et al. (2000) reported useful strategies which emanated from their work together with other emerging researches and these were structured as indicated in Table 4.

Several studies have shown that soil structure has impact on germination, root growth and development which consequently influence crop yield, as the formation of stable aggregates considered as the basic unit of soil structure strongly depends on both the nature and the content of organic matter (Chaney and Swift 1986; Chenu 1993; Cheshire 1979; Elustondo et al. 1990; Reid and Goss 1981; Alami et al. 2000). The soil aggregation process, organic matter inputs and the dynamics of the sand-sized macro-organic matter are among the important factors involved in the maintenance and regulation of organic matter functioning in soil (Carter 2002). According to Carter (2002), organic matter fractions (e.g., macro-organic matter, light fraction, microbial biomass), and mineralisable carbon describes the *quality* of soil organic matter. Soil organic matter influences soil compactibility, friability, and soil water-holding capacity while aggregated soil organic matter has major implications for the functioning of soil in regulating air and water infiltration, conserving nutrients. Also, influencing soil permeability and erodibility. All these aforementioned factors determine the fertility level of a given soil.

Table 4 Agronomic practices and policies promulgated to preserve soil structure (From Powlson et al. 2000)

Factor	Practice required	Polices to achieve desired practice	Observations
Organic matter, climate change, physical properties and water	Encourage practices to maintain or increase SOC content in agricultural soils including return of crop residues, animal manures, other organic residues (if commensurate with human and animal health considerations)	Providing information to farmers	Increasing soil organic carbon content (depending on how this is achieved) can contribute to C sequestration, thus justifying climate change. Recognise conflict with provision of extra land for food production
Increasing soil organic matter content is beneficial for almost all soil properties and functions. Even small changes in soil organic carbon (SOC) can have terribly large impacts on soil physical properties		This could be in the form of monetary incentives or regulations to promote specific practices	
	Promote collection of organic 'wastes' for re-use especially in peri-urban areas		
Tillage practices	Encourage minimum tillage where appropriate	Providing information to farmers and rendering possible financial assistance to small scale farmers to acquire new farm implements	Using available information to evaluate the applicability of reduced tillage–not always appropriate. Reduced tillage often claimed to bring climate change benefits through soil carbon sequestration. It is important to be conscious that these claims may be exaggerated and evaluate balance of soil C increase with possible increased N_2O emissions using the available relevant information
Reducing tillage practices and or practicing zero tillage can contribute to improved soil structure and functioning in several soil types and cropping systems. The advantages include improved water infiltration and decreased erosion through increased soil carbon near surface and improved animal or faunal activity			

This section reviewed that soils with similar characteristics including texture, depth or similar vegetation and climate but different structure react differently under similar conditions. Soil structure has a strong impact on the processes influencing crop yield, as it influences soil water movement and retention, erosion, crusting, nutrient recycling, root penetration. Processes including runoff, surface- and ground-water pollution and CO_2 emissions are also influenced by soil structure.

3 Soil Fertility in Africa

Crop production in Africa particularly around the sub-Saharan regions of the continent is impinged on by an increasing soil productivity crisis. In a review of literature, Akinrinde (2006) indicated that tropical and sub-tropical soils could guarantee sustainable production of important arable/field and permanent/tree crops such as maize-*Zea mays*; cowpea-*Vigna unguiculata*; soyabean-*Glycine max*; cassava-Manihot *species*; yams-*Dioscorea species*; cocoa-*Theobroma cacao*; oil palm-Elaeis *guineenensis*; rubber-*Hevea brasilensis* to mention a few, without external input of nutrients, before the advent of shifting cultivation system of farming. It is so unfortunate that this postulation continue to hang in the dark as the diverse nature of African soils limits the use of land for crop production purpose. As pointed out by Akinrinde (2006), this could be associated with the widespread nutrient deficiency, which limits crop uptake, growth and yield due to fixation of nutrients within soil environments in recent times, as most of the plant essential nutrients, especially phosphorus (P) and potassium (K) occur in complex forms in the soil and substantial proportions may be transformed into fixed states, making them relatively unavailable for plants absorption. According to Dakora and Keya (1997), nutrient deficiencies involving phosphorus, potassium, sulphur, molybdenum and zinc are quite common and in some of the highly weathered soils, such as oxisols. The major soil constraints include Al and Mn toxicity, low pH and unpredictable response to liming. Similar opinion was pointed out by Akinrinde (2006) who postulated that most of the soils in sub-Saharan Africa are acidic ($pH < 5.5$) in nature; having high aluminium (Al), iron (Fe) and manganese (Mn) ions level and or levels which readily fix nutrient elements in soils. As indicated by Dakora and Keya (1997), many savannah soils in Africa are also low in nitrogen, phosphorus and organic matter. According to Dakora and Keya (1997), in a report provided on the studies carried out 50 years ago, Nye and Greenland indicated that the savanna soils of West and East Africa contained amounts of N ranging from a low 0.02 to 0.07 %, and forest soils from 0.17 to 0.30 %. The report also indicated a low level of organic matter content in African savannah soils which ranged from 0.23 to 1.36 % carbon as compared to that of forest soils which had its organic matter with its carbon ranging from 1.80 to 2.90 %. As a result of the continued cultivation of arable farmlands, time after time in Africa, Dakora and Keya (1997) opined that the content of soil nitrogen and organic matter will remain on a declining trend, which is liable to the low crop yields obtained by farmers in recent times. Apart from the nutrient deficiency problems in African soils, which are considered to be due to intensive and exploitative use of agricultural lands, other factors that hampers crop productivity in the sub-Saharan Africa regions include acidity and salinity. According to Conyers (1986), all environments with high level of precipitation ranging from 1,500 mm and above are predisposed to soil acidity problems as appreciable amount of exchangeable bases are often leached from the soils in these environments. In a study carried out to determine the response of okra (*Abelmoschus esculentus*) to lime and phosphorus fertilisation in an acidic soil, Oluwatoyinbo et al. (2005) indicated that acid soils cover about 17 million hectares of land (representing about 18 % of total land area) in Nigeria alone. Sanchez (1997) reported that Ultisols and Oxisols, particularly have problems associated with Al toxicity, low nutrient status, nutrient imbalance and multiple nutrient deficiencies. This has similarity with the report provided by Dakora and Keya (1997) who postulated that in some of the highly weathered soils which include oxisols, the major soil constraints include Al and Mn toxicity, low pH coupled with unpredictable response to the addition of lime (Calcium carbonate-$CaCO_3$), which is normally incorporated to acidic soils to balance the alkalinity level with its counterpart. Part of which had been indicated earlier on.

While factors such as nutrient deficiencies and soil acidity continue to hinder increased crop productivity, soil salinity stress was reported by Ali et al. (2007) to be one of the world's oldest and the most serious environmental problems, which substantially continue to hinder crop productivity in arid and semi arid regions of many parts of Africa. According to Szabolcs (1994), salinity affects about 95 million hectares of land worldwide. As pointed out by Abari et al. (2011), salt stress adversely affects plant growth and productivity during all developmental stages.

High salinity decreases substrate water potential and thus restricts water and nutrient uptake by the roots. High salinity may also cause ionic imbalance and toxicity in plants (Larcher 1995; Lambers et al. 1998; Houle et al. 2001). Neumann (1995) reported that saline soils contain highly soluble salts that suppress plant growth through a series of interacting factors such as osmotic potential effect, ion toxicity and antagonism, which induce nutrient imbalances in soils. As postulated by Saboora et al. (2006), a dangerous trend of 10 % yearly increase in soil salinity level is possible throughout the globe. Problems imposed by salinity was reported not to only directly affect crop productivity but also indirectly affects the availability of animal feeds in arid and semi-arid regions of the world. This phenomenon imposes serious environmental peril that affects grassland cover which grazing animals depends on (El-Kharbotly et al. 2003).

Several plant species reach an optimum growth at salinities of 5–25 % of standard salt water level (Súarez and Medina 2005) and the range of salinity which plants survive varies according to the species (Ball 1988; Súarez and Medina 2005). According to Súarez and Medina (2005), growth may be affected by the excessiveness of NaCl available in nutrient sources considering the growth medium used for several plant species. Ball (1988); Ball and Pidsley (1995) pointed out that the leaf yield of plants decreases as salinity level in soil increases. Evidence of salt stress is shown in Plate 6, where manure from goat kraal applied to low fertility subsoil

Plate 6 Leafy Amaranth exhibiting reduced growth at application rates above critical level due to salt stress

indicated a declining trend in the growth of *Amaranthus cruentus* L. from 90 t ha^{-1} as a result of accumulation of salt.

Schuman and McCalla (1976) reported that the large soluble salts contained in nutrient sources interfere with seed germination, plant growth, water uptake and water infiltration when applied to the soil. As pointed out by Greenway and Munns (1980); Munns and Termaat (1986), accelerated plant leaf mortality rate is accompanied by a marked decrease in the leaf production rate from a plant, consequently resulting to the death of such plant.

Research findings reported by scientists from studies on the effect of soil salinity on crop productivity have resulted to the emergence of strategies or practices that could be used to manage salinity problems. For example, Aslam (2006) pointed out tree planting as one of the cheapest and most simple natural biological approach of controlling soil salinity. The utilisation of halophytic plants in the production of pasture and fodder crops was pointed out by Yeo and Flowers (1980). In addition, as a solution that is economical and readily available for the prevention and control of soil salinity problem. The application of conventional plant physiology, genetics and biochemical approaches involving the modification and breeding of salt stress tolerant varieties or cultivars for cultivation in saline soils was also suggested as a means of managing salinity problem (Parvaiz and Satyawati 2008). Considering these aforementioned ways of managing soil salinity problem, the need to carry out analysis on the properties of a given soil and taking into account the salt stress tolerance level of any crop or crops to be cultivated becomes relevant in order to obtain substantial yield. In line with this, Marcal et al. (1999) provided information (modified from the research carried out by Marcal and Khanna) on the properties of saline, sodic and waterlogged soils relevant to plant survival and growth for use as guide to avoid problems that hinders high crop productivity level due to salinity stress (Table 5).

In addition to the foregoing, other agronomic practices for soil conservation revealed by researchers include the cultivation of plants capable of replenishing the soil environment with nutrients, particularly nitrogen, as a result of forming symbiotic association with nitrogen fixing bacteria in their root nodules; the appropriate incorporation of organic matter to the soil through the addition of organic nutrient sources and the adequate use of inorganic nutrient sources particularly when the time lag required for compost to be processed could not be waited for or when the time lag required for nutrient release through manure decomposition and mineralisation could be a delay or when a high crop yield is targeted to meet up with the increasing demand for food. For example, Dakora and Keya (1997) indicated the biological process of nitrogen (N_2) fixation as the cheapest and probably the most effective tool for the maintenance of sustainable yield in African agriculture, as most of the nitrogen required for crop productivity in the continent fundamentally comes from an incisive management of biological N_2 fixation in conventional cropping systems. Apparently, it was considered that 65 % of the N input into global agriculture comes from N fixation. Suggestions given on the improvement of nutrient efficiency by Carranca (2012) include intercropping, crop rotation and a precision nitrogen management such as the appropriate use of chemical fertilisers. Also, Okorogbona and Adebisi (2012) indicated the use of organic materials such as animal manure for augmenting the fertility of soils in African smallholder cropping systems. The intercropping, as shown in Plate 7, normally involves the cultivation of a legume such as cowpea and non-legume-for example maize, due to the nitrogen fixing nature of leguminous crop.

4 Nitrogen Fixation and Its Impacts on Crop Productivity

Nitrogen is the most growth and yield limiting nutrient and the most applied nutrient element in terms of quantity for crop production (Nyamangara et al. 2005; Fageria 2009). Shortage of nitrogen reduce the growth of all plant organs, roots, stems, leaves, flowers and fruits (including seeds) (Barker and Pilbeam 2007; Fageria 2009; Sharifi and Taghizadeh 2009). According to Barker and Pilbeam (2007), a nitrogen deficient plant appears stunted due to restricted growth of the vegetative organs and the foliage gives a pale colour of light green or yellow with a consequential loss of the green colour uniformly across the leaf blade. Deficiency of nitrogen throughout the life cycle of a plant results in the entire plant being stunted or spindly but if the deficiency develops during the growth cycle, the nitrogen will be mobilised from the lower leaves and translocated to young leaves causing the lower leaves to become pale coloured and in the case of severe deficiency,

Table 5 Properties of saline, sodic and waterlogged soils (After Marcal et al. 1999)

Soil	Properties							
	EC_e[a] (dSm^{-1})	ESP[b]	pH	Major products	Physical structure	Soil water	Essential nutrients	Other
Saline	>2–4	<15[c]	<8.2	Sodium (Na), Chlorine (Cl), Sulphate (SO_4)	Flocculated	Likelihood of osmotically-induced water stress	Inconsistent	High Na: Calcium (Ca)
Sodic	<2–4	>15[c]	>8.2	Sodium (Na), Carbonate (CO_3), Hydrogen carbonate (HCO_3)	Dispersed	Reduced access to subsoil moisture likely due to impeding layers	Inconsistent	High Na: Ca
Water logged	Not applicable	Not applicable	pH varies	End products of Anaerobic respiration	Variable: low oxygen (O_2) concentrations	Excess supply	Inconsistent	Not applicable

[a]ECe = electrical conductivity of water extracted from a saturated soil paste
[b]ESP exchangeable sodium percentage
[c]Under Australian conditions, sodic soils have ESP > 6

Plate 7 Maize intercropped with cowpea

to brown and abscise. However, excessive or over application of nitrogen creates high accumulation of nitrogen in the vicinity of the soil where absorption takes place and this causes lavish consumption resulting to a toxic situation that is detrimental to plant growth and yield (Goyal et al. 2005; Barker and Pilbeam 2007). The symptoms of nitrogen toxicity are inclined with excess ammonium (NH_4^+) and less often excess nitrate (NO_3^-) which results in a consequent delay of crop development into maturity stage (Tisdale and Nelson 1975; Fageria 2009). Excess nitrogen in plants results in soft growth which makes some plants to be susceptible to attack by diseases and insect pests (Tisdale and Nelson 1975) and to damage by drought and cold as indicated by (Tshikalange 2007).

Barker and Pilbeam (2007) pointed out that the concentrations of nitrogen in plants reflects the supply of nitrogen in the root medium and yield increase as internal concentration of nitrogen increases. Fageria (2009) defined concentration as the uptake per unit dry weight of plant tissue and it is generally expressed as a percentage, g kg^{-1} or mg^{-1}.

In their review, Dakora and Keya (1997) pointed out N nutrition as one of the major determinants of crop yield in Africa. Nitrogen in soils occurs in organic and inorganic forms but because the soil has little capacity to retain oxidised form of N, most of it becomes associated more with its inert organic form in agricultural surface soils (Jones 1979; Davidescu and Davidescu 1972; Soon 1985). The inorganic form of nitrogen include ammonium (NH_4^+), nitrate (NO_3^-), nitrous oxide (NO), elemental nitrogen (N_2) and nitrite (NO_2^-). A small amount is found in soluble organic form. According to Davidescu and Davidescu (1972), it results from microbial activity or from decomposition of organic material in the soil solution. The process of N attraction by microbial activity is termed N fixation. Nitrogen fixation is the conversion of atmospheric nitrogen to N compounds by bacteria which forms symbiotic association with plant roots in the soil. Vance and Graham (1995) reported that about 65 % of N input into crop productivity worldwide comes from N_2 fixation. As such, most of the N required for crop productivity in African conservative cropping system originates from perceptive management of biological N fixation (Dakora and Keya 1997). According to Dakora and Keya (1997), in Africa, sole cropping, crop rotation and intercropping of legumes and cereals are the dominant cultural practices and crop rotation has been indicated as the most superior, followed by monoculture and lastly intercropping. In line with this, yields of sole cropped and intercropped maize were found to be only 35 % and 38 % of the yields obtained from crop rotation in Africa savanna respectively. Consequently, rotational cropping involving legumes and cereals were reported to be a more sustainable system for increasing food production in Africa than intercropping (Dakora and Keya 1997). Hitherto, over 90 % of cowpea is grown as intercrop with sorghum or pearl millet in West Africa (Dakora and Keya 1997). Under low N conditions, a companion cereal directly improves its N nutrition and yields from nitrogenous solutes excreted by nodulated roots of the associated legume with intercropping (Dakora and Keya 1997). According to Dakora and Keya (1997), sole crop legumes contributes more to subsequent cereal yields than intercropped legumes from studies carried out in the savanna zone of west Africa.

In a study carried out on crop rotation by Dakora et al. (1987), where cowpea-maize and groundnut-maize were rotated, maize yields increased by 95 % and 89 % respectively when grown after cowpea and groundnut. Consequently, it was reported that N benefit to maize from cowpea and groundnut residues containing 125 and 43 kg N ha^{-1} respectively, was equivalent to 60 kg N ha^{-1} of $(NH_4)_2SO_4$. As postulated by Dakora and Keya (1997), researches carried out on N dynamics in crop rotation system revealed the recovery of 27 % and 60 % N from cowpea and groundnut vestiges.

Despite the fact that N fixation play significant role in crop productivity in African Agro-ecological system by contributing to soil fertility, the process is hindered by factors such as drought and high temperature due to the inability of leguminous crops to tolerate these phenomena. In addition to the draw-back caused by drought and high temperature, it is relevant to also point out that the quantity of nutrient from biological fixation are far less than the amount that could recompense for agricultural production to meet up with the increasing demand for food as a result of rising human population (FAO 1998; Okorogbona and Adebisi 2012). The low quantity of N from biological fixation is due to reasons associated with their slow release pattern. In addition, the ability to fix nitrogen is not common to all crops but a factor associated mainly to leguminous crops (Stacey et al. 1992; Tikhonovich et al. 1995; Postgate 1998; Okorogbona and Adebisi 2012). In order to supply agricultural production activities with nutrients to produce the amount of crops that would meet up with the increasing demand for food, farmers have to supply nutrients through mineral fertiliser and organic nutrient sources (Okwu and Ukanwa 2007).

5 Mineral Fertiliser and Its Impact on Crop Productivity

As described by Okorogbona and Adebisi (2012), mineral fertilisers are inorganic substances applied to soil, irrigation water or a hydroponic medium to supply plants with nutrients. These are also referred to as chemical fertilisers. According to Dakora and Keya (1997), the increased use of N from chemical fertilisers has resulted in significant increase in crop productivity throughout the globe. The nutrients in chemical fertilisers are soluble and this makes them immediately available for plant root absorption. Consequently, the effect is usually direct and fast after incorporation and soil irrigation. Only relatively small amounts of chemical fertilisers are required for crop growth due to their high nutrient content.

It is no doubt that the use of chemical fertiliser increases crop yield and that its incorporation to soil for crop growth has tremendously improve crop productivity as far as farming is concerned. A situation which leads to the provision of food to the world's increasing human population. Nevertheless, the excessiveness and over-application of inorganic fertiliser has detrimental effect on crop growth, crop yield and even crop quality during storage. *For example,* Steyn and du Plessis (2012) *pointed out that the excessive application of nitrogen to soil used for Potato cultivation negatively affects the keeping quality of the crop tuber; lowers the tuber's specific gravity (starch content) and also lead to hollow heart in the tuber which is synonymous to the potato cultivar called* **BP1**-*a* South African *variety of Potato.* Apart from the negative impact the over application of inorganic fertiliser poses on crop growth and yield, the over use of these nutrient source also has detrimental effect on human environment. *For example, where nutrients washed from farms across the Mississippi Basin caused unexpected environmental impacts in the Gulf of Mexico which resulted to oxygen starvation that led to suffocation in the area* (Malakoff 1998). One of the negative effects that excessive application of chemical fertiliser poses on crop plant at pre-harvest stage is reduced growth and development. Evidence of this is shown in Plate 8 which shows the leaf sizes of Chinese cabbage grown in same soil amended with chemical fertilisers at the rates of 120 kg N ha^{-1}, 80 kg P ha^{-1}, 80 kg K ha^{-1} and 60 kg N ha^{-1}, 40 kg P ha^{-1}, 40 kg K ha^{-1}.

Applying inorganic or synthesised fertilisers in excess of plant absorbable rates twist these nutrient sources to be probable environmental pollutants particularly when consideration is given to the high leaching nature of nitrogenous fertilisers that results in ground water pollution that affect the quality of water for domestic use. With regard to water quality, farmers need to give considerable attention to the after effect of over-applied fertiliser especially excessive application of nitrate fertilisers to both surface and ground water. In line with the foregoing, the need for crop growers to confront the negativity posed by the over-application of chemical

Plate 8 Chinese cabbage plant showing the growth of the crop in terms of leaf size in pot amended with chemical fertilisers using two different application rates at the final harvesting stage of the crop plant. *NPK*-nitrogen, phosphorus and potassium fertilisers

fertiliser becomes imperative. This could be achieved by employing economically and environmentally sound management nutrients use practices relating to a huge challenge of giving consideration to efficiently tested application rates of inorganic nutrient sources which produced considerable crop yield while fertiliser input remain cost effective and at the same time avoiding the damage caused by fertiliser applied above plant absorbable level. Table 6 provides information on fertiliser application rates used to obtained considerable yield from different crops in African subsistence crop farming schemes.

The benefit of incorporating chemical fertiliser into soils used for crop cultivation is well known. The main positive effect of applying this nutrient source is that it improves growth and yield of crops as a result of plant uptake of the nutrients immediately available for use after addition to plant growth media. Also important, is the improvement of crop production capacity to considerable level that meets up with increasing food demand associated with human population increase across the globe. Despite all these benefits derived from inorganic nutrient sources, it is highly unfortunate that the use of chemical fertiliser is limited among resource poor farmers of developing countries due to its high cost and inaccessibility. These factors have made the use of animal manure a prominent soil amendment source among African low marginal input farmers. Also, the awareness created on the healthiness of organically cultivated crop produce in recent times, which necessitates the need to promote organic farming worldwide, has reawaken the use of animal manure as soil amendment source by organic food producers.

Table 6 Fertiliser rates applied to obtain considerable yield from different crops

Crop	Application rate of fertiliser	Soil type	Crop growth condition	Reference
Leafy Amaranth (*Amarathus cruentus* L.), Pumpkin (*Cucurbita maxima*), Spider flower (*Cleome gynandra*), Cowpea (*Vigna unguiculata*), Nightshade (*Solanum retroflexum* Dun.), Jew's mallow (*Chorchorus olitorus*), Tsamma melon, Chinese cabbage (*Brassica rapa*)	80 kg N ha^{-1}; 30 kg P ha^{-1}; 40 kg K ha^{-1}	Clayey soil	Field condition	Van Averbeke et al. (2012)
Potatoes	60–240 kg N ha^{-1}; 30–240 kg P ha^{-1}; 55–450 kg K ha^{-1}	These ranges from dry lands; under irrigation of soils of different clay contents, different soil analyses methods; soils with a CEC less than or greater than 6 $mol_c kg^{-1}$	Field	Steyn and du Plessis (2012)
Pumpkin (*Cucurbita maxima*)	85 kg N ha^{-1}; 45 kg P ha^{-1}; 60 kg K ha^{-1}	Sandy clay loam	Greenhouse condition	Azeez et al. (2010)
Nightshade (*Solanum retroflexum* Dun.)	85 kg N ha^{-1}; 45 kg P ha^{-1}; 60 kg K ha^{-1}	Sandy clay loam	Greenhouse condition	Azeez et al. (2010)
Grain Amaranth	45 kg N ha^{-1}	Low fertility soil	Field	Olaniyi et al. (2008)
Rice	150 kg N; 150 kg K ha^{-1};	Silt loam soil	Field	Jang et al. (2008)
Maize	175 kg N ha^{-1}; 73 kg P ha^{-1}; 17 kg K ha^{-1}	Low fertility soil	Field	FSSA (2004)
Wheat	28 kg N ha^{-1}; 18 P kg ha^{-1}; 3 kg K ha^{-1}	Low fertility soil	Field	FSSA (2004)
Sugar cane	92 kg N ha^{-1}; 57 kg P_2O_5 ha^{-1}; 133 kg K_2O ha^{-1}	Low fertility soil	Field	FSSA (2004)
Solanum villosum	100 kg N ha^{-1}	Clayey soil	Field	Kipkosgei et al. (2003)

N nitrogen, *P* phosphorus, *K* potassium, *CEC* cation exchange capacity

[a]Van Averbeke et al. (2012) opined that the application of nutrients to soils used for the cultivation of leafy vegetables should be managed carefully and therefore suggested that chemical fertilisers could be safely applied in the neighbourhood of 20 kg N ha^{-1}, 30 kg P ha^{-1}, 40 kg K ha^{-1} in the form of (2:3:4) fertiliser in planting furrow. Hence, it was strongly recommended that the given application rate should not be exceeded when considering the use of band application method at planting in order to avoid the burning of plants. In the case of application of chemical fertiliser to low fertility soils at higher rates, Van Averbeke et al. (2012) recommended that the remainder of fertiliser mixture should be broadcast

6 Animal Manure and Its Impact on Crop Productivity

Animal production in sustainable agriculture has generated income to farmers; provided food to human; provided hides and skin for the leather industry, with a substantial contribution to economic growth in all agrarian societies but despite this, manure from domestic animals has raised serious concerns as its erroneous disposal and excessive use as soil fertility material has some draw-backs to man and his environment. Unease over the effect of excessive land application of animal manure on crop growth, crop yield and water quality has always been raised as far as research on the organic nutrient source is concerned in crop productivity. As the soil science school of thought continues to view the use of animal manure as a nutrient amendment source in soil and other plant growth media-a situation which significantly has a positive effect on cost reduction. The waste management and environmental conservation schools of thought concomitantly opined that using these wastes from domestic animals help to solve disposal problems. Although, manure from poultry, goats, cattle, swine, and even abattoir wastes such as the wastes removed from the stomach content of ruminants after the evisceration of ruminant animals in slaughter houses, are useful by-products in some of the first world countries, where they are used for the production of methane gas but it is highly unfortunate that this situation is contrary in most developing countries where the method of slaughtering remains rudimentary, as these recyclable wastes are being disposed off in pits and running stream at times, thereby causing environmental pollution and contaminating the ground water (Okorogbona 2011).

However, the ideology from agriculturists to continue coming up with modalities which include agronomic management practices on the use of manure as soil amendment source; the use of manure as source of food in fish ponds; the use of manure for the growth of maggots used as protein source in livestock feeds, in order to prevent soil contamination, water and air pollution, have been transforming the view of animal manure as a waste to a resourceful product in African sustainable farming. For the purpose of this chapter, emphasis will be on its use as soil amendment source.

The benefits of animal manure utilisation as soil amendment source include improving the growth and yield of crops (Maerere et al. 2001; Gosh et al. 2004; Kihanda et al. 2004); raising organic matter content of agricultural soils (Fatunbi and Ncube 2009); increasing the populations of microfauna of soils and alleviating micronutrient deficiencies in soils (Wilkinson 1979). However, applying animal manure at rates that are higher than plant absorbable level result in negative effect on crop growth and yield as this causes soil salinity and alkalinity which hinders plant growth and development (Lu and Edwards 1994; De Campos et al. 2004). Excessive application of the organic nutrient sources is also known to cause environmental pollution including the contamination of groundwater (Sims and Bitzer 1988).

The efficient use of nutrients available in animal manure for soil fertility is essential to avoid the negative effect of crop growth and yield, soil and water contamination. Hence, giving consideration to the analysis of the nutrient contents of all types of manure before use as soil amendment material is imperative to obtain targeted crop yield.

Considering the reduction in crop growth rate and yield together with the contamination of both surface and ground water caused by excessive incorporation of animal manure to soil or improper disposal to the environment, the need to give attention to the after effect of inefficient use of the organic nutrient material becomes relevant. This is for crop growers to achieve considerable yield from their production activities. Consequently, it is of high significance for crop growers to employ economically and environmentally sound management manure utilisation practices by relating their proposed selected quantity of manure to be used to application rates of manure that had practically been used and found to provide considerable crop yields without causing any negative effect to crop growth and development. Table 7 indicates information on animal manure application rates in which crop yields comparable to yields obtained from inorganic nutrient sources were obtained (Tables 8 and 9).

Apart from the fact that applying animal manure at rates that are above plant critical absorption level results to negative effect on plant growth and yield, there has also been lack of confidence as to the use of manure among farmers, even if this organic nutrient source are applied at optimum level for plant growth and development because users have for all time commented that crops are usually affected by diseases and the nutrient release pattern in manure is too slow: reason being that animal manure is always considered as a haven for disease causing agents, weed seeds and the fact that manure contains materials such as fibres and other constituents that retards the rapid process of decomposition and minerilisation in the soil. Research carried out to investigate the growth of Non-heading Chinese cabbage using dried and pulverised chicken, cattle and goat manures by Okorogbona et al. (2011) indicated that the processed form of these organic nutrient sources could be used to obtain considerable yield of vegetables without any negative effect caused by disease(s). Evidence of healthy and disease free leafy vegetable plant growing in soils amended with inorganic and processed manures from poultry and ruminant is shown in Plate 9. Plants growing in the manure amended soils appear to have remarkably large and healthy leaves comparable to the leaves of the Chinese cabbage grown in the soil amended with lime ammonium nitrate (LAN), single superphosphate and potassium chloride.

In their study, Okorogbona et al. (2011) found out that pulverisation of manure increased the surface area of nutrients availability in the manures and suggested that this made the nutrients almost immediately available soon after irrigation followed the incorporation of the organic material into soil. In spite of this, warning was given on the adverse effect of excessive application of manure on plant growth. Table 10 provides information on different scenarios that entailed the excessive application of animal manure on different occasions.

Table 7 Animal manure application rates used to produce considerable yield from different leafy vegetables

Crop	Type of animal manure	Application rate of animal manure (t ha^{-1})	N P K (kg ha^{-1})	Reference
Leafy Amaranth (*Amaranthus cruentus* L.)	Chicken manure	24	895 kg N ha^{-1}, 357 kg P ha^{-1} and 433 kg K ha^{-1}	Van Averbeke et al. (2012)
	Cattle manure	180	3,063 kg N ha^{-1}, 757 kg P ha^{-1} and 3,026 kg K ha^{-1}	
	Goat manure	90	1,999 kg N ha^{-1}, 387 kg P ha^{-1} and 3,621 kg K ha^{-1}	
Pumpkin (*Cucurbita maxima*)	Chicken manure	12	448 kg N ha^{-1}, 178 kg P ha^{-1} and 216 kg K ha^{-1}	Van Averbeke et al. (2012)
	Cattle manure	180	3,063 kg N ha^{-1}, 757 kg P ha^{-1} and 3,026 kg K ha^{-1}	
	Goat manure	60	1,335 kg N ha^{-1}, 257 kg P ha^{-1} and 2,417 kg K ha^{-1}	
Chinese cabbage (*Brassica rapa*)	Chicken manure	24	895 kg N ha^{-1}, 357 kg P ha^{-1} and 433 kg K ha^{-1}	Van Averbeke et al. (2012)
	Cattle manure	210	3,574 kg N ha^{-1}, 883 kg P ha^{-1} and 3,530 kg K ha^{-1}	
	Goat manure	90	1,999 kg N ha^{-1}, 387 kg P ha^{-1} and 3,621 kg K ha^{-1}	
Nightshade (*Solanum retroflexum* Dun.)	Chicken manure	24	895 kg N ha^{-1}, 357 kg P ha^{-1} and 433 kg K ha^{-1}	Van Averbeke et al. (2012)
	Cattle manure	180	3,063 kg N ha^{-1}, 757 kg P ha^{-1} and 3,026 kg K ha^{-1}	
	Goat manure	90	1,999 kg N ha^{-1}, 387 kg P ha^{-1} and 3,621 kg K ha^{-1}	

[a]These safe application rates suggests that composted, dried and pulverised chicken manure can be applied at the range of 12–24 t ha^{-1} for the growth of leafy vegetables in soils with low nutrient availability and low biological activities, without the addition of any form of inorganic nutrient source, depending on the vegetable. Cattle manure can be applied between 180 and 210 t ha^{-1}. Goat manure can be applied from 60 to 90 t ha^{-1}. It is important to give consideration to the sources from which manures are obtained as storage conditions of this organic material affect the quality

Table 8 Animal manure application rates used to obtain considerable yield from fruit vegetables

Crop	Type of animal manure	Application rate of animal manure t ha^{-1}	N P K (kg ha^{-1})	Reference
Tomato	Chicken manure	30		Adekiya and Agbede (2009)
Water melon	Chicken manure	9.9	369 kg N ha^{-1}, 147 kg P ha^{-1} and 179 kg K ha^{-1}	Duada et al. (2008)

[a]It is important to take cognisance of the fact that the 30 t ha^{-1} chicken manure was applied at the period of heavy rainfall in an environment where the average annual rainfall ranges from 1,000 to 1,240 mm and the soil in the area include Alfisol classified as Oxic Tropuldalf (USDA) or Luvisol (FAO) derived from quartz, gneiss and schist (Agbede 2006)

Table 9 Animal manure application rates used to produce considerable yield from other crops

Crop	Type of animal manure	Application rate of animal manure t ha^{-1}	N P K (kg ha^{-1})	Reference
Collard	Chicken manure	26	969 kg N ha^{-1}, 386 kg P ha^{-1} and 469 kg K ha^{-1}	Lu and Edwards (1994)
Strawberry	Chicken manure	10.8	403 kg N ha^{-1}, 160 kg P ha^{-1} and 195 kg K ha^{-1}	Farren et al. (1993)
Grape production	Chicken manure	12.8	478 kg N ha^{-1}, 190 kg P ha^{-1} and 231 kg K ha^{-1}	Farren et al. (1993)

[a]These application rates were all identified as safe rates to soils with low fertility levels used for the cultivation of the crops listed in the table

Plate 9 Chinese cabbage grown in nutrient amended soils. *PL* poultry litter, *GKM* goat kraal manure

Table 10 Animal manure application rates reported to be above critical level

Crop	Manure	Application rate (t ha^{-1})	Effect on crop	Reference
Chinese cabbage	Goat manure	150	Reduced growth and drastic collapse in yield	Van Averbeke et al. (2012)
Pigweed, Nightshade and Pumpkin	Goat manure	150	No growth	Van Averbeke et al. (2012)
Chinese cabbage and Nightshade	Chicken manure	35	Reduced leaf yield	Van Averbeke et al. (2012)
Amaranth	Chicken manure	24	Reduced leaf yield	Van Averbeke et al. (2012)
Pumpkin	Chicken manure	18	Reduced leaf yield	Van Averbeke et al. (2012)
Pumpkin	Cattle manure	210	Reduced leaf yield	Van Averbeke et al. (2012)
Tomato	Chicken manure	40	Reduced fruit yield	Adekiya and Agbede (2009)
Fluted pumpkin	Chicken manure	80	Yield reduction	Philip et al. (2009)
Collard	Chicken manure	58	Seedlings died	Lu and Edwards (1994)
Maize	Chicken manure	90	Grain yield reduction	Shortall and Liebhardt (1975)

[a]From an experimental perspective, the different information retrieved in this review indicates that for production of the crops featuring in Table 10, the manure of ruminants including cattle and goats can be applied at higher rates than the manure obtained from monogastric such as chicken. The results obtained on the chicken manure applied above threshold points indicate the need for a cautious approach to the use of poultry litter as a fertiliser and the limited effectiveness of this particular animal manure

7 Conclusion

The costs of production in farming and even agronomic research are high in recent times due to the increasing cost of agricultural inputs and the down turn in global economy. This situation stimulates the interest in earmarking strategies to manage the various problems home gardeners, smallholders, emerging and commercial farmers encounter in their day to day operational activities. For agricultural sustainability to be achieved in the African context of farming, the need to assemble information from various scientists involved in research aimed at food security today and in the future, for use becomes imperative. This is for use as guide in crop cultivation and research purposes in order for crop growers and agronomic researchers to succeed and this review was aimed at such development.

From the various information retrieved so far, it could be tentatively concluded that for crop productivity improvement in sustainable agriculture to be achieved, consideration need to be given to factors which are crucial to crop yield increase. Among these factors are soil texture, soil structure, soil constituents and critical attention to these greatly determine the targeted bumper output always focused at in modern agriculture, which has always aimed at meeting up with the increasing demand for food, as a result of continued increase in human population throughout the globe.

Improvement of crop productivity in African sustainable agriculture has always been hindered by low soil fertility which is associated with nutrient deficiencies, high soil acidity and alkalinity levels together with soil salinity which remains the most serious bottle-neck in African crop farming systems, particularly in the sub-Saharan regions of the continent. Tree planting, use of halophytic plants, application of plant physiology, genetics and biochemical approaches involving the modification and breeding of salt stress tolerant varieties or cultivars are among the remedies pointed out as means of alleviating the soil fertility crisis associated with salinity. The biological process of nitrogen (N_2) fixation, precision nutrient management and the use of organic nutrient source are among the agronomic practices mentioned by scientists as the effective tools for maintaining sustainable yield in African agriculture. Estimate of the rate of soil formation indicated that about 2.5 cm of soil is formed in every 150 years, which shows that soil is non-renewable within the human-life-span. Henceforth, it is in the interest of the farmer, and human population as a whole, to ensure that good soil management is practiced so that this resource is preserved for continued use by the current and future generations.

As making sure information reach those in need of it remains the priority of today's research world, hopefully this review work would play a significant role in fulfilling this function.

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References

Abari AK, Nasr MH, Hojjati M, Bayat D (2011) Salt effects on seed germination and seedling emergence of two *Acacia* species. Afr J Plant Sci 5(1):52–56

Adekiya AO, Agbede TM (2009) Growth and yield of tomato (*Lycopersicon esculentum* Mill) as influenced by poultry manure and NPK fertilizer. Emir J Food Agric 21(1):10–20

Agbede TM (2006) Effect of tillage on soil properties and yam yield on an Alfisol in southwestern Nigeria. Soil Till Res 86:1–8

Akinrinde EA (2006) Strategies for improving crops' use-efficiencies of fertilizer nutrients in sustainable agricultural systems. Pak J Nutr 5(2):185–193

Alami Y, Achouak W, Marol C, Heulin T (2000) Rhizosphere soil aggregation and plant growth promotion of sunflowers by an exopolysaccharide-producing *Rhizobium* sp. strain isolated from sunflower roots. Appl Environ Microbiol 66(8):3393–3398

Ali Z, Salam A, Azhar FM, Khan IA (2007) Genotypic variation in salinity tolerance among spring and winter wheat (*Triticum aestivum* L.) accessions. S Afr J Bot 73:70–75

Andrade A, Wolfe DW, Fereres E (1993) Leaf expansion, photosynthesis and water relations of sunflower plants grown on compacted soil. Plant Soil 149:175–184

Aslam Z (2006) Saline agriculture. In: Quraishi MAA, Zia MA, Quraishi MS (eds) Pakistan agriculture management and development. A-One publishers, Lahore, pp 210–221

Azeez JO, Van Averbeke W, Okorogbona AOM (2010) Differential responses in yield of pumpkin (*Cucurbita maxima* L.) and nightshade (*Solanum retroflexum* Dun.) to the application of three animal manures. Bioresour Technol 101:2499–2505

Ball MC (1988) Salinity tolerance in the mangroves *Aegiceras corniculatum* and *Avicennia marina*. I. Water use in relation to growth, carbon partitioning, and salt balance. Aust J Plant Physiol 15:447–464

Ball MC, Pidsley SM (1995) Growth responses to salinity in relation to distribution of two mangrove species, *Sonneratia alba* and *S. lanceolata*, in northern Australia. Funct Ecol 9:77–85

Barker AV, Pilbeam DJ (2007) Handbook of plant nutrition. Taylor and Francis Group, LLC, Boca Raton

Bassiri R (2005) Nutrient acquisition by plants: an ecological perspective. Springer, New York

Bill and Melinda Gates Foundation (2011) Agricultural development: strategy overview. Global Development Program, pp 1–10

Björklund J, Limburg KE, Rydberg T (1999) Impact of production intensity on the ability of the agricultural landscape to generate ecosystem services: an example from Sweden. Ecol Econ 29:269–291

Boone FR (1986) Towards soil compaction limits for crop growth. Neth J Agric Sci 34:349–360

Brereton JC, McGowan M, Dawkins TCK (1986) The relative sensitivity of spring barley, spring field beans and sugar beet crops to soil compaction. Field Crop Res 13:223–237

Bronick CJ, Lal R (2005) Soil structure and management: a review. Geoderma 124:3–22

Carranca C (2012) Nitrogen use efficiency by annual and perennial crops. In: Lichtfouse E (ed) Farming for food and water security. Springer, Dordrecht

Carter MR (2002) Soil quality for sustainable land management: organic matter and aggregation interactions that maintain soil functions. Agron J 94:38–47

Chaney K, Swift RS (1986) Studies on aggregate stability. I. Reformation of soil aggregates. J Soil Sci 37:329–335

Chauvin ND, Mulangu F, Porto G (2012) Food production and consumption trends in sub-Saharan Africa: prospects for the transformation of the agricultural sector. Working paper, UNDP

Chenu C (1993) Clay or sand polysaccharide associations as models for the interface between micro-organisms and soil: water-related properties and microstructure. Geoderma 56:143–156

Cheshire KV (1979) Nature and origin of carbohydrates in soils. Academic Press, New York

Cohen JE (1995) Population growth and earths human carrying capacity. Science 261(5222): 341–346

Conway G (1997) The doubly green revolution: food for all in the twenty-first century. Penguin, London

Conyers M (1986) The relationship between average annual rainfall exchangeable between average annual rainfall exchangeable aluminium in soils of south eastern New South Wales. Aust J Exp Agric 26:587–590

Dakora FD, Keya SO (1997) Contribution of legume nitrogen fixation to sustainable agriculture in sub-Saharan Africa. Soil Biol Biochem 29(5/6):809–917

Dakora FD, Aboyinga RA, Mahama Y, Apaseku J (1987) Assessment of N_2 fixation in groundnut (*Arachis hypogea* L.) and cowpea (*Vigna unguiculata* L. Walp) and their relative N contribution to a succeeding maize crop in Northern Ghana. MIRCEN J 3:389–399

Davidescu D, Davidescu V (1972) Evaluation of fertility by plant and soil analysis. Abacus, Kent

De Campos AB, Reichert JM, Huang C (2004) Near-surface hydrology, runoff production, and soil loss from surface-applied liquid swine manure. In: Conserving soil and water for society: sharing solutions: 13th international soil conservation organisation conference, Brisbane, held in Australia on 4–8 July 2004

Devkota A, Jha PK (2009) Variation in growth of *Centella asiatica* along different soil composition. Bot Res Int 2(1):55–60

Dickson EL, Rasiah V, Groenevelt PH (1990) Comparison of four prewetting techniques in wet aggregate stability determination. Can J Soil Sci 71:67–72

Dinel H, Levesque PEM, Jambu P, Righi D (1992) Microbial activity and long-chain aliphatics in the formation of stable soil aggregates. Soil Sci Soc Am J 56:1455–1463

Duada SN, Ajayi FA, Ndor E (2008) Growth and yield of water melon (*Citrullus lanatus*) as affected by poultry manure. J Agric Soc Sci 4:121–124

El-Kharbotly A, Maghloub O, Al-Subhi A, Al-halhali A (2003) Indigenous grass species with potentials for maintaining rangeland and livestock feeding in Oman. Agric Ecosyst Environ 95:623–627

Elustondo JD, Angers AM, Laverdière R, N'Dayegamiye A (1990) Etude comparative de l'agrégation et de la matière organique associée aux fractions granulométriques de sept sols sous culture de maïs en prairie. Can J Soil Sci 70:395–402

Fageria NK (2009) The use of nutrients in crop plants. CRC Press, New York

Farren MT, Al-Assir IA, Sabra AS, Rubetz IG (1993) Layer and broiler poultry manure as nitrogen fertilizer sources for cabbage production. Commun Soil Sci Plant Anal 24(13 and 14):1583–1589

Fatunbi AO, Ncube L (2009) Activities of effective microorganism (EM) on the nutrient dynamics of different organic materials applied to soil. Am Eur J Agron 2(1):26–35

Fernandez-Illescas CP, Porporato A, Laio F, Rodriguez-Iturbe I (2001) The ecohydrological role of soil texture in a water-limited ecosystem. Water Resour Res 37(12):2863–2872

Food and Agriculture Organization (FAO) (1998) Guide to efficient plant nutrition management. Land and Water Development Division, Rome

Food and Agriculture Organization (FAO) (1999) Soil physical constraints to plant growth and crop production. Land and Water Development Division, Rome

Food and Agriculture Organization (FAO) (2011) Save and grow: a policy maker's guide to the sustainable intensification of smallholder crop production. FAO, Rome

Foster MAS, Lufafa A (2002) Agricultural intensification: 'feeding ourselves and sustaining Africa' land resources in the new millennium. Afr J Food Nutr Sci 2(1):5–21

Friend JA (1992) Achieving soil sustainability. J Soil Water Conserv 47:156–157

FSSA (2004) FSSA Journals, 2000 to 2004. President's reports. Fertilizer Society of South Africa, Pretoria

Fulginiti LE, Perrin RK (1998) Agricultural productivity in developing countries. Agric Econ 19:45–51

Gosh PK, Ramesh P, Bandyopadhyay KK, Tripathi AK, Hati KM, Misra AK, Acharya CL (2004) Comparative effectiveness of cattle manure, poultry manure, phosphocompost and fertilizer-NPK on three cropping systems in vertisols of semi-arid tropics. Bioresour Technol 95:77–84

Goyal SS, Tischner R, Basra A (2005) Enhancing the efficiency of nitrogen utilization in plants. The Harworth Press Inc, New York

Greenway H, Munns R (1980) Mechanisms of salt tolerance in nonhalophytes. Annu Rev Plant Physiol Plant Mol Biol 31:149–190

Houle G, Morel L, Christina ER, Siegel J (2001) The effect of salinity on different developmental stages of an endemic annual plant, *Aster laurentianus* (*Asteraceae*). Am J Bot 88(1):62–67

James C (2009) Executive summary-global status of commercialised biotech/GM crops: 2009. The International Service for the Acquisition of Agri-biotech Applications (ISAAA), Ithaca. http://www.isaaa.org/resources/publications/briefs/41/executivesummary/default.asp

Jang S, Hamayun M, Sohn E, Kang S, Choi K, Shin D, Lee I (2008) Growth and gibberellins level of two rice cultivars as influenced by different nitrogen containing compounds. J Crop Sci Biotechnol 11(4):223–226

Jonas N, Chianu EM, Nkonya FS, Mairura J, Chianu N, Akinnifesi FK (2011) Biological nitrogen fixation and socioeconomic factors for legume production in sub-Saharan Africa: a review. Agron Sustain Dev 31(1):139–154

Jones US (1979) Fertilizers and soil fertility. Prentice-Hall, Reston

Kates RW (1996) Ending hunger: current status and future prospects. Consequences 2:3–12

Katsunori S (2003) Sustainable and environmentally sound land use in rural areas with special attention to land degradation, Asia Pacific forum for environment and development expert meeting, 23 January, Gullin, China

Kihanda FM, Warren GP, Attwal SS (2004) The influence of goat manure application on crop yield and soil nitrate variations in semi–arid eastern Kenya. In: Bationo A (ed) Managing nutrient cycles to sustain soil fertility in sub-Saharan Africa. Academy Science Publishers, Nairobi, pp 174–186

Kipkosgei LK, Akundabweni LSM, Hutchinson MJ (2003) The effect of farmyard manure and nitrogen fertilizer on vegetative growth, leaf yield and quality attributes of *Solanum villosum* (Black nightshade) in Keiyo district, rift valley. Afr Crop Sci Con Proc 6:514–518

Lal R (1991) Soil structure and sustainability. J Sustain Agric 1:67–92

Lambers H, Chapin ES III, Pons TL (1998) Plant physiological ecology. Springer, New York

Larcher W (1995) Physiological plant ecology, 3rd edn. Springer, New York

Liverpool-Tasie LS, Kuku O, Ajibola A (2011) A review of literature on agricultural productivity, social capital and food security in Nigeria. The Nigeria strategy support program (nssp) working papers. IFPRI, Abuja

Lu N, Edwards JH (1994) Poultry litter quantity influences collard growth in pots and affects cabbage growth and nutrient uptake. J Hortic Sci 29(10):1143–1148

Maerere AP, Kimbi GG, Nonga DLM (2001) Comparative effectiveness of animal manures on soil chemical properties, yield and growth of *Amaranthus* (*Amaranthus cruentus* L). Afr J Sci Technol 1(4):14–21

Malakoff D (1998) Coastal ecology: death by suffocation in the Gulf of Mexico. Science 281:190–192

Marcal N, Ismails S, Hossain A, Ahmad R (1999) Trees, shrubs and grasses for saltlands: an annotated biography, ACIAR monograph no 56. Australian Centre for International Agricultural Research, Canberra, 316 p

Masle J (1998) Growth and stomatal responses of wheat seedlings to spatial and temporal variations in soil strength of bi-layered soils. J Exp Bot 49:1245–1257

Masle J, Passioura JB (1987) The effect of soil strength on the growth of young wheat plants. Aust J Plant Physiol 14:643–656

Mulholland BJ, Black CR, Taylor IB, Roberts JA, Lenton JR (1996) Effect of soil compaction on barley (*Hordeum vulgare* L.) growth. I. Possible role for ABA as a root-sourced chemical signal. J Exp Bot 47:539–549

Munns DN, Franco AA (1981) Soil constraints to legume production. In: Graham PH, Harris S (eds) Biological nitrogen fixation technology for tropical agriculture. Centro lnternacional de Agricultura Tropical, Cali, pp 133–152

Munns R, Termaat A (1986) Whole-plant responses to salinity. Aust J Plant Physiol 13:143–160

Nellemann C, MacDevette M, Manders T, Eickhout B, Svihus B, Prins AG, Kaltenborn BP (eds) (2009) The environmental food crisis–the environment's role in averting future food crises: a UNEP rapid response assessment. United Nations Environment Programme and GRID-Arendal, Arendal

Neumann PM (1995) Inhibition of root growth by salinity stress. In: Baluska F, Ciamporova M, Gasporikova O, Barlow PW (eds) Structure and function of roots. Kluwer Academic Publishers, Dordrecht, pp 299–304

Nyamangara J, Mudhara M, Giller KE (2005) Effectiveness of cattle manure and nitrogen fertilizer application on the agronomic and economic performance of maize. S Afr J Plant Soil 22(1):59–63

Okorogbona AOM (2011) Changes in the quality of slaughtered cattle gastro-intestinal wastes as affected by period of decomposition and the effect of treatments on decomposers of the abattoir wastes. Glob Vets 6(2):140–151

Okorogbona AOM, Adebisi LO (2012) Animal manure for smallholder agriculture in South Africa. In: Lichtfouse E (ed) Farming for food and water security. Springer, Dordrecht

Okorogbona AOM, Van Averbeke W, Ramunsadiwa TD (2011) Growth and yield response of Chinese cabbage as affected by nutrient availability in air-dried and pulverised different types of animal manure using low biological activity soil. World J Agric Sci 7(1):1–12

Okwu DE, Ukanwa NS (2007) Nutritive value and phytochemical contents of fluted pumpkin (*Telfaria occidentalis* Hook F.) vegetable grown with different levels of turkey dropping: African crop science proceedings, held in Egypt, Al Minya, El- Minia

Olaniyi JO, Adelasoye KA, Jegede CO (2008) Influence of nitrogen fertilizer on the growth, yield and quality of grain Amaranth varieties. World J Agric Sci 4(4):506–513

Oluwatoyinbo FI, Akande MO, Adediran JA (2005) Response of okra (*Abelmoschus esculentus*) to lime and phosphorus fertilization in an acid soil. World J Agri Sci 1:178–183

Oyinlola EY, Jinadu SA (2012) Growth, yield and nutrient concentration of tomato as affected by soil textures and nitrogen. Asian J Agric Res 6(1):39–45

Parvaiz A, Satyawati S (2008) Salt stress and phyto-biochemical responses of plants- a review. Plant Soil Environ 54(3):89–99

Passioura JB (2002) Soil conditions and plant growth. Plant Cell Environ 25:311–318

Philip B, Shittu AM, Aiyelaagbe IOO, Adedokun T (2009) Economic potentials of plantain and fluted pumpkin intercropping as a poverty reduction strategy in south-western Nigeria. World J Agric Sci 5(5):525–534

Plucknett DL (1993) International agricultural-research for the next century. Bioscience 43:432–440

Postgate J (1998) Nitrogen fixation, 3rd edn. Cambridge University Press, Cambridge, UK

Powlson DS, Gregory PJ, Whalley WR, Quinton JN, Hopkins DW, Whitmore AP, Schoenholtza SH, Van Miegroet H, Burger JA (2000) A review of chemical and physical properties as indicators of forest soil quality: challenges and opportunities. For Ecol Manag 138:335–356

Reid JB, Goss MJ (1981) Effect of living roots of different plant species on the aggregate stability of two arable soils. J Soil Sci 32:521–541

Saboora A, Kiarostami K, Behroozbayati F, Hashemi SH (2006) Salinity (NaCl) tolerance of wheat genotypes at germination and early season growth. Pak J Biol Sci 9(11):2009–2021

Sanchez PA (1997) Changing tropical soil fertility paradigms: from Brazil to Africa and back. In: Moniz AC, Furlani AMC, Schaffert RE, Fageria NK, Rosolem CA, Cantarella H (eds) Plant-soil interactions at low soil pH: sustainable agriculture and forestry production. Brazilian Soil Science Society, Campians, pp 19–28

Schoenholtz SH, Van Miegroet H, Burger JA (2000) A review of chemical and physical properties as indicators as indicators of forest soil quality: challenges and opportunities. For Ecol Manag 138:335–356

Schuman GE, McCalla TM (1976) Effect of short-chain fatty acids extracted from beef cattle manure on germination and seedling development. Appl Environ Microbiol 31(5):655–660
Sen A (1981) Poverty and famines: an essay on entitlement and deprivation. Clarendon, Oxford
Sharifi RS, Taghizadeh R (2009) Response of maize (*Zea mays* L.) cultivars to different levels of nitrogen fertilizer. J Food Agric Environ 7(3&4):519–521
Shortall JG, Liebhardt WC (1975) Yield and growth of corn as affected by poultry manure. J Environ Qual 4(2):186–191
Sims JT, Bitzer CC (1988) Estimating the availability of poultry manure through laboratory and field studies. J Environ Qual 17(1):47–54
Soon YK (1985) Soil nutrient availability: chemistry and concept. Macmillan, Ontario
Stacey G, Burris RH, Evans HJ (1992) Biological nitrogen fixation. Chapman and Hall, New York
Steyn JM, du Plessis HF (2012) Fertilisation and nutrient deficiencies of potatoes. In: Denner FDN, Venter SI, Niederwieser JG (eds) Guide to potato production in South Africa. ARC-VOPI, Pretoria
Stirzaker RJ, Passioura JB, Wilms Y (1996) Soil structure and plant growth: impact of bulk density and biopores. Plant Soil 185:151–162
Stoorvogel JJ, Smiling EMA (1990) Volume 1: Main report. In: Assessment of soil nutrient depletion in sub-Saharan Africa: 1983–2000, vol 2, Report 28. Winand Staring Centre, Wageningen
Súarez N, Medina E (2005) Salinity effect on plant growth and leaf demography of the mangrove, Avicennia germinans L. Trees 19:721–727
Szabolcs I (1994) Prospects of soil salinity for the 21st century. 15th world congress of soil science, Acapulco, 10–16, pp 123–141
Tikhonovich IA, Provorov NA, Ramonov VI, Newton WE (1995) Nitrogen fixation- fundamentals and applications. In: Proceedings of the 10th international congress on nitrogen fixation held in Russia in May 1995, St Petersburg
Tilman D, Kenneth GC, Matson PA, Rosamond N, Polasky S (2002) Agricultural sustainability and intensive production practices. Nature 418:671–677
Tisdale SL, Nelson WL (1975) Soil fertility and fertilizers, 3rd edn. Macmillan, New York
Tshikalange TE (2007) Response of *Brassica rapa* L. subsp. *chinensis* to nitrogen, phosphorus and potassium in pots. M.Tech. dissertation, Tshwane University of Technology, Pretoria
Ukpore BA (2009) Sustainable development in Nigeria: roles of women and strategies for their improvement. J Sust Dev Afr 10(4):268–283
Van Averbeke W, Chabalala MP, Okorogbona AOM, Ramusandiwa TD, Azeez JO, Slabbert MM (2012) Plant nutrient requirements of African leafy vegetables. In: Oelofse A, Van Averbeke W (eds) Nutritional value and water use of African leafy vegetables for improved livelihoods. WRC, Pretoria
Vance CP, Graham PH (1995) Nitrogen fixation in agriculture: application and perspectives. In: Tikhonovich IA, Provorov NA, Ramonov VI, Newton WE (eds) Nitrogen fixation: fundamentals and applications: proceedings of the 10th international congress on nitrogen fixation held in Russia in May 1995, St Petersburg, pp 77–86
Waggoner PE (1995) How much land can ten billion people spare for nature? Does technology make a difference? Technol Soc 17:17–34
Wilkinson SR (1979) Plant nutrient and economic value of animal manures. J Anim Sci 48(1):123–133
Wolfe DW, Topoleski DT, Gundersheim NA, Ingall BA (1995) Growth and yield sensitivity of four vegetable crops to soil compaction. J Am Soc Hortic Sci 120:956–963
Yeo AR, Flowers TJ (1980) Salt tolerance in the halophyte *Suaeda maritima* L. Dum: evaluation of the effect of salinity upon growth. J Exp Bot 31:1171–1183

Drought Stress Tolerant Horse Gram for Sustainable Agriculture

Jyoti Bhardwaj and Sudesh Kumar Yadav

Abstract Sustainable development is successful development that can be maintained. In agriculture sustainable development is seen as the new paradigm of economic development. Around three billion people living in rural areas are dependent on agriculture as their main source of income. It is not easy to sustain agricultural development given the various kinds of ever changing environmental challenges like metal toxicity, drought, cold, and salinity stresses that crops have to face. Drought stress is one of the most devastating environmental stress severely affecting crop growth, development and yield. To withstand drought stress and sustain the agricultural productivity we need to identify and maintain the natural crop resources.

Horse gram (*Macrotyloma uniflorum*) is a highly drought tolerant yet underexploited tropical legume, commonly known as 'kulthi'. The U.S. National Academy of Sciences in 1978 identified horse gram as potential food source for the future. Insurmountable drought tolerance and pest resistance together make it agriculturally an attractive crop. Besides normal cultivation *in vitro* regeneration of horse gram is an asset. Its high iron, low lipid, low sodium content and slow digestible starch make it a preferred choice for diabetic and obesity patients. Dark coloured seeds of horse gram in form of sprouts usually have higher antioxidant capacity as proven by various *in vitro* antioxidant assays. Seed extracts from horse gram contain isoflavones and glucopyranosides which show *in vitro* antilithic activity leading to reduced stone formation. When different processing techniques are used in combination they act synergistically reducing most of the antinutritional factors like polyphenols, lectins protease inhibitors in horse gram. However, some of the antinutrients have also been projected as health-promoting factors having anticancerous and antioxidant properties. Here we review the major points about horse gram: (1) morphology and physiology, (2) cultivation (3) composition, (4) medical implications, (5) antinutritional factors and their processing. To enhance horse gram's utilization potential future research areas are highlighted. Also, an effort has been made to compile updated research activities conducted on horse gram from 1968 to 2013.

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Keywords Horse gram • Nutrition • Drought tolerance • Antinutritional factors • Medicinal importance • Poor man's pulse

Abbreviations

UNWWDR	United Nations World Water Development Report
GDP	Gross Domestic Production
OECD	Organisation for Economic Cooperation and Development
NAS	National Academy of Sciences
LOX	Linoleate: Oxygen Oxidoreductase
TNAU	TamilNadu Agricultural University
PER	Protein Efficiency Ratio
EC	Enzyme Commission
Kcal	Kilo Calorie
NaCl	Sodium Chloride
Na_2CO_3	Sodium Carbonate
EC_{50}	Half Maximal Effective Concentration
IDDM	Insulin Dependent Diabetes Milletus
NIDDM	Non-Insulin Dependent Diabetes Milletus
ROS	Reactive Oxygen Species
DPPH	1,1-Diphenyl-2-Picryl-Hydrazyl
ABTS	2,2′-Azino-bis (3-Ethylbenz-thiazoline-6-sulfonic acid)
FRAP	Ferric Reducing Antioxidant Power
BBI	Bowman-Birk
HTST	High Temperature Short Time
EDTA	Ethylenediaminetetraacetic acid
TIA	Trypsin Inhibitor Activity

1 Introduction

Agriculture sustains the world. It was estimated in 2007 that around three billion people living in rural areas are dependent on agriculture as their main source of income. Rainfed agriculture is responsible for 80 % of the world's total cultivated land and 60 % of the crop production while remaining is occupied by irrigated agriculture. Sustaining the agriculture is important for the present and future generations which are increasing at an alarming rate. It is estimated that developing countries will have more (95 %) of the growth in urban population which may double between the years 2000–2030 (UNWWDR 2008). Unlike conventional agriculture, sustainable agriculture encompasses global, economic, social and environmental issues (Lichtfouse et al. 2009). All the issues are highly interrelated. Agriculture is directly related to global issues like climate change and global warming which involve temperature, rainfall, weather and increase in green house gases. All these factors can immensely affect agriculture (El-Ramady et al. 2013). Besides this, agriculture sector is an indispensible part of the economy of any country. Agricultural productivity is essential to be maintained not only in terms of quantity but also quality as it is directly proportional to better human health, which is one of the major concerns for any country (Lele 1991). Social issues like quality and accessibility of food are of prime importance to general masses. It is estimated that 1.4 billion people around the world live on just $1.25 a day (World Bank 2008). Increasing food prices intend a pun on the poor people leave alone the quality of food. The price hike of main agricultural commodities like wheat, corn and rice has risen to 41 % in the international market within a year (2007–2008). While poultry prices have doubled, butter and milk prices have tripled within the years (2000–2008). This has caused the number of people suffering from hunger to rise from 850 to 963 million in the present scenario (UNWWDR 2008). Balancing out the food resources in urban and rural areas remains a challenge in developing countries where the rich become richer and poor become poorer.

Environmental problems arise due to the actions of man and/or nature. Human actions are more and frequent in contributing towards such problems. Humans challenge nature through desertification and industrialization leading to a cascade of problems like pollution, soil fertility, soil erosion, declining resources of water, biodiversity and ecosystems. These all ultimately lead to climate change issues (El-Ramady et al. 2013). On the other hand, nature challenges flora and fauna with abiotic and biotic stresses. The cost of such disasters has been estimated to be 14 % of the GDP in poor countries (annual GDP per capita less than $760) while in rich countries (annual GDP per capita more than $9361) this rate is around 4 %. Since 1970, more than 7000 major disasters have been recorded worldwide with $2 trillion of damage. For example, in Kenya alone floods and drought has caused an annual loss of 22 % of GDP (Samuel et al. 2006). We particularly emphasize on drought stress as major abiotic stress to cope up with because it infinitely affects crops, animals, humans, land, soil and water resources (Bhardwaj and Yadav 2012a). A study has shown that 85 % of the world's population resides in the drier half of the earth and the use of water resource is uneven (WHO 2008). The 10 largest water users across globe are India, China, the United States, Pakistan, Japan, Thailand, Indonesia, Bangladesh, Mexico and the Russian Federation. It is estimated that although rural populations in Asia may remain stable but urban population is expected to increase by 60 % before 2025 leading to water scarcity (Ti 2007). Already, rapid population growth over the last 50 years has increased the water usage over three times. Therefore, it is predicted that in 2030, 47 % of the world population may live in areas of high water stress (OECD 2008). This all is going to ultimately affect the agriculture as it is the main user of water. Therefore, water is highly alarming danger for plants because it affects them more easily than humans. Not all plants are able to survive through drought periods, some being very sensitive and others somewhat tolerant. It is not very common to find crops that can not only survive such stresses but are also able to resist it effectively. Given the magnitude of the problem, we suggest naturally drought tolerant plants like horse gram as a part of answer to this major problem (Fig. 1).

Fig. 1 The 'sustainable agriculture' as sustained by the natural drought or other abiotic stress tolerant plants like horse gram. The progress and happiness of whole society is interdependent on various issues like social, economic, agricultural and global. Maintenance of such agriculturally important plants is of long term benefit for human society

Horse gram is a eudicot member among 236 genera of fabaceae (pea) family of legumes. It is thought to have been domesticated in India since prehistoric times. Its binomial name is *Macrotyloma uniflorum* (Lam.) Verdc., named after its authors Jean-Baptiste Pierre Antoine de Monet, Chevalier de La Marck, a naturalist and Bernard Verdcourt, a botanist. Its scientific synonym is *Dolichos biflorus* (auct.) or *Dolichos uniflorus* (Lam.). There are about 25 known species of horse gram most of which are located in Africa (Brink 2006; Verdcourt 1982).

It is commonly used for feeding horses and hence named horse gram. It is known by different regional names in India such as, gahat or kulath, kurti kalai, kulith (Maharashtra), ulavalu (Andhra Pradesh), hurali (Karnataka), madras bean or gram (Chennai), kollu (Tamil Nadu) and muthira (Kerala) (Brink 2006; Kawsar et al. 2009; Muthu et al. 2006). Being a drought tolerant legume having medicinal uses, horse gram carries potential importance which is underexplored. The U.S. National Academy of Sciences has identified horse gram as potential food source for the future (NAS 1978).

This review focuses on horse gram as a pulse crop of economic, agricultural and medicinal importance. Here, we have revived the fragmentary information available in the literature touching various aspects about horse gram so as to create a resurgence of interest and hence, improve its utilization as an important agricultural crop.

2 Morphology and Physiology

Morphology of horse gram seed and developmental stages of plant is shown in Fig. 2. Horse gram is herbaceous autogamous plant with twining annual branches. The stem is 60 cm tall, leaf arrangement is alternate, three foliolate, leaflets are

Fig. 2 The figure shows the horse gram plant at different stages of its development. (**a**) This part shows the variations in seed color of horse gram seeds; (a) pale brown color, (b) brown color, (c) black color and (d) spotted grey color. (**b**) horse gram seedlings after 7-day of germination in nutrient medium. (**c**) Uprooted horse gram plant showing roots. (**d**) Mature plant showing green pods. (**e**) Horse gram plants showing varietal difference in growth habit. (**f**) Fully mature and dry horse gram plants showing matured pods

ovate-rhombic or elliptical (2.5–5 cm), flowers are bisexual, cream or yellow in color with a purple blotch and fruit is a linear-oblong pod (3–8 cm) with 5–8 seeds inside. The seeds of horse gram are small rhomboidal, trapezoidal or oblong, pale to dark reddish brown, grey, all black or mottled with black or brown spots having a shining testa (Brink 2006; Kawsar et al. 2009; Duke and Reed 1981). It is a short day plant and requires an average temperature of 20–30 °C, but does not tolerate frost or water logging. It can be grown in areas with less than 980 mm annual rainfall and on poor soils with pH 5–7.5. It is cheap and good source of proteins, antioxidants and minerals. It is used as food and fodder in developing countries (Brink 2006; Bravo et al. 1999).

Different parts of the horse gram plant are used as medicine for the treatment of medical ailments such as heart conditions, asthma, bronchitis, leucoderma, urinary discharges and kidney stones (Ghani 2003). It is commonly prescribed for persons suffering from jaundice, common cold, cough, body pain, tiredness and obesity. It is thought to be helpful for iron deficiencies and maintaining body temperature in winter season (Ghani 2003; Bazzano et al. 2001). Seeds of this tropical legume are the richest source of lipoxygenase (LOX; linoleate:oxygen oxidoreductase, EC 1.13.11.12) activity known for its insurmountable pest resistance (Roopashree et al. 2006). However, it is affected by yellow mosaic virus (HgYMV), anthracnose (*Colletotrichum lindemuthianum*), leaf spot (*Cercospora dolichi*), rust (*Uromyces appendiculatum*) and root rot (*Pellicularia filamentosa*). Pests that can affect horse gram growth and yield are gram caterpillar (*Azazia rubricans*) and the green pod-boring caterpillar (*Etiella zinckenella*). The crop is also susceptible to iron chlorosis which greatly reduces its yield (Brink 2006; Sankar et al. 2002).

Horse gram requires minimum management and resources yet grows well under aberrant weather conditions. It grows well on poor soils with less than 980 mm of annual rainfall. Insurmountable drought tolerance and pest resistance together make it agriculturally an attractive crop.

3 Cultivation

Drought is one of the most devastating abiotic stresses affecting the productivity of pulses (Bhardwaj and Yadav 2012a). Therefore, in the remunerative export market of pulses, arid legumes have gained status. Despite being drought tolerant and deep rooted, horse gram (*Macrotyloma uniflorum*) still suffers low productivity. This is so because the area under kulthi (0.7 million ha) is quite less than that for well known legumes like chickpea (6 million ha), soybean (7 million ha) and mungbean (1 million ha). Consequently, the production for this little known legume also remains low as compared to the renowned legumes like chickpea, soybean and mungbean (Kharkwal and Gupta 2003; Fourth Advanced Estimates 2011).

Horse gram is largely cultivated in Southeast Asia especially in dry areas of Australia, Burma, India, Bangladesh, Sri Lanka and Tropical Africa. It is usually grown as crop for late *kharif* season and under aberrant weather conditions. This crop is used as green manure in the soil at the time of flowering to improve the soil fertility. The cultivation of horse gram is not only of dietary importance but also holds an economic value (Duke and Reed 1981; Brink 2006; Kawsar et al. 2009).

Horse gram should not be cultivated in a field previously sown with horse gram in order to avoid the mixing between varieties and occurrence of endemic pathogens. Loamy soil with neutral pH is best suited for horse gram (Brink 2006; TNAU agritech portal 2008). The organic matter content of the soil should not be very high as it will lead to vigorous seed production. The quality of the seeds sown should be genetically and physically pure. This is achieved by isolating the seed crop while sowing from other varieties to avoid cross pollination or by sowing some other crop in between (Sankar et al. 2002; TNAU agritech portal 2008). In India, horse gram is usually sown as sole crop but sometimes it is intercropped with finger millet, maize, chickpea and groundnut. Since horse gram does not tolerate frost, it is sown in warm season and harvested in cool dry climate (Brink 2006; TNAU agritech portal 2008).

Use of compost, urea, super phosphate, fungicide and rhizobium inoculation is considered as good practice during basal application in the field. Although horse gram is a drought tolerant crop it requires irrigation during flowering, pod formation and seed development (TNAU agritech portal 2008). Upon maturation the plants are uprooted, dried in the sun and threshed. Horse gram is harvested about 6 weeks after sowing for use as fodder. Horse gram is propagated by seeds sown in rows 20–90 cm apart and 1–2.5 cm in depth. Besides cultivation in fields, *in-vitro* regeneration of horse gram has been achieved by direct organogenesis using shoot tips, cotyledonary node explants and by somatic embryogenesis through cell suspension culture of callus induced on leaf explants (TNAU agritech portal 2008; Mohamed et al. 2004).

Drought is one of the most devastating abiotic stresses. Arid legume like horse gram has emerged as an important crop against such environmental stresses. There is a need to increase the area and productivity for horse gram. Besides normal cultivation *in vitro* regeneration of horse gram can be an asset.

4 Consumption

Pulses are useful for vegetarian class of society where animal proteins are usually considered costly and sometimes unsafe on health grounds. In India food is considered as medicine. Horse gram is considered as a pulse with medical value in ayurvedic science. Therefore, it forms an integral part in cuisines. It is consumed as whole seeds, dhal and sprouts by a large population in rural areas of India (TNAU Agritech portal 2008).

Whole seeds are cooked and consumed by frying in oil or used for the preparation of curry. Thick soup of horse gram is prepared by adding seeds in boiling water and stirred for some time to make thick slurry. This is commonly used for the treatment of cough and bronchitis in the rural areas of India. The cotyledon known as 'dhal' obtained after dehulling of horse gram is consumed in a diversity of forms depending on region. It has faster cooking time, increased digestibility and reduced antinutrient levels as compared to whole seeds. The splits can be soaked in water for 30–60 min and used for the preparation of curry. Besides this, it is often used to make special palatable dishes by frying in oil with onion and other spices (Kadam and Salunkhe 1985; Singh et al. 1991; Sreerama et al. 2010). Horse gram is sometimes germinated before it is used as human food. The seeds are soaked in water overnight and the soaked seeds are allowed to germinate for some hours. The sprouted seeds are either used for the preparation of curry or fried in oil with onion, chilli powder and other spices to make it tasty. This preparation is often used as a vegetable in many parts of India (Kadam and Salunkhe 1985).

The use of dry seeds of horse gram is very fragmentary due to its poor cooking quality. Nonetheless, the consumption of horse gram seeds, after processing such as soaking or dry heating followed by cooking along with rice, sorghum or pearl millet is a common practice among the rural people in India (Siddhuraju and Manian 2007). The sauce prepared from the cooking liquors of coloured beans, contains mainly the seed coat pigments such as dietary tannins and non-tannin phenolics and is very popular among the village people in certain parts of India (Siddhuraju and Manian 2007; Sreerama et al. 2010). Horse gram flour is used for the preparation of certain recipes by mixing it with other cereal flours. Supplementation of horse gram dhal with sesame flour (8 %) significantly improved the growth rate and protein efficiency ratio (PER) suggesting better utilization of horse gram protein (Vijaylaksmi and Venkatrao 1977). Ash content is an index of the quality of feeding materials used for poultry and cattle feeding. Because the ash content of embryonic axe and seed coat fractions of horse gram is lower, these fractions are suitable for feed materials in livestock production farms. Horse gram fodder being rich in protein is widely used as a feed to milch animals and horses (Maheri-Sis et al. 2007; Ravindran and Sundar 2009; Sreerama et al. 2010).

Horse gram is consumed in many forms both by humans and animals. It is consumed as whole seeds, dhal and sprouts by a large population in rural areas. Consuming processed or cooked horse gram is more desirable and beneficial as it has reduced antinutritional factors as compared to the raw horse gram. Besides providing a protein rich diet, horse gram has good amount of fiber and low ash content and hence it is used as feed and fodder for livestock maintenance.

5 Composition

Pulses are rich in lysine but deficient in tryptophan and sulfur containing essential amino acids (methionine and cysteine). Whereas cereals have sulfur containing amino acids but they are lacking in lysine. It is for this reason that cereals and pulses are taken in combination so as to provide us with complete protein diet. Horse gram occupies a very important place in human nutrition of many developing countries because it is nutritious yet inexpensive and abundantly available food source (Singh and Singh 1992).

Legume seeds have three major components; seed coat, cotyledon, and embryo. Seed coat fractions in comparison to cotyledons, usually have higher dietary fiber content (26–38 %) mainly consisting of insoluble dietary fiber (Sreerama et al. 2010). The cotyledon is the most nutritious part of the seed and contains water (6–9 %), protein (17.9–25.3 %), carbohydrates (51.9–60.9 %), energy (200–300 kcal), fiber (3–4 %), lipids (0.58–2.06 %) and dehulled ash (1–2.92 %) (Sosulski and Young 1979; Sreerama et al. 2010; Sudha et al. 1995; Messina 1999). The embryo is about 2–5 % of grain weight. Although it is rich in proteins but being the smallest part of the seed it cannot contribute significantly to the nutritive value (Kadam and Salunkhe 1985). Data on composition of various nutrients in horse gram is presented in Table 1.

Table 1 General compositional and functional information regarding horse gram shows it nutritive value for humans and livestock

S. No.	Nutrients/functional properties/antinutrients	Value
1.	Protein (%)	23.6
2.	Fat (%)	1.36
3.	Carbohydrates (%)	61.9
4.	Crude fibre (%)	5.3
5.	Ash (%)	3.3
6.	Water absorption capacity (g/g)	2.0
7.	Oil absorption capacity (g/g)	2.0
8.	Foaming capacity (% volume increase)	23.6
9.	Polyphenols (%)	1.60
10.	Phytic acid (%)	0.66
11.	Trypsin inhibitor activity (U/g flour)	94.0
12.	Hemagglutinins (HU/g seed)	2.6
13.	Oligosaccharides (%)	0.5–3

5.1 Proteins

Pulses are most important source of proteins. Many comprehensive reviews dealing with the legume proteins are available (Millerd 1975; Boulter and Derbyshire 1978; Messina 1999). Amongst legume family, the proximate composition may be similar but the protein content varies to a good extent (Mushtari-Begum et al. 1977). Seeds of horse gram contain 23.6 % protein which is much higher in content than whole egg protein (7–13 %). But characteristically like other legumes, horse gram cannot match the essential amino acid composition of an egg protein (Kadam and Salunkhe 1985; USDA 2011). As compared to animal proteins utilization of legume proteins is less because of their low digestibility and poor cooking quality. However, ungerminated horse gram protein has been found to be more digestible than other legume proteins. Seed coat accounts for 13.7 % of the whole seed in horse gram with a crude protein content ranging between 7 and 9 % (Kadam and Salunkhe 1985; Satwadhar et al. 1981). Extraction studies on horse gram protein have shown sodium chloride (NaCl) and sodium carbonate (Na_2CO_3) salts to be the best medium for extracting proteins (Borhade et al. 1984a). Water and oil absorption functions were observed to be better after such extractions. The protein content in horse gram is increased to a certain extent as an adaptive mechanism against drought stress conditions (Khandpal et al. 1981; Bhardwaj and Yadav 2012b).

5.2 Lipids

Lipid content of legumes depends upon variety, origin, location, soil and environmental conditions. With few exceptions like peanut, soy bean and winged bean, most legumes are low in total lipid content (1.0–7.2 %) (Sessa and Rackis 1977;

Mahadevappa and Raina 1978). Palmitic and linoleic acids are the principal fatty acids in horse gram but inconspicuous amounts of these fatty acids cannot directly and significantly contribute towards human health. Horse gram contains 2.2–2.36 % of lipids (Mahadevappa and Raina 1978). However, unique 10 % lipid content in IC 212722 line, belonging to a new species of wild horse gram has been reported (Yadav et al. 2004). This wild species of horse gram was identified from the Garhwal Himalayas and was named as *Macrotyloma sar-garhwalensis* Gaur and Dangwal (Gaur and Dangwal 1997). The low lipid content in horse gram might be advantageous for shelf life and weight restriction diets but is disadvantageous for their utilization in food products with respect to flavor, structure binding, oil retention and loss of fat during the formulation (Sreerama et al. 2010).

5.3 Carbohydrates

Horse gram contains 50–60 % carbohydrates comprising mainly of sugars and starch. Among sugars, oligosaccharides like galactose (0.1–0.5 %), glucose (0.1–7 %), sucrose (0.1–1.5 %), raffinose (0.7–6.2 %), stachyose (2.0–14.5 %) and verbascose (3.1–4.1 %) predominate in horse gram. The raffinose family oligosaccharides are notorious for flatulence production when ingested (Kadam and Salunkhe 1985; Sreerama et al. 2010). Many prebiotic oligosaccharides are marketed in form of products such as aerated drinks, cookies, cereals, candies, and infant foods (Tomomatsu 1994; Nakakuki 2003). Hence, carbohydrates from horse gram when enzymatically or chemically treated find an application here. In a study on extraction of carbohydrates from horse gram, non-digestible carbohydrates were found to be 24 %, 28–37 % starch and 3.6 % free sugars. Husk contains mostly cellulose and no ethanol soluble sugar or starch (Faki et al. 1983; Asp et al. 1996).

5.4 Vitamins and Minerals

Vitamins as the name suggests are vital in very small concentrations for over all human growth and development. Deficiency of vitamins is associated with occurrence of diseases like, night blindness (vitamin A), beri-beri (vitamin B). Legumes are good source of vitamins particularly thiamin, riboflavin, and niacin. However, horse gram in comparison to other legumes has lower amount of thiamin (0.42 mg/100 g seed), riboflavin (0.2 mg/100 g seed) and niacin (1.5 mg/100 g seed) (Kadam and Salunkhe 1985; Sreerama et al. 2010).

Horse gram contains more minerals than vitamins like potassium (762 mg/100 g seed), calcium (105 mg/100 g seed), zinc (3.4 mg/100 g seed), magnesium (172 mg/100 g seed), iron (11.9 mg/100 g seed) and phosphorus (310 mg/100 g seed) (Kadam and Salunkhe 1985; Sreerama et al. 2010). However, as compared to animals, plant minerals are less readily available due to the complex formation between phenols or phytates and minerals. Horse gram has fairly good amount of phosphorus and iron. The phosphorus is mainly associated with phytic acid. In the case of horse gram, the phytate phosphorus accounts for about 55 % of the total phosphorus (Borhade et al. 1984b; Turner et al. 2002). Horse gram contains higher amount of iron (11.9 mg/100 g) than other legumes and has significantly lower sodium content (25–35 mg/100 g) which makes it nutritionally advantageous in diets for people suffering with high blood pressure (Kadam and Salunkhe 1985; Bravo et al. 1999).

Horse gram is a nutritious yet inexpensive and abundantly available pulse legume. Cotyledon is the most nutritious part of the seed. Like other legumes horse gram is low in total lipid content. However, a unique 10 % lipid content in IC 212722 line, belonging to a new species of wild horse gram (*Macrotyloma sar-garhwalensis* Gaur and Dangwal) has been reported.

6 Medical Implications

Oxidative and other kind of stresses generated due to diseased body state are combated by antimicrobial and anticancer agents like carotenoids, vitamins and phenolic compounds that are naturally present in vegetables, fruits and pulses (Rates 2001; Lee et al. 2004; Kintzios 2006). People are becoming more aware about health benefits from the pulses which are easily accessible and cheap source of human nutrition. Horse gram is one such pulse crop carrying mammoth medicinal importance (Table 2). Experiments and clinical trials relating horse gram to cytotoxicity, antimicrobial and hemolytic activity have been conducted. The different extracts (ethyl acetate, dichloromethane, aqueous and butanol extracts) of horse gram were found to be non-toxic in the cytotoxicity test (brine shrimp lethality assay) with the conclusion that they could be potential therapeutic agents. The ethyl acetate and dichloromethane extract showed antimicrobial activity while aqueous and butanol extracts did not show any such significant activity. The 1-butanol extract of horse gram with EC_{50} value 200 μg/ml for hemolytic assay was considered to be the most active of all the other extracts (Kawsar et al. 2003, 2008a, b, 2009).

6.1 Diabetes and Obesity

Diabetes (milletus) is a chronic disease with elevated levels of sugar (glucose) in blood. It has nothing to do with diabetes insipidus (caused by deficiency of vasopressin or antidiuretic hormone) except for the similarity in the first name and very few of the symptoms. However, it is potentially associated with obesity through a unique signaling molecule named resistin (Steppan et al. 2001) Insulin is a hormone produced by pancreas to control blood sugar. Diabetes milletus could arise due to too little production of insulin (Type I diabetes or Insulin Dependent

Table 2 Summary of health benefits of horse gram

S. No.	Part of horse gram plant	Major components	Health benefits	Miscellaneous benefits
1.	Seed coat	Insoluble dietary fiber	Improves bowel movement	Food and fodder
		Calcium	Strengthens bones	Food product formulations
		Phenolics	Reduces oxidative stress related to heart diseases, cancer and inflammation	Endogenous antioxidants
		Ash content	Low ash content index of feeding quality	Livestock maintenance
2.	Seeds	Carbohydrates	Slow digestible starch, galacto-oligosaccharides help in growth of intestinal bifidobacteria, linked with reduced risk of diabetes, obesity and heart diseases.	oligosaccharides used as prebiotics in various products as aerated drinks, candies, infant food etc.
		Proteins	Cheaper and safer protein source on health grounds, improves protein efficiency ratio, reduces plasma low density lipoprotein	
		Lipids	Improves shelf life, used in weight restriction diets, possesses hypolipidemic activity	
		Vitamins	Overall growth and development	
		Minerals	Low sodium and high iron advantageous for high blood pressure	
		Bioactive peptides	Antioxidant activity, antihepatotoxic activity	
		Trypsin inhibitors	Suppression of carcinogenesis	
3.	Dark coloured seeds	Higher phenolics	High ferric reducing antioxidant power (FRAP)	Elevated levels of antioxidative enzymes
4.	Soup of horse gram seeds	Isoflavones Glucopyranosides	Potential against cold, throat infections, fever, generates heat and possesses antilithic activity, inhibits calcium oxalate crystallization	

Different parts of horse gram carry different medicinal properties which are considered beneficial for humans in or other medical ailments. Different parts of horse gram, their main constituents and the health benefit derived out of it is mentioned correspondingly

Diabetes Milletus (IDDM) or juvenile diabetes), resistance to insulin (Type II diabetes or Non-Insulin Dependent Diabetes Milletus (NIDDM) or adult-onset diabetes) and gestational diabetes. Type I usually occurs in children and teenagers when body makes little or no insulin at all. Although diabetes cannot be cured, daily injections of insulin are required for survival. Type II is most common and occurs in adulthood because of high rates of obesity. During gestational diabetes high blood glucose levels occur in pregnant ladies who never had diabetes before (ADAM 2011).

Diabetes has become a very common and serious problem across the globe. The composition of legumes like horse gram besides providing good amount of protein has been linked to reduce the risks of medical ailments like diabetes and obesity (Kadam and Salunkhe 1985; Bazzano et al. 2001; Pittaway et al. 2008). It possesses slow digestible starch, which is considered to have low postprandial glucose response when consumed by diabetic patients (Bravo et al. 1998). Acarbose is a synthetic amylase inhibitor which is used to control type II diabetes. It inhibits the starch-digesting enzymes, reducing starch digestion and absorption which lowers the postprandial hyperglycaemic response. This hypoglycaemic effect is favourable as it alleviates the course of type II diabetes (Chiasson et al. 2002). Phenolic compounds present in seed coat fractions of horse gram may mimic this action of acarbose against α-amylase. Low lipid content in horse gram is useful in weight restriction diets which help to fight obesity (Sreerama et al. 2010).

6.2 Antioxidants

Antioxidants can be referred to as reductants which inactivate reactive oxygen species (ROS) generated at higher levels in body under stressful conditions. These ROS create oxidative stress and sequentially prove to be toxic at high levels. Horse gram has good antioxidant capacity against ROS. Dark coloured seeds of horse gram usually have higher antioxidant capacity. Horse gram has higher DPPH (1,1-diphenyl-2-picryl-hydrazyl), ABTS [2,2′-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid)] radical scavenging activity and FRAP (Ferric Reducing Antioxidant Power) than other legumes (Marathe et al. 2011; Siddhuraju and Manian 2007). Various *in vitro* antioxidant assays like reducing power assay, DPPH assay, total phenolic assay and total antioxidant assays have shown that sprouts rather than the seeds of horse gram have higher antioxidant capacity (Ramesh et al. 2011). Aqueous extract of horse gram has reducing and stabilizing effect. The polyphenols of horse gram are shown to reduce the gold and silver ions (Vidhu et al. 2011; Aswathy Aromal et al. 2012). Horse gram flour has highest concentration of total phenolics and total flavonoids compared to cowpea and chickpea flour. It shows significantly higher hydrogen peroxide scavenging activity, reducing power and metal chelating ability than chickpea flour. Furthermore, hyperglycemia and hypertension inhibitory activities are relatively higher in horse gram flour than other two flours viz. chickpea and cowpea flour (Sreerama et al. 2012; Siddhuraju and Manian 2007).

Administration of methanolic extracts from horse gram to rabbits with oxidative stress induced by a high fat diet, led to improvement in antioxidant enzymes activity such as superoxide dismutase, catalase and increase in reduced glutathione concentration (Muthu et al. 2006). Therefore, the study of the importance and role of non-nutrient compounds particularly phenolic acids, flavonoids and high molecular tannins of legumes as natural antioxidants have greatly increased. These studies suggested that phenolic substances from raw seeds as well as from processed seeds of horse gram are potent antioxidants. In an another study on plants exposed to lead toxicity antioxidant enzymes like superoxide dismutase, catalase, peroxidase, glutathione reductase, glutathione-s-transferase were shown to play significant role against oxidative injury (Reddy et al. 2005b).

6.3 Hypercholestrol

Hypercholesterolemia is usually an asymptomatic metabolic disorder caused due to abnormalities in the levels of lipids (hyperlipidemia) or lipoproteins (hyperlipoproteinemia) which ultimately lead to elevated levels of cholesterol in the blood. This disorder is related to cardiovascular diseases, diabetes, obesity and thyroid problems. Hereditary hypercholesterolemia is called as familial hypercholesterolemia and it may lead to development of atherosclerosis. In addition to its nutritional impact, legume protein has been shown to reduce plasma low density lipoprotein when consumed (Zech and Hoeg 2004; Bhatnagar et al. 2008).

The methanolic extract of whole plants of horse gram has shown hypolipidemic activity in rats fed with high fat diet. The globulin fraction from horse gram also has hypolipidemic action in rats fed with a high-fat-high-cholesterol diet (Muthu et al. 2005). Effect of certain pulses on the total cholesterol and phospholipid levels of the serum, liver and aorta in rats fed with hypercholesterolaemic and normal diets had been compared. Black gram (*Phaseolus mungo*), red gram (*Cajanus cajan*) and horse gram (*Dolichos biflorus*) showed a remarkable cholesterol-lowering effect in serum, liver and aorta of rats. Serum phospholipid levels were also found to be lowered (Saraswathy and Kurup 1970). These studies suggest that horse gram could be potentially effective against hypercholesterol.

6.4 Kidney Stones

Kidney or urinary stones, also known as renal calculi are formed as solid aggregations in the kidney from the dietary minerals like sodium and calcium oxalate present in the urine. These are classified according to their location and composition. Small kidney stones are passed in the urine without pain but become problematic if they grow above the size of 3 mm (Finkielstein and Goldfarb 2006; Coe et al. 2008; Johri et al. 2010). The soup obtained after boiling horse gram seeds with or without spices has the potential to dilute the kidney stones by generating heat (Siddhuraju and Manian 2007). Seed extracts from horse gram contain isoflavones and glucopyranosides which show *in vitro* antilithic activity (Mitra et al. 1983; Garimella et al. 2001). Recently, extracts of horse gram have been found to be effective *in vitro* in inhibition of calcium oxalate crystallization which could lead to reduced stone formation (Das et al. 2005).

6.5 Miscellaneous

The physicochemical properties and chemical components of dietary fibres in horse gram show various physiological effects in the gastrointestinal tract of humans. The galacto-oligosaccharides found in horse gram promote the growth of intestinal bacteria especially bifidobacteria which are important for maintenance of human health (Alles et al. 1999).

Horse gram is recommended to women having abnormal menstrual cycle. *In vivo* and *in vitro* models have shown horse gram trypin inhibitors to be associated with suppression of carcinogenesis (Sreerama et al. 2010; Sreerama and Gowda 1997). The phenolic compounds and phytic acid in horse gram enhance the enzyme activity of antioxidant pathway as well as those related to starch, lipid and protein metabolism (Sreerama et al. 2010). Processes like apoptosis, blood vessel dilation, platelet aggregation and carcinogen detoxification are also affected by these compounds leading to better human health (Shahidi and Wanasundara 1992; Tapiero et al. 2002). The rats intoxicated with paracetamol were cured after feeding diet rich in horse gram seeds. This recovery was attributed to antihepatotoxic activity of horse gram (Laskar et al. 1998). *In vivo* experiments with rats have shown healing activity of dietary lipids from horse gram (Jayaraj et al. 2000).

Experiments and clinical trials relating horse gram to cytotoxicity, antimicrobial and hemolytic activity have suggested it as a source of potential therapeutic agents. Its slow digestible starch and low lipid content are important for diabetic and obesity patients. Dark coloured seeds of horse gram in form of sprouts usually have higher antioxidant capacity as proven by various *in vitro* antioxidant assays. As compared to other legumes hyperglycemia and hypertension inhibitory activities have been found to be relatively higher in horse gram. Seed extracts from horse gram contain isoflavones and glucopyranosides which show *in vitro* antilithic activity which could lead to reduced stone formation.

7 Antinutritional Factors of Horsegam

Compared to other sources (cereals and vegetables) utilization of food legumes like horse gram in human diet is rather low due to the presence of many antinutritional factors like phytates, polyphenols, protease inhibitors, lectins, hemagglutinins and flatulence-causing factors. These factors lower the nutritional quality of any food legume. The antinutritional factors of horse gram are discussed below.

7.1 Phytates and Polyphenols

Like other food legumes, horse gram possesses phytochemicals like phytates and polyphenols that are known to form complexes with minerals and proteins thereby reducing their availability during digestion. Phytic acid contains most of the total phosphorus present in the horse gram and mostly located in the cotyledon fraction of seeds. It reduces the availability of minerals like calcium, zinc, iron and magnesium. Hence, phytic acid is mainly responsible for poor digestibility of proteins and hard-to-cook nature of horse gram (Turner et al. 2002; Borhade et al. 1984b).

Phenolic compounds belonging to secondary metabolites group are abundant in horse gram seed coat (Siddhuraju and Manian 2007). The phenolic compounds have been reported to act as endogenous antioxidants thereby preventing oxidative stress that is related to diseases such as coronary heart disease, some types of cancer and inflammation (Tapiero et al. 2002). It has also been reported that phenolic compounds and their inhibitors are higher in seed coat while phytic acid, oligosaccharides and proteinaceous inhibitors are higher in the cotyledons of horse gram. The ability to inhibit digestive enzymes like trypsin and α-amylase is attributed to these compounds (Sreerama et al. 2010; He et al. 2006). Earlier, many chemical compounds belonging to different categories like phytochemicals, flavonoids and sterols have been isolated from horse gram (Incham et al. 1981; Handa et al. 1990). *In vitro* and *in vivo* studies project these phytochemicals with antinutrient effects as health-promoting and disease-preventing factors. Antioxidant, metal ion-chelating and free radical scavenging activities are frequently attributed to phenolic compounds. Presence of tannins in horse gram may have a positive role as an antioxidant and anticancer agent (Borhade et al. 1984b). This attracts more and more interest from both researchers and food manufacturers.

7.2 Protease Inhibitors

Protease inhibitors of horse gram have the ability to inhibit proteases like trypsin and chymotrypsin. Some of the suggested functions of the protease inhibitors include their role as storage proteins, regulation of endogeneous proteases and as protective agents against insect or microbial predators (Ryan and Green 1974).

Bowman-Birk (BBI) and kunitz are the two most well studied of the serine protease inhibitors from horse gram. Despite being notorious for reducing the digestibility of proteins some research work attributed anticarcinogenic property to bowman-birk type of protease inhibitors (Ikenaka and Norioka 1986; Prakash et al. 1996). A double headed BBI has been isolated from horse gram (Sreerama and Gowda 1998). Similar, BBI type isoinhibitors have been isolated and their structure/reactive site determination has been carried out on horse gram (Mehta and Simlot 1982). Recently, profiles of BBIs during germination and seed development have been studied in horse gram and their presence has been shown in the leaf, husk and flower (Sreerama and Gowda 1998).

Other widely studied groups of inhibitors are trypsin, chymotrypsin and trypsin-chymotrypsin inhibitors. Presence and role of these inhibitors in horse gram has been shown. Several studies on the appearance and disappearance of electrophoretically distinct protease inhibitors during germination in horse gram have been conducted. Presence of these distinct inhibitors at very early stages of seed maturation and vegetative parts may be vital for natural plant defense (Sreerama and Gowda 1997, 1998).

7.3 Lectins

Lectins are proteins that have affinity for specific carbohydrate moieties and are used as a model system for studying protein–carbohydrate interactions. They bind to glycoproteins in the peritrophic matrix lining midgut disrupting the digestive processes and nutrient assimilation. Lectins are particularly abundant in the seeds of legumes and are located in protein bodies. They constitute up to 10 % of the soluble protein in the seed extracts of horse gram (Ranjekar et al. 2003; Talbot and Etzler 1978a). They play an important role in seed maturation, cell wall assembly, rhizobial nodulation of legume roots and defence mechanisms (Gardner 1991; Porta and Rocha-Sosa 2002).

LOX proteins contribute to plant growth, maturation, senescence and metabolic responses to pathogen attack. LOX activity has been observed to be associated with a lectin from horse gram. The hydrophobic site and presence of Mn^{2+} ion on the tetrameric lectin accounts for the observed LOX activity of horse gram seed. LOX activity associated with this molecule provides a new dimension to lectin functions of horse gram. This dual function lectin may help to explain the pest resistance of horse gram (Roopashree et al. 2006). Studies on purification and characterization of lectins from horse gram seeds has been conducted (Kocourek et al. 1977; Roopashree et al. 2006). *In vivo* biosynthetic studies on horse gram lectins have revealed the events that take place during the synthesis of lectins (Toress-Pinedo 1983; Quinn and Etzler 1989).

In humans, lectins bind to human gut cell wall lining causing blood clotting, loss of epithelial cells, diminished digestion and absorption, interference with hormones and signalling factors, and shift in microbial flora. If after rupturing the gut cell lining, lectins leak into the circulation they can cause allergic reactions and immune system disruption (Pusztai et al. 1993). However, lectins in moderate concentrations could be non-toxic. Also processing methods like cooking and fermentation can affect them substantially. Their resistance to gut proteolysis and binding to membrane cells in digestive tract makes them potential tools for drug targeting applications thus suggesting their application in medicine and research (Roopashree et al. 2006).

7.4 Hemagglutinins

Hemagglutinins compounds have the ability to agglutinate the red blood cells. Hemagglutinins from horse gram are known to be composed of four identical subunits. Lectins purified from horse gram has shown erythroagglutinating activity which was enhanced by ions like, Ca^{2+}, Co^{2+}, Ni^{2+} and Mg^{2+} but inhibited by galactosamine and EDTA (Subbulaksmi et al. 1976; Manage et al. 1972). Experiments regarding oral administration of hemagglutinins from horse gram to rats have shown retarded growth (Anisha and Prema 2008). Fractionated crude extracts from aerial parts of horse gram have shown significant hemolytic activity in mouse erythrocytes (Kawsar et al. 2009). The toxicity of these compounds can be reduced by treatments like moist heat, cooking and germination (Khader and Rao 1981).

7.5 Flatulence Producing Factors

Oligosaccharides like galactose, glucose, sucrose, raffinose, stachyose, and verbascose have been implicated in flatulence production in man and animals. Most of these are located in the cotyledon fractions of horse gram (Sreerama et al. 2010). Their ingestion by humans requires the activity of α-galactosidase enzyme which is not present in the upper gut. Hence, incomplete hydrolysis of these sugars leads to production of gases. The non-digestible oligosaccharides from horse gram have been found to be reduced by external enzymatic treatment with galactosidase (Sreerama et al. 2009; Faki et al. 1983). These studies indicate towards large scale production of flatulence free horse gram flour. Another study suggests that increasing the rate of digestion of carbohydrates may not reduce the flatulence caused by horse gram. The enzymatic treatment before dehulling reduces non-starch polysaccharides but does not alter the cooking properties of horse gram (Jyoti and Reddy 1981).

The antinutritional factors in horse gram can be reduced by various processing methods thus making horse gram a nutritious food choice. Besides, some of these antinutrients have also been projected as health-promoting factors having anticancerous and antioxidant properties. These factors have been found to be associated with pest resistance, natural plant defense and could be utilized in drug targeting applications.

8 Processing

Most of the pulses have antinutrients like protease inhibitors, lectins, polyphenols, phytates, and oligosaccharides which need to be reduced if not eliminated before human consumption. The physical separation of different morphological parts in legumes has the greatest impact on the nutrient and antinutrient contents of the grain legumes (Sreerama et al. 2010). Therefore, processing of pulses is conducted so as to make them appealing by enhancing their digestibility and nutritive value. The processed food products of pulses are highly nutritive and delicious, hence very popular. Not only dhal but their by-products are also potential ingredients for specialty food products. There is no common processing method for all pulses. However, general procedures are discussed in successive paragraphs.

8.1 Commercial Processing

8.1.1 Milling

Pulses are usually converted into dhal by milling procedures before being used in human diet. The removal of outer husk and splitting of grain into two equal halves is known as milling of pulses. The traditional milling of pulses involves preconditioning of pulses for loosening of husk and subsequent dehulling and splitting. This can be achieved by wet milling and dry milling processes (Kadam and Salunkhe 1985).

Wet Milling

The wet method involves mixing of legume seeds with small quantities of water sequentially. The water is allowed to be absorbed for several hours followed by sun-drying where in the seeds are spread in thin layers in drying yards with periodical stirring. This process helps in shrinkage of the endosperm more than that of the husk, resulting in a “bubbled” husk which can be easily removed by the shearing action of the shelling machine. Moreover, prolonged soaking of grains in water forces the cotyledons to cave in at the surface of fusion and touch with each other at the periphery. This allows the sheller machine to simultaneously split the cotyledons while removing the husk. However, this method is laborious, time-consuming, and dependent on climatic conditions. Mechanical drying can be an option to make it independent of climatic conditions but results in higher cost of energy and equipment (Sreerama et al. 2010; TNAU agritech portal 2008).

Dry Milling

This method is more popular and used in commercial mills. It is suitable for operational convenience, greater turnover and production economy. General operations under dry milling are the same i.e. preconditioning and loosening of husk followed by dehulling and splitting of seeds. The steps involved are of cleaning, grading, pitting, oiling, moistening and drying of seeds (TNAU agritech portal 2008).

Preconditioning and Loosening of Husk

Pulses are cleaned from extraneous materials like dust, grits and graded according to size where in the filled seeds are separated from broken seeds by a rotating sieve type cleaners. Such sieves of different sizes are easily available in the market. A sieve size of 8/64″ (3.2 mm) is recommended for horse gram seeds. The clean pulses are passed through an emery roller known as gota machines where the husk is cracked and scratched by friction between pulses and emery. This is known as pitting or rolling. The pitted pulses are oiled with some edible oil like linseed oil for shine and better appeal. Oil penetrates through the husk into the gum layer and releases its binding under mild heat of the sun. Oiling prevents excessive loss of moisture even on prolonged sun-drying (Kadam and Salunkhe 1985; TNAU agritech portal 2008).

Watering or moisture addition is conducted in a mixer called 'worm mixer' or 'screw conveyer'. This is to facilitate the subsequent oil penetration process for the loosening of husk. The pulses are kept on the floor for about a day so that the diffusion of the oil can occur smoothly. Conditioning of pulses is done by alternate wetting and drying. After sun drying for a certain period, moisture is added to the pulse and it is again dried in the sun. Addition of moisture (3–5 %) to the pulses can be accomplished by allowing water to drop from an overhead tank on the pulses being passed through a screw conveyor. The whole process of alternate wetting and drying is continued for 2–4 days until all pulses are sufficiently conditioned. Pulses are finally dried for some time till moisture content of about 10–12 % is achieved (TNAU agritech portal 2008).

Dehulling and Splitting

Dehulling of legumes in general results in variations in the content of nutrients and antinutrients in different milled fractions because the nutrients and antinutrients in legumes are unevenly distributed in the seed (Shahidi et al. 2001; Sreerama et al. 2010). Pulses like arahar, urad, moong and lentil are difficult to dehull as a result of it repeated operations by dehulling rollers are required. Roller mills are also used but cause a loss of kernel by scouring of the surface on the dehulled grain which also leads to a loss of surface proteins. These losses are particularly high if the grains are not well graded by size. To obtain complete dehulling of the grains large abrasive force is applied which results in high losses in the form of broken pieces and powder. Yield of split and pulses is only 65–75 % due to the above losses while the expected potential yield is 82–85 % (Kurien 1977; TNAU agritech portal 2008).

Dehulled pulses are split into two parts by burr grinders. The husk and powder from the products is aspirated off by blowers and the split pulses are separated by sieving. The tail pulses and unsplit dehulled pulses are again conditioned and milled as above. The whole process is repeated two to three times until the remaining pulses are dehulled and split. Splitting causes loss of embryo (amounting to 2–5 % of grain weight) and breakage of the edges of the cotyledon. Non uniform loosening of the husk due to inadequate premilling treatments results in splits with patches of husk. Improved milling technology with over 95 % yield in a single operation has been developed at the Central Food Technological Research Institute, Mysore, in India (Sreerama et al. 2010; TNAU agritech portal 2008).

Generally, horse gram seeds are most often ground into meal. This is used for a number of preparations either alone or in combination with cereal grains or oilseeds. This is utilized for the preparation of thick soup, mostly by the poor in sections of India. The grinding is done in a mortar/stone grinders or hammer mills. Mechanized mortars are available for this purpose where larger quantities are handled (TNAU agritech portal 2008).

8.2 Processing at Home

8.2.1 Germination

Germination has been suggested as an inexpensive and authenticated processing technique for improving the nutritional quality of legumes by enhancing their digestibility; increasing the level of amino acids and reducing the content of antinutritional factors. Pulses in raw form have low protein digestibility due to non absorbance of the nitrogen present in pulses (Burr 1975). During germination the free amino acid content and *in vitro* protein digestibility is increased probably due to hydrolysis of proteins (Subbulaksmi et al. 1976). Although the mineral content of germinated seeds of legumes have been lowered than the original seeds but increased content of vitamin B complex like niacin, riboflavin, choline, biotin contents and ascorbic acids has been observed (Sathe et al. 1984). Germinated and sprouted pulses have been used to prevent and cure scurvy (Venugopal and Rao 1978). Increase in the activity of starch degrading enzymes reduces the flatulence production and results in higher reducing sugars content during germination (Subbulaksmi et al. 1976; Jyoti and Reddy 1981). Besides affecting the nutrients, germination process reduces or eliminates most of the antinutritional factors like polyphenols and phytates probably by their hydrolysis, leaching and phytase activity resulting in higher availability of bound nutrients (Lolas and Markakis 1975; Borhade et al. 1984b).

8.2.2 Cooking

Correct application of heat during cooking pulses can eliminate most toxic factors without impairing the nutritional value, increase the palatability and contribute towards pulse digestibility. Prolonged heating can lead to loss of nutrients. The cooking time is considerably reduced if the legumes are dehulled and split into dhal. The growth rate of rats and digestibility of proteins of cooked horse gram dhal were higher as compared with the cooked whole seed (Vijaylaksmi and Venkatrao 1977).

Cooking has been reported to decrease the protease inhibitors and hemagglutinin activity and hence, improve the protein digestibility (Liener 1976). Heat causes denaturation of the proteins responsible for trypsin inhibition and hemagglutination. Improved digestibility of starch from horse gram has been reported after cooking hence, lowering the flatulence production (Jyoti and Reddy 1981). Some studies suggest that reduction in flatulence is observed after cooking as compared to the raw seeds (Venkataraman and Jaya 1975). However, contradicting studies are available regarding flatulence production from legumes indicating that cooking or increasing the rate of digestion of horse gram starch may not be correlated to flatulence production (Khader and Rao 1981). It has also been reported that decrease in the antinutrient factors during processing is not the actual decrease in their content but occurs due to leaching or the formation of insoluble complexes between these factors and other components in the seed which make them incapable of chemical colour reaction (Butler et al. 1979).

The mode of application of heat is important. It has been reported that cooking alone can reduce the antinutrient factors like phytic acid and polyphenols but when combined with other processing procedures like soaking or germination, they tend to act synergistically (Kumar et al. 1978). Autoclaving and soaking followed by heating are effective. Studies on thermal stability of trypsin inhibitor from horse gram showed that it is not affected by dry heat as much as by high temperature short time (HTST), autoclaving and boiling water (Sreerama et al. 2008).

8.2.3 Soaking and Roasting

During soaking, hydration of seeds occurs which markedly reduces the cooking time and leads to decrease in antinutrients like polyphenols, phytic acid and trypsin inhibitor activity. Soaking treatment is known to soften the pellicle of the grain, improve the *in vitro* digestibility, solubilize the proteins and starch components. Soaking in water is the first step in most methods of preparing pulses for consumption. However, better soak solutions than water consisting of sodium bicarbonate, sodium carbonate, sodium chloride, sodium polyphosphate and citric acid have been recommended for better results. Sodium bicarbonate is thought to provide smooth structure to the legume grain by facilitating uniform and central penetration of the soaking solution but concentration above 2 % in the solution is not beneficial. Therefore, right concentration and ratio of components is essential for a good soaking solution. Soaking reduces the oligosaccharides of the raffinose family hence, contributing towards reduction in flatulence production (Rockland and Metzler 1967; Bongirwar and Srinivasan 1977).

Roasted legumes are incorporated in snack preparations commonly consumed by poor sections of the population. Roasted legumes like those of bengal gram and peas are widely used in India which is not so in the case of horse gram. Studies have revealed that roasting of horse gram seeds improved the growth rate, PER, and digestibility in rats as compared with cooked horse gram seeds (Kadam and Salunkhe 1985). Roasting also renders the husk to be separated easily (Vijaylaksmi and Venkatrao 1977). The processing of food pulses by fermentation increases their digestibility, palatability and nutritive value. Fermentation process improves the availability of essential amino acids and hence, the nutritional quality of protein. In general, the nutritive value of the legume based fermented foods has been shown to be higher than their raw counterparts. Such studies on horse gram require further investigation (Reddy and Salunkhe 1980).

Processing techniques like germination reduces most of the antinutritional factors like polyphenols and phytates probably by their hydrolysis. Cooking improves the protein digestibility by decreasing the protease inhibitors and hemagglutinin activity. Soaking causes hydration of seeds to occur improving the *in vitro* digestibility. When different processing procedures are combined with one another they tend to act synergistically.

9 Uses and Future Perspectives

9.1 As Nutraceuticals

A nutraceutical is a substance that is a food or part of a food that provides health benefits (Shahidi et al. 2001). Horse gram fractions with variations in the contents of fibre might find application in different specialty food products for target populations. The processed seed coat fractions of horse gram containing phenolic compounds could be a valuable addition to diets supplemented with cereal proteins. Phenolic compounds which inhibit the activities of α-amylases and proteases provide an attractive target for the development of potential therapeutic agents to treat variety of diseases. Researchers have studied polyphenolic constituents of various legume seeds and reported that they contain potential nutraceutical properties (Siddhuraju and Manian 2007; Sreerama et al. 2010). Hence, these could advance the nutraceutical research.

Utilization of horse gram and its products is limited due to the presence of antinutritional components, poor functional and expansion properties. Enzymatic treatment was used to improve the expansion and functional properties of horse gram to facilitate its use as an ingredient in functional foods (Jyoti and Reddy 1981). Xylanase-mediated depolymerization of cell wall polysaccharides of horse gram lead to the development of a new expanded horse gram. These results suggest the potential utility of under-utilized expanded horse gram grains or their flours in legume composite flours as ready-to-eat breakfast foods. Horse gram fractions having higher trypsin inhibitor activity (TIA) could be used as functional food ingredient similar to soybean BBI concentrate (Blanca et al. 2009). Furthermore, seed coat fractions of legumes with high fiber and low protein may be useful in food product formulations (Table 3).

Table 3 The summary of the various processing methods used for pulses including horse gram

S. No.	Commercial processing		S. No.	Processing at home	
	Type of processing	Process summary		Type	Associated benefits
1.	Milling	Removal of husk by preconditioning of pulses for subsequent dehulling and splitting	1.	Germination	Increases free amino acid content, *in vitro* protein digestibility and starch degrading enzymes, lowers flatulence and antinutritional factors
1a.	Wet milling	Mixing of water and seeds followed by hours of sun drying	2.	Cooking	Increases palatability, digestibility, Lowers toxic factors
		Method is laborious, time consuming and climate dependent			
		Less costly			
1b.	Dry milling	Pulses are cleaned, graded, pitted, moistened and dried, dehulled and split into dhal	3.	Soaking	Increases *in vitro* protein digestibility, reduces cooking time, flatulence production and antinutrient factors
		Used for large scale production, greater turnover, more economic	4.	Roasting	Increases growth rate, digestibility and protein efficiency ratio
		More costly	5.	Fermentation	Increases palatability, nutritive value, digestibility and availability of amino acids

These processing methods are commercial as well as non commercial that is used at home for processing. All these processing methods help in reducing the antinutritional constituents and effects thereof from pulses like horse gram

9.2 *High Yielding Pedigrees*

There are no suitable early and synchronous maturing pedigrees of horse gram that could provide high grain yield, agronomic traits and biomass which is desired for dry land agriculture. The crop if exploited properly could help in reducing the dependence on the major pulses which are not only costly but also less in supply. There is a great need to increase the productivity and stabilise the yields of horse gram by exploring suitable pedigrees which are resistant to stresses. As a result, attention is being focussed on programs at genetic level involving collection and conservation of horse gram germplasm. Characterization and correlation studies on variability of horse gram germplasm in India have been conducted. This unique legume is of much importance to breeders engaged in crop breeding programs (Chahota et al. 2005; Sankar et al. 2002).

Since it is an autogamous plant, it might have meager possibilities of genetic variability. Conventional breeding practices for improvement in this crop have not been very fruitful (Kishore et al. 2007). Genetic variability is primary prerequisite for crop improvement. The role of induced mutations in crop improvement is evident from a large number of improved high yielding varieties of several crops developed through mutation breeding within the shortest possible time. Desirable mutations can be created through gamma rays (Bolbhat and Dhumal 2009). Two improved varieties of horse gram *viz*. KBHG-1 and BJPL-1 have been developed in India but their overall potentialities are yet to be tested before they are approved at the national level (Prakash et al. 2002, 2008).

9.3 Miscellaneous Uses

Series of natural products from higher plants have become clinical agents. The chemical compounds isolated from horse gram can be used as possible chemotaxonomic markers (Kawsar et al. 2009). Horse gram could be utilized as ration in poultry farms (Ravindran and Sundar 2009). One of the most interesting strategies is to develop selective low molecular weight inhibitors from natural sources like horse gram. Designing of smaller protease inhibitors as cancer preventive agents can be employed a by utilizing the studies on reactive sites and antigenic determinants of inhibitors from horse gram (Kennedy 1998). Horse gram flour is used as supplement in ethanol production for fuel generation (Reddy and Reddy 2005). All the research activities conducted on or related to horse gram (1968–2012) have been complied in Table 4.

Table 4 Various research activities conducted on or related to horse gram since the year 1968 to 2013 with its references

S. No.	Nature of research activity conducted on horse gram	References
1.	Comparative MSAP analysis of horse gram	Bhardwaj et al. (2013)
2.	Biochemical parameters and antioxidant activity	Bhardwaj and Yadav (2012b)
3.	Computational identification of miRNAs	Bhardwaj et al. (2010)
4.	Nutrients and antinutrients in milled fractions	Sreerama et al. (2010)
5.	Expansion properties and ultrastructure	Sreerama et al. (2009)
6.	Bioactive peptides with antioxidant activity	Chand (2009)
7.	Purification and characterization of cysteine protease	Jinka et al. (2009)
8.	Gamma mutations	Bolbhat and Dhumal (2009)
9.	Haemolytic activity	Kawsar et al. (2009)
10.	Supplement for poultry feed	Ravindran and Sundar (2009)

(continued)

Table 4 (continued)

S. No.	Nature of research activity conducted on horse gram	References
11.	Effect of enzyme pre-treatments	Sreerama et al. (2009)
12.	Nutritional implications and flour functionality	Sreerama et al. (2008)
13.	Identification of horse gram varieties for Karnataka	Prakash et al. (2008)
14.	Non-digestible oligosaccharides	Anisha and Prema (2008)
15.	Isolation of phenolic compounds	Kawsar et al. (2008a)
16.	Cytotoxic activity	Kawsar et al. (2008b)
17.	Antimicrobial activity	Kawsar et al. (2008b)
18.	Free radical scavenging capacity of phenolic extracts	Siddhuraju and Manian (2007)
19.	Variability and correlation studies	Kishore et al. (2007)
20.	Dual function lectin	Roopashree et al. (2006)
21.	Antioxidant potential of methanolic extract	Muthu et al. (2006)
22.	Inhibition of calcium oxalate crystallization	Das et al. (2005)
23.	Lead induced changes in antioxidant activity	Reddy et al. (2005b)
24.	Improvement in ethanol production	Reddy and Reddy (2005)
25.	Hypolipidemic effect in methanolic extract	Muthu et al. (2005)
26.	Alfatoxin contamination	Reddy et al. (2005a)
27.	Characterization of germplasm from Himachal	Chahota et al. (2005)
28.	Isolation of thermostable lipoxygenase	Roopashree and Rao (2004)
29.	Protein and oil rich wild horse gram	Yadav et al. (2004)
30.	*In vitro* plant regeneration	Mohamed et al. (2004)
31.	Phytochemical studies	Kawsar et al. (2003)
32.	Effect of yellow mosaic virus on seed yield	Sankar et al. (2002)
33.	Identification of suitable varieties	Prakash et al. (2002)
34.	Performance on medium black soils	Parameshwarappa and Lamani (2002)
35.	Storage protein degradation	Jinka and Rao (2002)
36.	In vitro antilithic activity	Garimella et al. (2001)
37.	Healing activity against duodenal ulcers	Jayaraj et al. (2000)
38.	Genetic parameters	Prakash and Khanure (2000)
39.	Novel hydrophobic binding site in lectin	Hamelryck et al. (1999)
40.	Plant type for yield based on selection indices	Nagaraja et al. (1999)
41.	Bowman-Birk type proteinase inhibitor	Sreerama and Gowda (1998)
42.	Antihepatotoxic activity	Laskar et al. (1998)
43.	Reactive site determination for inhibitors	Sreerama and Gowda (1997)
44.	New horse gram species	Gaur and Dangwal (1997)
45.	Genetic variability performance	Samal and Senapati (1997)
46.	Structure determination of HGI-III isoinhibitor	Prakash et al. (1996)
47.	Performance in sub-humid zone of Nigeria	Omokanye (1996)
48.	Role of disulfide linkages of protease inhibitor	Ramasarma et al. (1995)
49.	Leaf growth in relation to seed yield	Sahaul et al. (1995)
50.	Nutrients and antinutrients	Sudha et al. (1995)
51.	Effect of nitrogen, phosphorous and zinc	Choudhary and Singh (1994)

(continued)

Table 4 (continued)

S. No.	Nature of research activity conducted on horse gram	References
52.	Effect of fluoride on germination	Dhanunjayanath et al. (1993)
53.	Effect to powdery mildew	Patil and Moghe (1993)
54.	Characterization of adenine binding sites	Gegg et al. (1992)
55.	Starch mobilization during germination	Karunagaran and Rao (1991)
56.	Water deficit factor limiting leaf area	Narayanan and Anuradha (1991)
57.	Proline metabolism	Reddy and Veeranjaneyulu (1991)
58.	Axial control of protease development	Karunagaran and Rao (1990)
59.	Isolation of pyroglutaminylglutamine (diuretic principle)	Handa et al. (1990)
60.	Effect of water stress on tissue ionic changes	Reddy and Veeranjaneyulu (1990)
61.	Axial control of protease development	Karunagaran and Rao (1990)
62.	*In vivo* biosynthetic studies	Quinn and Etzler (1989)
63.	Digestion of seed from hard seeded selections	Nortron et al. (1989)
64.	Genotypes times environment interactions	Henry and Daulay (1988)
65.	Primary structure of seed lectin	Schnell and Etzler (1987)
66.	*In vitro* synthesis of seed lectin	Schnell et al. (1987)
67.	Nature of lectins	Kumar and Rao (1986)
68.	Solubilization properties	Borhade et al. (1984a)
69.	Subcellular localization of lectins	Etzler et al. (1984)
70.	Development and distribution of seed lectins	Roberts and Etzler (1984)
71.	Changes in phytate phosphorus and minerals	Borhade et al. (1984b)
72.	Carbohydrate composition	Faki et al. (1983)
73.	Isoflavone from seeds	Mitra et al. (1983)
74.	Acid stable trypin-chymotrypsin inhibitor	Mehta and Simlot (1982)
75.	Structural comparision of lectin subunits	Roberts et al. (1982)
76.	Isolation of dolichin A, B and pterocarpans	Incham et al. (1981)
77.	Monoclonal antibody against seed lectin	Borrebaeck and Etzler (1981)
78.	Effects of germination on polyphenols	Satwadhar et al. (1981)
79.	Digestibility of carbohydrates	Khader and Rao (1981)
80.	Carbohydrate binding properties	Etzler et al. (1981)
81.	Effect of germination and cooking	Jyoti and Reddy (1981)
82.	Improvement in cooking quality	Kadam et al. (1981)
83.	Carbohydrate binding activity of glycoprotein	Etzler and Borrebaeck (1980)
84.	Yield and functional properties	Sosulski and Young (1979)
85.	Radioimmuno assay of lectin	Talbot and Etzler (1978a)
86.	Isolation of a protein that cross reacts with antibody	Talbot and Etzler (1978b)
87.	Protein quality of sprouted horse gram	Chandrasekhar and Chitra (1978)
88.	Properties of lectins	Kocourek et al. (1977)
89.	NH_2- terminal sequences of lectins	Etzler et al. (1977)
90.	Varietal difference in protein	Mushtari-Begum et al. (1977)
91.	Effect of germination on carbohydrates	Subbulaksmi et al. (1976)
92.	Dietary component in chick ration	Banerjee and Mandal (1975)
93.	Circular dichroism and conformational transition	Pere et al. (1975)

(continued)

Table 4 (continued)

S. No.	Nature of research activity conducted on horse gram	References
94.	Isolation and characterization of predominant lectin	Carter and Etzler (1975a)
95.	Isolation of cyanogens bromide fragments	Carter and Etzler (1975b)
96.	Isolation of subunits of lectins	Carter and Etzler (1975c)
97.	Proximate composition and amino acid makeup	Manage and Sohonie (1972)
98.	Toxicity to rats	Manage et al. (1972)
99.	Effect on high fat and cholesterol diet on rats	Prema and Kurup (1973)
100.	Horse gram lectins	Etzler (1972)
101.	Effect on hypercholesterolaemic diet	Saraswathy and Kurup (1970)
102.	Isolation of plant hemagglutinin	Etzler and Kabat (1970)
103.	Determination of biological value	Ray (1970)
104.	Toxic factors	Ray (1969)
105.	Distribution of nutrients in anatomical parts	Singh et al. (1968)
Related reviews		
1.	Genetic mechanisms of drought stress tolerance	Bhardwaj and Yadav (2012a)
2.	Descriptor analysis, anthocyanin indexes and potential uses	Morris (2008)
3.	Genetic improvement of drought resistance	Mitra (2001)
4.	Nutritional composition, processing and utilization	Kadam and Salunkhe (1985)
5.	Legumes in human nutrition	Salunkhe (1982)
6.	General properties of pant proteins	Boulter and Derbyshire (1978)
7.	Biochemistry of legume seed protein	Millerd (1975)

Related review articles are also listed

10 Conclusion

Agriculture industry is on the verge of unparalleled advancement. This rapid expansion without competitive increase in the availability of new and better varieties of important plants would be a handicap. Therefore, it becomes mandatory to carryout research for identifying and evaluating inexpensive unconventional alternatives like horse gram for the future. Horse gram is an under exploited yet nutritionally one of the most important pulse crops. Its cultivation is cost effective and economic. It is called poor man's pulse as it sustains millions of people when everything else seems obscure Horse gram is used as food and fodder for humans as well as livestock. It carries medical importance against diseases like diabetes, kidney stones and hyperlipidemia. Processing is beneficial for its use in food, as it reduces the associated antinutritional factors. Horse gram has many future implications in neutraceuticals, functional foods and therapeutics.

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References

ADAM (2011) Medical encyclopedia, Diabetes

Alles MS, Hartemink R, Meyboom S, Harryvan JL, VanLaere K, Nagengast FM (1999) Effect of transgalactooligosaccharides on the composition of the human intestinal microflora and on putative risk markers for colon cancer. Am J Clin Nutr 69:980–991

Anisha GS, Prema P (2008) Reduction of non-digestible oligosaccharides in horse gram and green gram flours using crude R-galactosidase from *Streptomyces griseoloalbus*. Food Chem 106:1175–1179. doi:10.1016/j.foodchem.2007.07.058

Asp NG, Van Amelsvoort JMM, Hautvast JGAJ (1996) Nutritional implications of resistant starch. Nutr Res Rev 9:1–31. doi:10.1079/NRR19960004

Aswathy Aromal S, Vidhu VK, Philip D (2012) Green synthesis of well dispersed gold nanoparticles using *Macrotyloma uniflorm*. Spectrochim Acta Part A 85:99–104

Banerjee GC, Mandal L (1975) Metabolizable energy value of horse gram or Kulthi (*Dolichos biflorus*) and its usefulness as a dietary component in chick ration. Indian J Poult Sci 10:99–103

Bazzano L, He J, Ogden LG, Loria C, Vuputuri S, Myers L, Whelton PK (2001) Legume consumption and risk of coronary heart disease in US men and women. Arch Intern Med 161:2573–2578

Bhardwaj J, Mahajan M, Yadav SK (2013) Comparative analysis of DNA methylation polymorphism in drought sensitive (HPKC2) and tolerant (HPK4) genotypes of horsegram. Biochem Genet 51:493–502. doi:10.1007/s10528-013-9580-2

Bhardwaj J, Mohammad H, Yadav SK (2010) Computational identification of microRNAs and their targets from the expressed sequence tags of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.). J Struct Funct Genomics 11:233–240

Bhardwaj J, Yadav SK (2012a) Genetic mechanisms of drought stress tolerance, implications of transgenic crops for agriculture. Sustain Agric Rev 8:213–235. doi:10.1007/978-94-007-1905-7_9

Bhardwaj J, Yadav SK (2012b) Comparative study on biochemical parameters and antioxidant enzymes in a drought tolerant and a sensitive variety of horse gram (*Macrotyloma uniflorum*) under drought stress. Am J Plant Physiol 7:17–29. doi:10.3923/ajpp.2012.17.29

Bhatnagar D, Soran H, Durrington PN (2008) Hypercholesterolaemia and its management. BMJ 337:a993. doi:10.1136/bmj.a993

Blanca HL, Hsieh CC, Lumen BOD (2009) Lunacin and Bowman-Birk protease inhibitor (BBI) in US commercial soy foods. Food Chem 115:574–580

Bolbhat SN, Dhumal KN (2009) Induced macromutations in horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.). Legum Res 32:278–281

Bongirwar DW, Srinivasan A (1977) Development of quick cooking peas. J Food Sci Technol 4:17–23

Borhade VP, Kadam SS, Salunkhe DK (1984a) Solubilization and functional properties of moth bean (*Vigna aconitifilium* (Jacq.) Marechal and horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) proteins. J Food Biochem 8:229–235

Borhade VP, Kadam SS, Salunkhe DK (1984b) Changes in phytate phosphorus and minerals during germination and cooking of horse gram and moth bean. Qual Plant Plant Foods Hum Nutr 34:151–157. doi:10.1007/BF01094843

Borrebaeck CAK, Etzler ME (1981) Production and characterization of a monoclonal antibody against the seed lectin of the *Dolichos biflorus* plant. J Biol Chem 256:4723–4725

Boulter D, Derbyshire E (1978) The general properties, classification and distribution of plant proteins. In: Norton G (ed) Plant proteins. Butterworths, London, pp 3–24

Bravo L, Siddhuraju P, Saura-Calixto F (1998) Effect of various processing methods on the *in vitro* starch digestibility and resistant starch content of Indian pulses. J Agric Food Chem 46:4667–4674

Bravo L, Siddhuraju P, Saura-Calixto F (1999) Composition of underexploited Indian pulses. Comparison with common legumes. Food Chem 64:185–192

Brink M (2006) *Macrotyloma uniflorum* (Lam.) Verdc. In: Brink M, Belay G (eds) PROTA 1: Cereals and pulses/Céréales et légumes secs. (CD-Rom). PROTA, Wageningen

Burr HK (1975) Pulse proteins. In: Friedman M (ed) Protein nutritional quality of foods and feeds, vol 1. Marcel Dekker, New York, p 119

Butler LG, Hagerman AE, Price ML (1979) Biochemical effects of sorghum polyphenols. In: Hulse JH (eds) Proc Symp polyphenols in cereals and legume. IFT, St. Louis, p 36

Carter WG, Etzler ME (1975a) Isolation and characterization of subunits from the predominant form of *Dolichos biflorus* lectin. Biochemistry 14:2685–2689

Carter WG, Etzler ME (1975b) Isolation and characterization of cyanogen bromide fragments and a glycopeptide from the *Dolichos biflorus* lectin. Biochemistry 14:5118–5122

Carter WG, Etzler ME (1975c) Isolation, characterization and subunit structures of multiple forms of *Dolichos biflorus* lectin. J Biol Chem 250:2756–2762

Chahota RK, Sharma TR, Dhiman KC, Kishore N (2005) Characterization and evaluation of horse gram germplasm from Himachal Pradesh. Indian J Plant Genet Resour 18:221–223

Chand S (2009) Bioactive peptides isolated from alcalase hydrolysed horse gram (*Macrotyloma uniflorum*) protein exhibiting antioxidant activity. New Biotechnol 25:S167–S168

Chandrasekhar V, Chitra S (1978) Evaluation of the protein quality of sprouted horse gram and green gram on albino rat. Indian J Nutr Diet 15:223–227

Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M (2002) Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. Lancet 359:2072–2077

Choudhary LS, Singh I (1994) Effect of nitrogen, phosphorus and zinc on growth and yield attributes of rainfed horsegram (*Macrotyloma uniflorum*) in Rajasthan. Indian J Agric Sci 64:257–258

Coe FL, Evan A, Worcester E (2008) Kidney stone disease. In: Marks AR, Neill US (eds) Science in medicine: the JCI textbook of molecular medicine. Part II: Kidney and urinary tract. Jones and Bartlett Publishers, Sudbury, pp 898–908

Das I, Gupta SK, Ansari SA, Pandey VN, Rastogi RP (2005) *In vitro* inhibition and dissolution of calcium oxalate by edible plant *Trianthema monogyna* and pulse *Macrotyloma uniflorum* extracts. J Cryst Growth 273:546–554. doi:10.1016/j.jcrysgro.2004.09.038

Dhanunjayanath V, Karunagaran D, Rao RK (1993) Effect of fluoride on germination and starch mobilization of horse gram seeds. Indian J Plant Physiol 36:131–133

Duke JA, Reed CF (1981) *Macrotyloma uniflorum* (Lam). Verdc. In: Duke JA (ed) Handbook of legumes of world economic importance. Plenum Press, New York, p 146

El-Ramady HR, El-Marsafawy SM, Lewis LN (2013) Sustainable agriculture and climate changes in Egypt. In: Lichtfouse E (ed) Sustainable agriculture reviews, vol 12. Springer Science+Business Media, Dordrecht, pp 41–95. doi:10.1007/978-94-007-5961-9_2

Etzler ME (1972) Horse gram (*Dolichos biflorus*) lectin. Methods Enzymol 38:340–344

Etzler ME, Kabat EA (1970) Purification and characterization of a lectin (plant hemagglutinin) with blood group A specificity from *Dolichos biflorus*. Biochemistry 9:869–877

Etzler ME, Borrebaeck C (1980) Carbohydrate binding activity of a lectin like glycoprotein from stems and leaves of *Dolichos biflorus*. Biochem Biophys Res Commun 96:92–97

Etzler ME, Gupta S, Borrebaeck C (1981) Carbohydrate binding properties of the *Dolichos biflorus* lectin and its subunits. J Biol Chem 256:2367–2370

Etzler ME, Talbot CF, Ziaya PR (1977) NH_2-terminal sequences of the subunits of *Dolichos biflorus* lectin. FEBS Lett 82:39–41

Etzler ME, Macmillan S, Scates S, Gibson MD, James WD JR, Cole D, Thayer S (1984) Subcellular localizations of two *Dolichos biflorus* lectins. Plant Physiol 76:871–878

Faki HAE, Desikachar HSR, Paramahans SV, Tharanathan RN (1983) Carbohydrate make-up of chickpea, cowpea and horse gram. Starch 35:163–166. doi:10.1002/star.19830350505

Finkielstein VA, Goldfarb DS (2006) Strategies for preventing calcium oxalate stones. Can Med Assoc J 174:1407–1409. doi:10.1503/cmaj.051517

Fourth Advanced Estimates (2011) Department of Agriculture and Cooperation, Govt. of India database

Gardner HW (1991) Recent investigations into the lipoxygenase pathway of plants. Biochim Biophys Acta 1084:221–239

Garimella TS, Jolly CI, Narayanan S (2001) *In vitro* studies on antilithiatic activity of seeds of *Dolichos biflorus* Linn. and rhizomes of *Bergenia ligulata* Wall. Phytother Res 15:351–355

Gaur RD, Dangwal LR (1997) New species of *Macrotyloma* (Wight and Arn.) Verdc. (Fabaceae) from Garhwal Himalaya, UP, India. J Bombay Nat Hist Soc 94:381–383
Gegg CV, Roberts DD, Segel IH, Etzler ME (1992) Characterization of the adenine binding sites of two *Dolichos biflorus* lectins. Biochemistry 31:6938–6942
Ghani A (2003) Medicinal plants of Bangladesh: chemical constituents and uses. Asiatic Society of Bangladesh, Dhaka, pp 5–16
Hamelryck TW, Loris R, Boukaert J, Dao-Thi MH, Strecker G, Imberty A, Fernandez E, Wyns L, Etzler ME (1999) Carbohydrate binding, quaternary structure and a novel hydrophobic binding site in two legume lectin oligomers from *Dolichos biflorus*. J Mol Biol 286:1161–1177
Handa G, Singh J, Nandi LN, Sharma ML, Kaul A (1990) Pyroglutaminylglutamine – a new diuretic principle from *Dolichos biflorus* seeds. Indian J Chem 29:1156–1158
He Q, LV Y, Yao K (2006) Effects of tea polyphenols on the activities of α-amylase, pepsin, trypsin and lipase. Food Chem 101:1178–1182
Henry A, Daulay HS (1988) Genotype times environment interaction for seed yield in horse gram (*Macrotyloma uniflorum*). Indian J Agric Sci 58:684–686
Ikenaka T, Norioka S (1986) Bowman-Birk family of serine proteinase inhibitors. In: Barrett AJ, Salvenson G (eds) Proteinase inhibitors. Elsevier, Amsterdam, pp 361–374
Incham JL, Keen NT, Markham KK, Mulheirn LJ (1981) Dolichins A and B, two pterocarpans from bacteria-treated leaves of *Dolichos biflorus*. Phytochemistry 20:807–809
Jayaraj AP, Tovey FI, Lewin MR, Clark CG (2000) Duodenal ulcer prevalence: experimental evidence for the possible role of dietary lipids. J Gastroenterol Hepatol 15:610–616
Jinka R, Ramakrishna V, Rao SK, Rao RP (2009) Purification and characterization of cysteine protease from germinating cotyledons of horse gram. BMC Biochem 10:28
Jinka R, Rao PR (2002) Storage protein degradation in germinating horse gram seeds. Indian J Plant Physiol 7:314–320
Johri N, Cooper B, Robertson W, Choong S, Rickards D, Unwin R (2010) An update and practical guide to renal stone management. Nephron Clin Pract 116:c159–c171
Jyoti E, Reddy PR (1981) The effect of germination and cooking on *in vitro* digestibility of starch in some legumes. Nutr Rep Int 23:799–804
Kadam SS, Salunkhe DK (1985) Nutritional composition, processing, and utilization of horse gram and moth bean. Crit Rev Food Sci Nutr 22:1–26. doi:10.1080/10408398509527407
Kadam SS, Subramanyam P, Jawale HK, Satwadhar PN, Jadhav SJ (1981) Improvement in cooking quality of horse gram *(Dolichus biflorus)* by pre-soaking treatment with salt solution. Plant Foods Hum Nutr 31:171–174
Karunagaran D, Rao PR (1990) Axial control of protease development in cotyledons of horse gram (*Macrotyloma uniflorum* Lam.) seeds during germination. Indian J Plant Physiol 33:232–238
Karunagaran D, Rao PR (1991) Mode and control of starch mobilization during germination of seeds of horse gram. Plant Sci 73:155–160
Kawsar SMA, Rahman MR, Huq E, Mosihuzzaman M, Nahar N, Mamun MR (2003) Studies of different extractives of *Macrotyloma uniflorum*. Dhaka Univ J Pharm Sci 2:81–84
Kawsar SMA, Huq E, Nahar N (2008a) Cytotoxicity assessment of the aerial parts of *Macrotyloma uniflorum* Linn. Int J Pharmacol 4:297–300
Kawsar SMA, Uddin MS, Huq E, Nahar N, Ozeki Y (2008b) Biological investigation of *Macrotyloma uniflorum* Linn. Extracts against some pathogens. J Biol Sci 8:1051–1056
Kawsar SMA, Mostafa G, Huq E, Nahar N, Ozeki Y (2009) Chemical constituents and hemolytic activity of *Macrotyloma uniflorum* L. Int J Biol Chem 3:42–48
Kennedy AR (1998) The Bowman-Birk inhibitor from soybean as an anticarcinogenic agent. Am J Clin Nutr 68:1406–1412
Khader V, Rao SV (1981) Digestibility of carbohydrates of raw and cooked bengal gram (*Cicer arietinum*), green gram (*Phaseolus aureus*) and horse gram (*Dolichos biflorus*). Food Chem 7:267–271
Khandpal RP, Vaidyanathan CS, Kumar MU, Sastry KSK, Rao NA (1981) Alterations in the activities of the enzymes of proline metabolism in Ragi (*Eleusine coracana*) leaves during water stress. J Biosci 3:361–370

Kharkwal MC, Gupta VP (2003) Advances in arid legumes research. Published for Indian Arid Legumes Society, Jodhpur, p 523

Kintzios SE (2006) Terrestrial plant-derived anticancer agents and plant species used in anticancer research. Crit Rev Plant Sci 25:79–113

Kishore N, Dogra RK, Thakur SR, Chahota RK (2007) Variability and correlation studies for some agro-morphological traits in horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.). Himachal J Agric Res 33:117–118

Kocourek J, Jamieson GA, Votruba T, Horejsi V (1977) Studies on phytohemagglutinins I. Some properties of the lectins of horse gram seeds (*Dolichos biflorus* L.). Biochim Biophys Acta 500:344–360

Kumar NS, Rao DR (1986) The nature of lectins from *Dolichos lablab*. J Biosci 10:95–109

Kumar KG, Venkatraman LV, Jaya TV, Krishnamurthy KS (1978) Cooking characteristics of some germinated legumes: changes in phytins Ca++, Mg++ and pectins. J Food Sci 43:85

Kurien D (1977) Grain legume milling technology. In: FAO expert consultation on grain legume processing. Central Food Technological Research Institute, Mysore, 14–18 Nov

Laskar S, Bhattacharyya UK, Sinhababu A, Basak BK (1998) Antihepatotoxic activity of kulthi (*Dolichos biflorus*) seed in rats. Fitoterapia 69:401–402

Lee J, Koo N, Min DB (2004) Reactive oxygen species, aging, and antioxidative nutraceuticals. Compr Rev Food Sci Food Saf 3:21–33

Lele SM (1991) Sustainable development: a critical review. World Dev 19:607–621

Lichtfouse E, Navarrete M, Debaeke P, Souchere V, Alberola C, Menassieu J (2009) Agronomy for sustainable agriculture. A review. Agron Sustain Dev 29:1–6. doi:10.1051/agro:2008054

Liener IE (1976) Legume toxins in relation to protein digestibility. J Food Sci 41:1071–1081

Lolas GM, Markakis P (1975) Phytic acid and other phosphorus compounds of beans (*Phaseolus vulgaris* L.). J Agric Food Chem 23:13

Mahadevappa VG, Raina PL (1978) Nature of some Indian legume lipids. J Agric Food Chem 26:1241–1243

Maheri-Sis N, Chamani M, Sadeghi AA, Mirza-Aghazadeh A, Safaei AR (2007) Nutritional evaluation of chickpea wastes for ruminants using *in vitro* gas production technique. J Anim Vet Adv 6:1453–1457

Manage L, Sohonie K (1972) Proximate composition, amino acid make up and *in vitro* proteolytic digestibility of horse gram *(Dolichus biflorus)*. J Food Sci Technol 9:35–36

Manage L, Joshi A, Sohonie K (1972) Toxicity to rats and mice of purified phytohemagglutinins from four Indian legumes. Toxicon 10:89–91

Marathe SA, Rajalakshmi V, Jamdar SN, Sharma A (2011) Comparative study on antioxidant activity of different varieties of commonly consumed legumes in India. Food Chem Toxicol 49:2005–2012. doi:10.1016/j.fct.2011.04.039

Mehta SL, Simlot MM (1982) An acid stable trypsin-chymotrypsin inhibitor from horse gram (*Dolichos biflorus*). J Biosci 4:295–306

Messina MJ (1999) Legume and soybeans: overview of their nutritional profiles and health effects. Am J Clin Nutr 70:439S–449S

Millerd A (1975) Biochemistry of legume seed protein*s*. Annu Rev Plant Physiol 26:53–72

Mitra J, Das A, Joshi T (1983) An isoflavone diglycoside from the seeds of *Dolichos biflorus*. Phytochemistry 22:1063–1064

Mohamed VS, Wang CS, Thiruvengadam M, Jayabalan N (2004) *In vitro* plant regeneration via somatic embryogenesis through cell suspension cultures of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.). In Vitro Cell Dev Biol Plant 40:284–289

Morris JB (2008) *Macrotyloma axillare* and *M. uniflorum*: Descriptor analysis, anthocyanin indexes and potential uses. Genet Resour Crop Evol 55:5–8

Mushtari-Begum J, Srihara P, Hiremath SR (1977) Varietal difference in protein of horse gram *Dolichus biflorus* (Linn.). Mysore J Agric Sci 11:521–524

Muthu AK, Sethupathy S, Manavalan R, Karar PK (2005) Hypolipidemic effect of methanolic extract of *Dolichos biflorus* Linn. in high fat diet fed rats. Indian J Exp Biol 43:522–525

Muthu AK, Sethupathy S, Manavalan R, Karar PK (2006) Antioxidant potential of methanolic extract of *Dolichos biflorus* Linn. in high fat diet fed rabbits. Indian J Pharmacol 38:131–132

Nagaraja N, Nehru SD, Manjunatha A (1999) Plant type for yield in horsegram as evidenced by path coefficient and selection indices. Karnataka J Agric Sci 12:33–37

Nakakuki T (2003) Development of functional oligosaccharides in Japan. Trends Glycosci Glycotechnol 15:57–64

Narayanan A, Anuradha M (1991) Water deficit as a factor limiting leaf area development in phosphorus deficient horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) plants. Indian J Plant Physiol 34:264–266

NAS (1978) Tropical legumes: resources for the future. National Academy of Sciences, Washington, DC

Nortron BW, Whitford C, Staples B (1989) Digestion of seed from hard-seeded selections of *Macrotyloma uniflorum* (Horse gram) by cattle. Trop Grassl 23:219–224

OECD (2008) Organisation for Economic Cooperation and Development. OECD Environment Outlook to 2030. Organisation for Economic Co-operation and Development Paris

Omokanye AT (1996) Performance of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.) in the sub-humid zone of Nigeria. Legume Res 19:52–54

Parameshwarappa SG, Lamani KD (2002) Performance of horsegram cultivars on medium black soils of Northern transitional zone of Karnataka. Karnataka J Agric Sci 15:627–629

Patil BG, Moghe PG (1993) Reaction of mung, urid, rajama and kulthi varieties to powdery mildew. J Maharashtra Agric Univ 18:467–468

Pere M, Bourrillon R, Jirgensons B (1975) Circular dichroism and conformational transition of *Dolichos biflorus* and *Robinia pseudoacacia* lectins. Biochim Biophys Acta 393:31–36

Pittaway JK, Robertson IK, Ball MJ (2008) Chickpeas may influence fatty acid and fiber intake in an ad libitum diet, leading to small improvements in serum lipid profile and glycemic control. J Am Diet Assoc 108:1009–1013

Porta H, Rocha-Sosa M (2002) Plant lipoxygenases: physiological and molecular features. Plant Physiol 130:15–21

Prakash B, Selvaraj S, Murthy MRN, Sreerama YN, Rajagopal Rao D, Gowda LR (1996) Analysis of the plant Bowman-Birk inhibitors. J Mol Evol 42:560–569

Prakash BG, Khanure SK (2000) Genetic parameters, correlation and path coefficient analysis in horsegram. Karnataka J Agric Sci 13:312–314

Prakash BG, Nayakar NY, Guled MB (2002) KBHG-1: a promising horsegram variety for Northern dry zone of Karnataka. Karnataka J Agric Sci 15:362–363

Prakash BG, Guled MB, Bhosale AM (2008) Identification of suitable horsegram varieties for northern dry zone of Karnataka. Karnataka J Agric Sci 21:343–345

Prema L, Kurup PA (1973) Effect of protein fractions from *Cajanus cajan* (red gram) and *Dolichos biflorus* (horse gram) on the serum, liver and aortic lipid levels in rats fed a high-fat-high-cholesterol diet. Atherosclerosis 18:369–377

Pusztai A, Ewen SW, Grant G, Peumans WJ, Van Damme EJ, Coates ME, Bardocz S (1993) Kidney bean lectin-induced Escherichia coli overgrowth in the small intestine is blocked by GNA, a mannose specific lectin. J Appl Bacteriol 75:360–368

Quinn JM, Etzler ME (1989) *In vivo* biosynthetic studies of the *Dolichos biflorus* seed lectin. Plant Physiol 91:1382–1386

Ramasarma PR, Rao AG, Rao DR (1995) Role of disulfide linkages in structure and activity of protinase inhibitor from horsegram (*Dolichos biflorus*). Biochim Biophys Acta 1248:35–42

Ramesh CK, Rehman A, Prabhakar BT, Vijay Avin BR, Aditya Rao SJ (2011) Antioxidant potentials in sprouts vs. seeds of *Vigna radiata* and *Macrotyloma uniflorum*. J Appl Pharm Sci 1:99–103

Ranjekar PK, Patankar A, Gupta V, Bhatnagar R, Bentur J, Kumar PA (2003) Genetic engineering of crop plants for insect resistance. Curr Sci 84:321–329

Rates SMK (2001) Plants as sources of drugs. Toxicon 39:603–613

Ravindran R, Sundar STB (2009) Nutritive value of horse gram (*Dolichos biflorus*) for egg-type chicks and growers. Tamilnadu J Vet Anim Sci 5:125–131

Ray PK (1969) Toxic factor(s) in raw horse gram *(Dolichos biflorus)*. J Food Sci Technol 6:207–211

Ray PK (1970) Nutritive value of horse gram *Dolichus biflorus.* III. Determination of biological value, digestibility and net protein utilization. Indian J Nutr Diet 7:71

Reddy BN, Brijitha N, Raghavender CR (2005a) Aflatoxin contamination in insect damaged seeds of horsegram under storage. Mycotoxin Res 21:187–191

Reddy AM, Kumar SG, Jyothsnakumari G, Thimmanaik S, Sudhakar C (2005b) Lead induced changes in antioxidant metabolism of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.) and bengalgram (*Cicer arietinum* L.). Chemosphere 60:97–104

Reddy LVA, Reddy OVS (2005) Improvement of ethanol production in very high gravity fermentation by horse gram (*Dolichos biflorus*) flour supplementation. Lett Appl Microbiol 41:440–444. doi:10.1111/j.1472-765X.2005.01767.x

Reddy NR, Salunkhe DK (1980) Changes in oligosaccharides during germination and cooking of black gram and fermentation of black gram rice blend. Cereal Chem 57:353–358

Reddy PS, Veeranjaneyulu K (1990) Effect of water stress on tissue ionic changes in horse gram, *Macrotyloma uniflorum* (Lam.). Geobios 17:154–156

Reddy PS, Veeranjaneyulu K (1991) Proline metabolism in senescing leaves of horse gram (*Macrotyloma uniflorum* Lam.). J Plant Physiol 137:381–383

Roberts DM, Etzler ME (1984) Development and distribution of a lectin from the stems and leaves of *Dolichos biflorus*. Plant Physiol 76:879–884

Roberts DM, Walker J, Etzler ME (1982) A structural comparison of the subunits of the *Dolichos biflorus* seed lectin by peptide mapping and carboxyl terminal amino acid analysis. Arch Biochem Biophys 218:213–219

Rockland LB, Metzler EA (1967) Quick cooking lima and other dry beans. Food Technol 21:344–348

Roopashree S, Rao AGA (2004) Thermostable iron containing low molecular weight lipoxygenase from *Dolichos biflorus*: purification and characterization. FASEB J 18:C13

Roopashree S, Singh SA, Gowda LR, AppuRao AG (2006) Dual-function protein in plant defence: seed lectin from *Dolichos biflorus* (horse gram) exhibits lipoxygenase activity. Biochem J 395:629–639. doi:10.1042/BJ20051889

Ryan CA, Green TR (1974) Proteinase inhibitors in natural plant protection. Recent Adv Phytochem 8:123–140

Sahaul DV, Dhonukshe BT, Navale PA (1995) Leaf growth in relation to crop yield in horse gram. J Maharashtra Agric Univ 20:10–12

Salunkhe DK (1982) Legumes in human nutrition: current status and future research needs. Curr Sci 51:387–398

Samal KM, Senapati N (1997) Genetic variability performance, correlation and coheritability of horse gram *(Macrotyloma uniflorum* Len. Verd) mutant lines. Legume Res 203:207–211

Samuel G, Davis R, Hirji R (2006) Climate variability and water resources degradation in Kenya: improving water resources development and management, World bank working paper series 69. World Bank, Washington, DC

Sankar GRM, Das ND, Reddy PR (2002) Effect of yellow mosaic virus on seed yield of horse gram (*Macrotyloma uniflorum*) pedigrees under rainfed alfisols. Ann PIant Prot Sci 10:95–98

Saraswathy KD, Kurup PA (1970) Effects of certain Indian pulses on the serum, liver and aortic lipid levels in rats fed a hypercholesterolaemic diet. Atherosclerosis 11:479–484

Sathe SK, Deshpande SS, Salunkhe DK (1984) Dry beans of phaseolus: a review. Crit Rev Food Sci Nutr 20:1

Satwadhar PN, Kadam SS, Salunkhe DK (1981) Effects of germination and cooking on polyphenols and *in vitro* protein digestibility of horse gram and moth bean. Plant Foods Hum Nutr 31:171–176

Schnell DJ, Etzler ME (1987) Primary structure of the *Dolichos biflorus* seed lectin. J Biol Chem 262:7220–7225

Schnell DJ, Alexander DC, Williams BG, Etzler ME (1987) cDNA cloning and *in vitro* synthesis of the *Dolichos biflorus* seed lectin. Eur J Biochem 167:227–231

Sessa DJ, Rackis JJ (1977) Lipid derived flavors of legume protein products. J Am Oil Chem Soc 54:468–473

Shahidi F, Wanasundara PK (1992) Phenolic antioxidants. Crit Rev Food Sci Nutr 32:67–103

Shahidi F, Chava UD, Naczk M, Amarowicz R (2001) Nutrient distribution and phenolic antioxidants in air-classified fractions of beach pea (*Lathyrus maritimus* L.). J Agric Food Chem 49:926–933

Siddhuraju P, Manian S (2007) The antioxidant activity and free radical-scavenging capacity of dietary phenolic extracts from horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) seeds. Food Chem 105:950–958. doi:10.1016/j.foodchem.2007.04.040

Singh U, Singh B (1992) Tropical grain legumes as important human foods. Econ Bot 46:310–321

Singh S, Singh HD, Sikka KC (1968) Distribution of nutrients in the anatomical parts of common Indian pulses. Cereal Chem 45:13–18

Singh U, Subrahmanyan N, Kumar J (1991) Cooking quality and nutritional attributes of some newly developed cultivars of chickpea (*Cicer arietinum*). J Sci Food Agric 55:37–46

Sosulski F, Young CG (1979) Yield and functional properties of air classified protein and starch fraction from eight legume flours. J Am Oil Chem Soc 56:292–295

Sreerama YN, Gowda LR (1997) Antigenic determinants and reactive sites of a trypsin/chymotrypsin double-headed inhibitor from horse gram (*Dolichos biflorus*). Biochim Biophys Acta 1343:235–242

Sreerama YN, Gowda LR (1998) Bowman-birk type proteinase inhibitor profiles of horse gram (*Dolichos biflorus*) during germination and seed development. J Agric Food Chem 46:2596–2600

Sreerama YN, Sasikala VB, Pratape VM (2008) Nutritional implications and flour functionality of popped/expanded horse gram. Food Chem 108:891–899

Sreerama YN, Sasikala VB, Pratape VM (2009) Expansion properties and ultrastructure of legumes: effect of chemical and enzyme pre-treatments. Food Sci Technol 42:44–49

Sreerama YN, Neelam DA, Sashikala VB, Pratape VM (2010) Distribution of nutrients and antinutrients in milled fractions of chickpea and horse gram: seed coat phenolics and their distinct modes of enzyme inhibition. J Agric Food Chem 58:4322–4330. doi:10.1021/jf903101k

Sreerama YN, Sashikala VB, Pratape VM (2012) Phenolic compounds in cowpea and horse gram flours in comparison to chickpea flour: evaluation of their antioxidant and enzyme inhibitory properties associated with hyperglycemia and hypertension. Food Chem 133:156–162. doi:10.1016/j.foodchem.2012.01.011

Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA (2001) The hormone resistin links obesity to diabetes. Nature 409:307–312

Subbulaksmi G, Ganeshkumar K, Venkatraman LV (1976) Effect of germination on carbohydrates, proteins, trypsin inhibitor, amylase inhibitor, and hemagglutinin in horse gram and moth bean. Nutr Rep Int 13:19–31

Sudha N, Mushtari-Begum J, Shambulingappa KG, Babu CK (1995) Nutrients and some antinutrients in horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.). Food Nutr Bull 16:81–83

Talbot CF, Etzler ME (1978a) Development and distribution of *Dolichos biflorus* lectin as measured by radioimmuno assay. Plant Physiol 61:847–850

Talbot CF, Etzler ME (1978b) Isolation and characterization of a protein from leaves and stems of *Dolichos biflorus* that cross reacts with antibodies to the seed lectin. Biochemistry 17:1474–1479

Tapiero H, Tew KD, Nguyen BG, Mathe G (2002) Polyphenols: do they play a role in the prevention of human pathologies? Biomed Pharmacother 56:200–207

Ti L-H (2007) Chief, water security section, United Nations Economic and Social Commission for Asia and the Pacific, based on information from the Asian River Restoration Network and the fresh and coastal waters session at the 3rd Southeast Asia Water Forum Regional Workshop, Kuala Lumpur

TNAU agritech portal (2008) Agriculture pulses

Tomomatsu H (1994) Health effects of oligosaccharides. Food Technol 48:61–66

Toress-Pinedo R (1983) Lectins and the intestine. J Pediatr Gastroenterol Nutr 2:588–594

Turner BL, Paphazy JM, Haygarth MP, Mckelvie DI (2002) Inositol phosphate in the environment. Online J R Soc 357:449–469

UNWWDR (2008) United Nations World water development report 3: water in a changing world. Taylor and Francis, Hoboken. Accessed 5 Aug 2013

USDA (2011) National nutrient database for standard reference, Release 25

Venkataraman LV, Jaya TV (1975) Gastro-intestinal gas production in rats fed on diets containing germinated legumes. Nutr Rep Int 12:387–398

Venugopal K, Rao GR (1978) Studies on black gram proteins. Indian Food Pack 32:44

Verdcourt B (1982) A revision of *Macrotyloma* (Leguminosae). Hooker's Icones Plantarum 38:1–138.

Vidhu VK, Aswathy Aromal S, Philip D (2011) Green synthesis of silver nanoparticles using Macrotyloma uniflorum. Spectrochim Acta Part A Mol Biomol Spectrosc 83:392–397

Vijaylaksmi D, Venkatrao S (1977) Effect of processing and supplementation with sesame on the quality of the proteins of Bengal gram, green gram and horse gram. In: FAO expert consultation on grain legume processing, held at Central Food Technological Research Institute, Mysore, 14–18 Nov

WHO (2008) World Health Organization and UNICEF (United Nations Children's Fund) Joint monitoring programme. A snapshot of sanitation in Africa. United Nations Children's Fund/ World Health Organization, New York/Geneva

World Bank (2008) World development report. Agriculture for development. World Bank, Washington, DC. http://go.worldbank.org/2DNNMCBGI0

Yadav S, Negi KS, Mandal S (2004) Protein and oil rich wild horsegram. Genet Res Crop Evol 51:629–633

Zech LA Jr, Hoeg JM (2004) Correlating corneal arcus with atherosclerosis in familial hypercholesterolemia. Lipids Health Dis 7:7. doi:10.1186/1476-511X-7-7

Essential Oils for Pest Control in Agroecology

Bhawana Srivastava, Anand Sagar, Nawal Kishore Dubey, and Lipika Sharma

Abstract Currently attention has been paid towards exploitation of higher plant products in plant pest management in view of the public concern over the level of pesticide residues in food and environment causing health and ecological problems. The indiscriminate use of synthetics in crop protection has also led to the development of resistant strains of pests. Amongst plant products, essential oils of different higher plants have been formulated for large scale application as botanical pesticides in eco-friendly management of different plant pests. These products have low mammalian toxicity and are cost effective. Such products of higher plant origin may be exploited as eco-chemical and bio-rational approach in management of crop pests. Unlike conventional insecticides that are based on a single active ingredient, plant-derived essential oils comprises an array of chemical compounds which act concertedly on both behavioral and physiological processes. Thus the chances of pests developing resistance to such substances are less likely. The current status and future prospects of botanical pesticides in eco-friendly management of different plant pests are reviewed and discussed.

Keywords Aflatoxins • Antifungal agents • Antimicrobials • Essential oils • Mycotoxins • Postharvest

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1 Introduction

The constant growth of the world's population requires substantial resources for the production of food. One of the greatest challenges of the world is to produce enough food for the growing population. Agriculture is the driving force for the broad-based economic growth in developing countries where situation is particularly critical because of the slow rate of net food production in relation to population growth. Tropical and sub-tropical regions of world have a greater potential for food production and can grow multiple crops annually. Owing to the congenial climatic conditions and specific environment, the agriculture in tropical and subtropical countries suffers severe losses due to pests (Varma and Dubey 2001; Roy 2003). During pre- and post-harvest period, foods are severely destroyed by fungi, bacteria, viruses and other pests. In spite of the use of all available means of plant protection, even today losses can lead to famine in some countries which are densely populated. Production of mycotoxins by several fungi has added a new dimension to the gravity of the problem. Food and Agriculture Organization estimates, 25 % of the world food crops are affected by mycotoxins each year.

2 Synthetic Chemicals and Their Side Effects

The current methods for management of pests depend heavily on synthetic pesticides. The use of synthetic chemicals as pesticides for the management of plant pathogens have undoubtedly increased crop protection but with considerable deterioration of environmental quality and human health (Cutler and Cutler 1999). Different synthetic pesticides used as agrochemicals in plant protection have been reported to incite adverse effects on different biological functions especially liver and kidney functions of human and animal beings and are indirectly animal toxic in nature (Marini-Bettolo 1977; Lingk 1991; Tripathi et al. 2004). Control of these pest populations around the world primarily depend upon continued applications of organochlorines, organophosphorus and pyrethroid pesticides and the fumigants viz. phosphine and methyl bromide (Lorini et al. 2007). Lindane, a persistent, highly toxic and bioaccumulative organochlorine insecticide, was used in agriculture beginning in the 1940s. Organophosphates, malathion, dichlorvos, iodafenphos chlorpyriphos and azinophos methyl cause residual toxicity to non-target organisms and environment (OHS 1991). On the other hand, some synthetic pyrethroids such as permethrin and deltamethrin have been reported for their genotoxicity and carcinogenicity (Tisch et al. 2002). Permethrin is classified by the US Environment Protection Agency as a human carcinogen, based on reproducible studies in which mice fed permethrin developed liver and lung tumors (USEPA 2006) (Fig. 1).

Fumigants are low molecular weight volatile chemicals which are used during storage to kill all insect stages residing in the produce. Phosphine (PH_3) is a formulated fumigant in the form of metal phosphide under trade name Celphos, Quickphos,

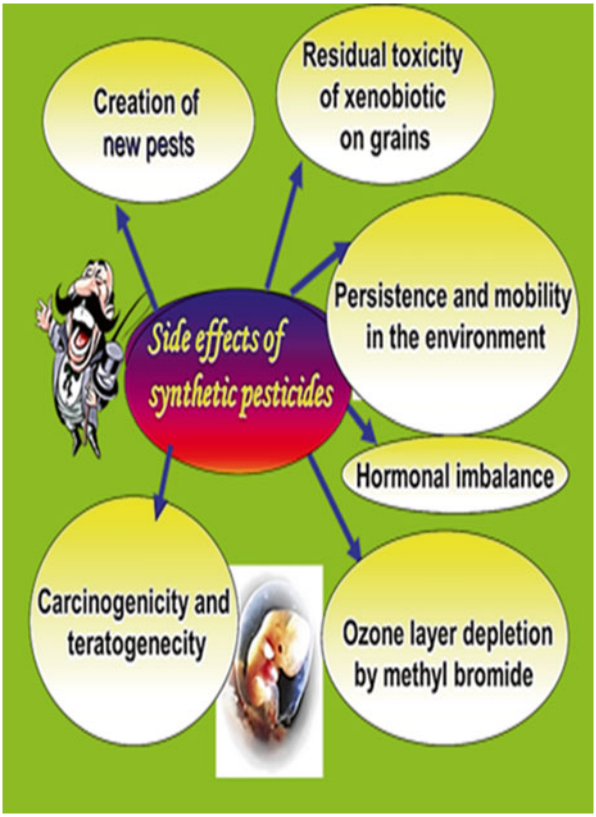

Fig. 1 Side effects of synthetic pesticides

Phosphotoxin etc.; commercially available as either tablets or pellets. Methyl bromide (CH_3Br), on the other hand, is gaseous in form and packed in a liquid form in pressurized steel bottles. Insect resistance to phosphine is a global issue/problem and its reduced efficacy has been reported in some countries (Pimentel et al. 2009). Methyl bromide, a broad-spectrum fumigant, has been declared an ozone-depleter and therefore, is being phased out completely. In addition, repeated use of certain chemical pesticides in packing houses has led to the appearance of resistant populations of storage pathogens (Brent and Hollomon 1998). In recent years there has been considerable pressure from consumers to reduce or eliminate chemical pesticides in foods. There is increasing public concern over the level of pesticide residues in food. Their uninterrupted and indiscriminate use has led to the health problems (Sharma and Meshram 2006). In a recent report of the World Health Organization (WHO), the annual number of cases of acute poisoning caused by synthetic pesticides have been estimated at three million, with 20,000 deaths every year (Dasgupta et al. 2007). Further, the use of synthetic chemicals to control the pests has been restricted due to their carcinogenicity, teratogenecity, high and acute residual toxicity, hormonal imbalance, long degradation period, environmental pollution and their adverse effects on food and side effects on humans (Feng and Zheng

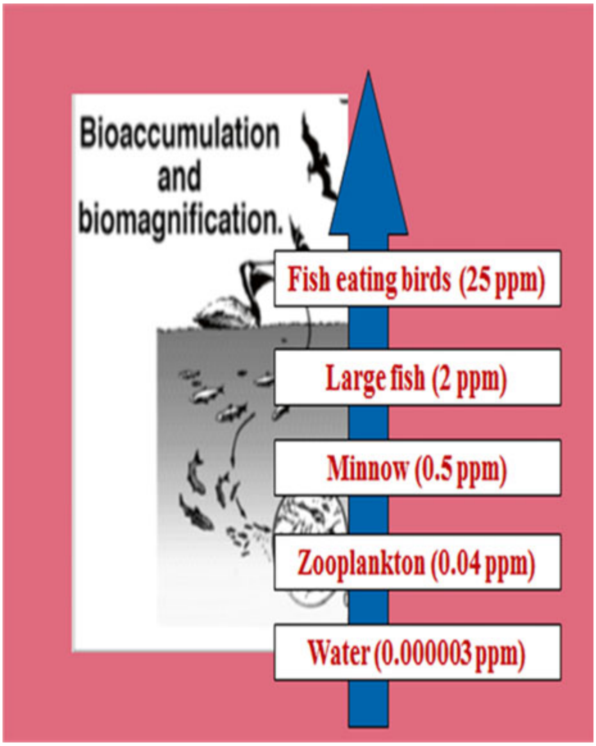

Fig. 2 Presentation of bio-magnification of synthetic pesticides in different trophic levels

2007; Unnikrishnan and Nath 2002). Different types of ecological problems have been reported from time to time by these xenobiotics as they lace the food with residue. The residual toxicity causes disturbances in food chain because of bioaccumulation and bio-magnification at different trophic levels. Effective pest control is no longer a matter of heavy application of pesticides, partly because of rising cost of petroleum-derived products but largely because excessive use of pesticide promotes faster evolution of resistant forms of pests, destroys natural enemies, turns formerly innocuous species into pests, harms other non-target species and contaminates food (Parmar and Devkumar 1993). It has also been estimated that hardly 0.1 % of the agrochemicals used against different pests affect the target organisms, leaving 99.9 % to enter in environment causing hazards to non-target organisms and environment (Varma and Dubey 1999) (Fig. 2).

The use of synthetic pesticides has undoubtedly resulted in increased crop production and achievement of green revolution by different countries. On the other hand, the intensive application of such synthetic pesticides has raised environmental and toxicological concerns, especially questions on hormonal imbalance (Pandey 2003), poisoning of applicators and wildlife, groundwater contamination and residue in food. Due to the development of resistance in a number of pathogenic fungi, failures of disease control have been reported for the benzimidazole fungicides such as benomyl, thiabendazole, carbendazim and thiophanate methyl (Wilson et al. 1997).

The number of insects resistant to certain pesticides has increased significantly over the last years. Therefore, there is an urgent need to find out safer alternatives of currently used pesticides which are environmentally and toxicologically safe and more selective and efficacious than synthetic pesticides.

3 Botanicals as Alternatives of Synthetic Pesticides

Recently, in different parts of the world, attention has been paid towards exploitation of higher plant products as novel chemotherapeutants in plant protection. Plants have a wide variety of biologically active chemicals that may contain some novel pesticidal compounds. Aside from their protective aspect, such chemicals have no other apparent purpose in the physiology of the plant, and they are, therefore, called secondary plant metabolites. Terrestrial plants produce a spectrum of natural products viz., terpenoids, phenolics and alkaloids. Many of these are thought to serve an ecological function for the plants producing them, serving to defend the plants from herbivores and pathogens (Isman and Akhtar 2007). Such defensive chemistry is thought to be extremely widespread among the plant kingdom. This is the foundation upon which many scientists have viewed higher plants as a valuable resource for the discovery of new pesticides or for novel structures that could serve as lead compounds in antimicrobial development. Currently, recognition of the important role of these compounds has increased, particularly in terms of resistance to pests and diseases. Naturally occurring biologically active compounds from plants are generally assumed to be more acceptable and less hazardous than synthetic compounds and represent a rich source of potential disease control agents. Different secondary metabolites of higher plants may be thus exploited as botanical pesticides and numerous opportunities exist to explore their usefulness in controlling pests and diseases (Tripathi et al. 2004). The pesticidal nature of some plant products has been known for centuries. Natural pest controls using botanicals are safer to the user and the environment because they break down into harmless compounds within hours or days in the presence of sunlight. They are also very close chemically to those plants from which they are derived, so they are easily decomposed by a variety of microbes common in most soils. Because of non-phytotoxicity, systemicity, easy biodegradability and stimulatory nature of host metabolism, plant products possess the potential in pest management (Mishra and Dubey 1994) (Fig. 3).

This rationale is based partly on the assumption that novel structures produced by plants may have novel modes-of-action (Hedin et al. 1997; Koul and Dhaliwal 2001; Regnault-Roger et al. 2005). Hence, there is growing interest in the use of safer alternatives for pest control. Research and development cost of botanical antimicrobials from discovery to marketing is reported to be less compared to chemical pesticides (Carlton 1998; Woodhead et al. 1990). As present-day farmers are showing greater interest in the use of green pesticides, scientists are encouraged to carry out more research on botanical pesticides and low-cost production technologies to cater to the demand of farmers and to protect the environment from harmful effects.

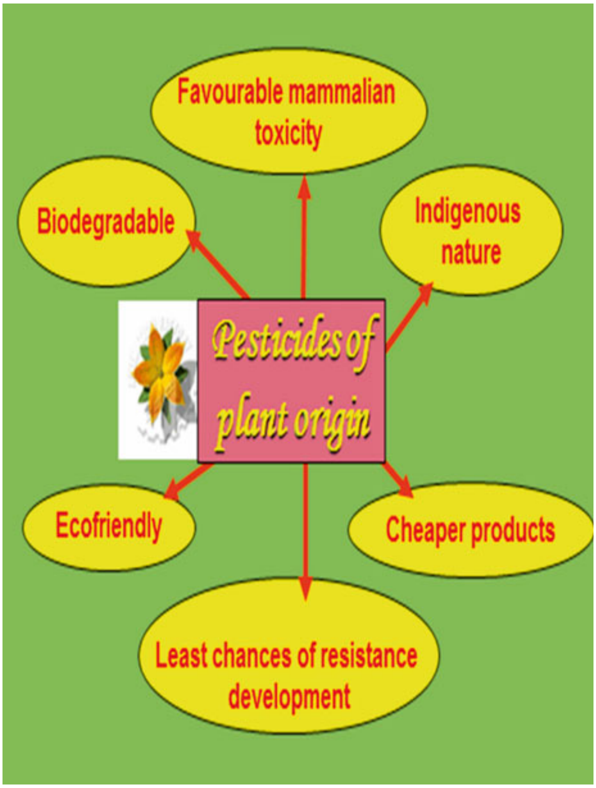

Fig. 3 Superiority of plant products over synthetic pesticides

Such plant products have been formulated for large scale application as botanical pesticides, which are used as alternatives to synthetic pesticides in crop protection. Some products of higher plant origin are being used as botanical pesticides like Azadirachtin from *Azadirachta indica* and pyrethrum from *Chrysanthemum cinerareafolium*. Carvone is a monoterpene standardised from essential oil of *Carum carvi* used as botanical pesticide with the trade name TALENT in The Netherlands. Carvone inhibits sprouting of potato tubers during storage and protects them from bacterial rotting without exhibiting mammalian toxicity. Thus, it enhances the shelf life of stored fruits and vegetables and inhibits microbial deterioration without altering the taste and odour of the fruits after treatment (Varma and Dubey 1999).

Different crude extracts and other plant materials rich in polyphenolics are becoming increasingly important in food industries because of their antifungal, antiaflatoxigenic and antioxidant activity (Kumar et al. 2008). Hence, such plant chemicals can improve shelf-life, quality and nutritional value of stored food commodities (Tripathi and Dubey 2004). Because of greater consumer awareness and concern regarding synthetic chemical additives, food preservation with plant based additives is becoming popular. A renewed interest in natural preservation appears to be stimulated by present food safety concerns, growing problems with microbial resistance, and a rise in production of minimal processed food joined with green

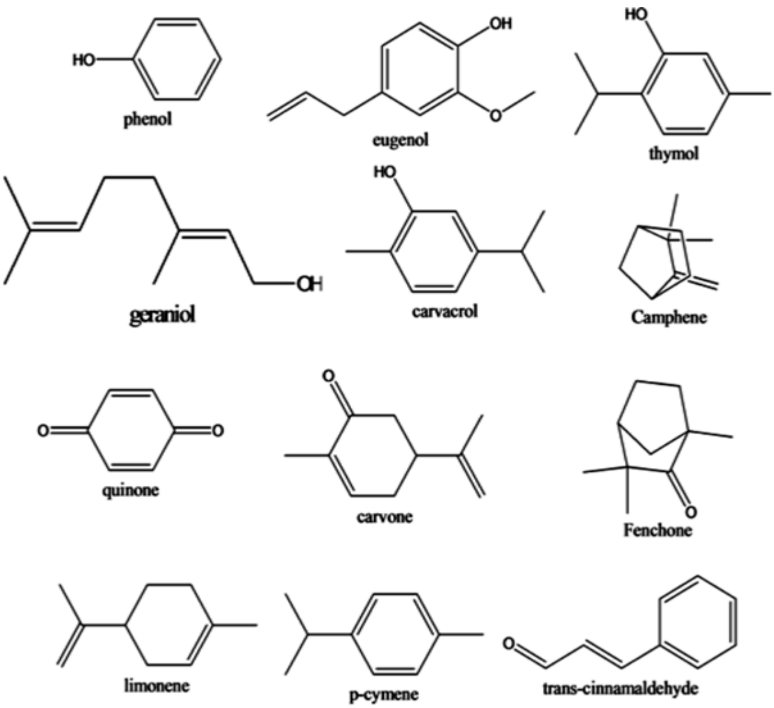

Fig. 4 Structures of some selected components of essential oils which are found to be highly active for pest control in agro-ecology

image policies of food industries. After severe setback arising from the use of chemical pesticides on living systems and the environment, the use of eco-friendly biopesticides is gaining momentum. The general antimicrobial activity of plant products is well documented (Deans and Ritchie 1987) and there have been some studies on the effects of plant products on pathogens (Fig. 4).

Recently some plant products have successfully been tested against a number of storage microbes. In the context of agricultural pest management, botanical pesticides are best suited for use in organic food production in industrialized countries and can play a much greater role in the production and post-harvest protection of food in developing countries (Isman 2006).

4 Essential Oils in Agriculture Pest Management

Among the different plant products, application of essential oils is an attractive method for controlling post-harvest diseases. Production of essential oils by plants is believed to be predominantly a defense mechanism against pathogens and pests (Oxenham 2003). Essential oils are mixtures of aromatic compounds in plants that are extracted by steam or solvent distillation. The volatility, ephemeral nature and biodegradability of flavour compounds of angiosperm will be especially advantageous if they are developed as pesticide. Essential oils and their components are gaining increasing interest because of their relatively safe status, wide acceptance by the consumers and their exploitation for potential multi-purpose functional use (Ormancey et al. 2007; Sawamura 2000). In addition, small quantities of oils would be required for their action. In the past few years, several studies have been focused on the potential use of essential oil applications in biological control of different pests. Studies have shown that essential oils are readily biodegradable (Baysal 1997) and less detrimental to non-target organisms as compared to synthetic pesticides. There may be least chance of residual toxicity by treatment of food commodities with volatile substances of higher plant origin. Concerns for residue of essential oil pesticides on food crops should be mitigated by the fact that different essential oil constituents acquired through the diet are actually beneficial to human health (Huang et al. 1994).

Action of essential oils as antimicrobial agents against pests has been extensively studied (Bazzoni et al. 2002) and essential oils produced by different plant genera are in many cases biologically active, endowed with antimicrobial, allelopathic, antioxidant and bio regulatory properties (Elakovich 1988; Vaughan and Spencer 1991; Caccioni and Guizzardi 1994; Holley and Patel 2005). Essential oils generally have a broad spectrum of bioactivity because of the presence of several active ingredients that work through several modes of action. Essential oils are made up of different volatile compounds and the make-up of the oil quite often varies between species (Mishra and Dubey 1994). The toxicity of individual oils or compounds often exerts differential effects depending on both the mode of action and the target pest (Isman 2000; Liu et al. 2006). The biological activity of essential oils may be due to synergistic effects of different active principles. They may impart different mode of action during their antimicrobial actions. These products may exhibit either for one particular biological effect or may have diverse biological effects (Varma and Dubey 1999). This in turn reduces the chances for multiple genomic mutations in pests and the subsequent development of resistance (Begon et al. 1999; Davies 2000).

4.1 Essential Oils as Antioxidants

In addition to pest spoilage, oxidation is the second most important cause of food spoilage during processing and storage. Important nutrients such as proteins, unsaturated lipids and vitamins can be lost through oxidation. Therefore control of oxidative processes in foods has been one of the highest priorities of the food industry (Diplock et al. 1998; Kumar 2005).

Lipid peroxidation is a complex process whereby polyunsaturated fatty acids in the phospholipids of cellular membrane undergo reaction with oxygen to yield lipid hydroperoxidases. Lipid hydroperoxidases and conjugated diens that are formed can decompose to form numerous other products including alkanals, alkenals, hydroxyalkenals, malondialdehyde (MDA) and volatile hydrocarbons. Oxidative free radicals are highly reactive molecules which appear to function in cellular defense mechanism and their accumulation may damage protein, lipids, carbohydrates and nucleic acids. Products of lipid peroxidation (i.e. free radicals) have been associated with the carcinogenesis, mutagenesis, ageing and atherosclosis, apoptosis by oxidising membrane lipid, cellular proteins, DNA and enzymes thus shutting down cellular respiration. Oxidative deterioration of food stuffs can result in alterations of organoleptic characteristics e.g. taste and aroma making them unacceptable to the consumer. Antioxidants may interact with free radicals to inactivate them and thus terminate the chain reaction causing tissue injury. A number of synthetic antioxidants such as BHA (butylated hydroxyl anisole), BHT (butylated hydroxytoluene) and TBHQ (tert-butylhydroquinone) added to food stuffs are suspected to be carcinogenic (Madhavi and Salunkhe 1995). Antioxidants not only used to fight against oxidation but themselves important for the maintenance and optimization of health and special efforts may be required to protect the antioxidant nutritional value of food (Diplock et al. 1998).

Thus, antioxidants may enhance shelf life of food commodities and protect food quality by inhibiting free radical oxidation of fat and oils thus resulting off flavour and odour (Holley and Patel 2005; Selvi et al. 2003). Among the various kinds of natural substances, essential oils are receiving particular attention as radical scavengers (Barrata et al. 1998; Ruberto et al. 2000).

4.2 Essential Oils as Aflatoxin Suppressors

Colonization of various moulds on food commodities reduces their shelf life and market value as well as renders them unfit for human consumption causing undesirable effects on human health due to secretion of different types of mycotoxins. Aflatoxins are potent toxic, carcinogenic, mutagenic, immunosuppressive and teratogenic agents produced as secondary metabolites by *Aspergillus flavus* and *A. parasiticus* (Kumar et al. 2007). Among 18 different types of aflatoxins identified, major members are aflatoxins B_1, B_2, G_1, G_2, M_1 and M_2. Aflatoxin B_1 are produced most abundantly and is also most toxic followed by G_1, B_2 and G_2. Besides their effect on health of humans and animals, aflatoxin also has an impact on the agricultural economy through the loss of crop production and the time and costs involved in monitoring and decontaminating efforts as FAO and WHO have imposed regulatory guidelines of 20 ppb of total aflatoxins as the maximum allowable limit in food or feed substrate. In some European countries, aflatoxin levels are regulated below 5 ppb. Knowing the hazards of aflatoxin exposure, the need for protection of foods and feedstuffs against aflatoxin is universally recognized and several approaches have been suggested. Aflatoxins chiefly produced by toxigenic strains of *A. flavus* are the most dangerous causing side effects to different systems of human beings. Hence, both qualitative as well as quantitative losses of food commodities have been reported due to fungal infestations. Reducing aflatoxin residue levels in food or feed can confer international trade advantages in developing countries and there may also be long-term benefits for the local population through health improvement (Dichter 1987).

Thyme, *Chenopodium* and *Anise* oils were able to inhibit production of aflatoxins, ochratoxin A and fumonisin respectively (Kumar et al. 2008; Soliman and Badeaa 2002). Five essential oils extracted from *Cymbopogon citratus*, *Monodora myristica*, *Ocimum gratissimum*, *Thymus vulgaris* and *Zingiber officinale* were investigated for their inhibitory effect against three food spoilage and mycotoxin producing fungi, *Fusarium moniliforme*, *Aspergillus flavus* and *Aspergillus fumigatus*. The essential oil from *O. gratissimum*, *T. vulgaris* and *C. citratus* were the most effective. Recently, the essential oils of *Alpinia galanga* (Srivastava et al. 2008), *Artabotrys odoratissimus* (Srivastava et al. 2009) *Cinnamomum camphora* (Singh et al. 2008a), *Cinnamomum tamala* (Srivastava et al. 2011), *Pelargonium graveolens* (Singh et al. 2008b), and *Thymus vulgaris* (Kumar et al. 2008), have been reported to inhibit aflatoxin B_1 secretion by different toxigenic strains of *A. flavus*. Such plant products which are efficacious in checking growth as well as aflatoxin secretion may provide complete quantitative and qualitative control of fungal deterioration of food commodities. These effects against food spoilage and mycotoxin producing fungi indicated the possible ability of essential oils as a food preservative and potent antimicrobial agents (Fig. 5).

The components of the oils may be acting by different mode of action for antifungal activity and aflatoxin inhibition. Hence, it is also advisable that during screening programs, the minimum inhibitory concentration of a product against growth of fungal mycelium as well as aflatoxin elaboration should be recorded so as to recommend it in the maintenance of the quality of stored food commodities.

4.3 Essential Oils as Fumigamts Against Pests

Essential oils are generally volatile substances composed of mono- and sesquiterpenoids, aldehydes, esters, acids, ketones, alcohols, coumarins. Their composition varies within the same species as a result of genetic and environmental factors. Essential oils have been tested against a wide range of pests and mycotoxigenic filamentous fungi (Knobloch et al. 1989) and pathogenic and dimorphic yeasts (Ghannoum 1988). Numerous studies have documented the antifungal and antibacterial effect of plant essential oils. Most of the components of essential oils are specifically active against particular insect groups (Isman 2000). Dubey et al. (1983) demonstrated the efficacy of essential oils of *Ocimum canum* and *Citrus medica* as volatile fungitoxicants for the protection of some spices against post-harvest fungal deterioration. Essential oils of *Cymbopogon citratus*, *Caesulia axillaris* and *Mentha arvensis* have shown *in vivo* fumigant activity for the control of storage fungi of some food commodities (Mishra et al. 1992; Varma and Dubey 2001).

Essential oils of *Thymus serpyllum* (rich in thymol and carvacrol) and *Origanum majorama* (rich in terpinen-4-ol) were effective as fumigants against the bean

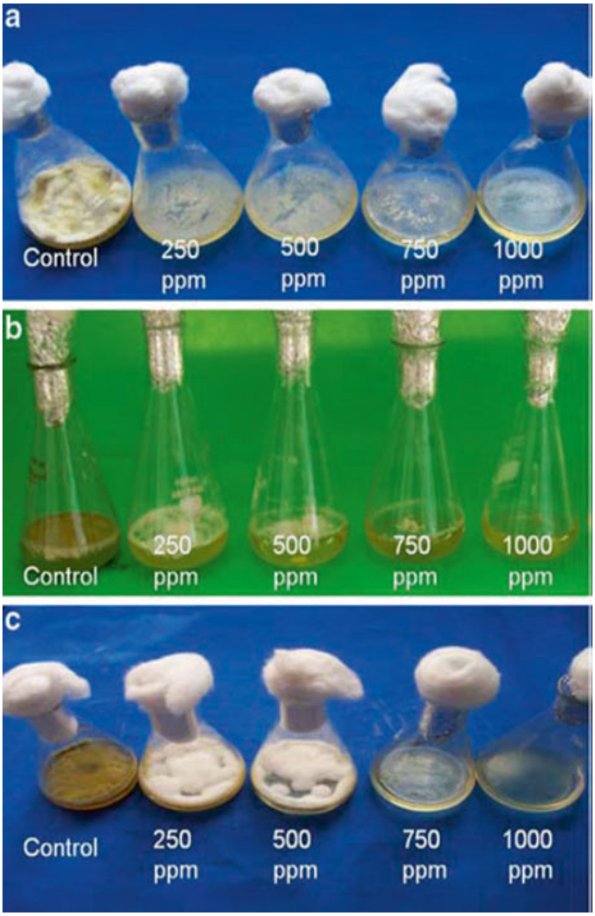

Fig. 5 Photograph showing inhibition of *A. flavus* growth by essential oils. (**a**) Inhibition by *C. camphora* oil, (**b**) inhibition by *A. galanga* oil, (**c**) inhibition by combination of *C. camphora* and *A. galanga* oils (Source: Srivastava et al. 2008)

weevil *Acanthoscelides obtectus* (Bruchidae) (Regnault-Roger et al. 2005). Sarac and Tunc (1995) investigated the fumigant activity of four essential oils against three species of stored product pests. Eugenol, the major constituent of oil of cloves and holy basil, *Ocimum suave*, was effective against coleopterans, *S. granarius* and *Prostephanus truncatus* (Obeng-Ofori and Reichmuth 1999). There is evidence that certain essential oils and their constituents are effective also against *Varroa jacobsoni*, an ectoparasite of the honey bee (Calderone et al. 1997). Recently, essential oils have received increased attention due to a growing interest in the need for alternative techniques to assure quality and safety of perishable food (Burt 2004; Holley and Patel 2005). Some of the essential oils are exempted from the usual data requirements for registration of pesticides particularly in the USA. American companies have recently introduced essential-oil-based pesticides to the market. Mycotech Corporation produces Cinnamite TM, as an aphidicide/miticide/fungicide for glasshouse and horticultural crops, and ValeroTM, as a miticide/fungicide for use in grapes, berry crops, citrus and nuts. Both products are based on cinnamon oil, with cinnamaldehyde (30 % EC formulation) as the active ingredient (Isman 2000).

4.4 *Efficacy of Essential Oils as Semiochemicals Against Agricultural Pests*

Chemicals that deliver behavioural messages in the pests rather than killing them are termed semiochemicals. They may be exploited to manage agricultural pests in different ways. These compounds may act as fumigants, contact insecticides, repellents, and antifeedants, and may affect some biological parameters such as growth rate, life span and reproduction.

4.4.1 Insect Growth Regulators

Insect growth regulators are chemical compounds that alter growth and development of insects. Instead of exhibiting a killing effect, insect growth regulators interfere with the normal mechanisms of development and cause the insects to die before reaching the adult stage. Insect growth regulators disrupt insect growth and development in three different ways: as juvenile hormones, as precocenes and as chitin synthesis inhibitors. Juvenile hormones disrupt insect maturation and emergence as adults. They include ecdysone (the molting hormone), juvenile hormone mimics, juvenile hormone analogues, and are known as their broader synonyms, juvenoids and juvegens. Precocenes interfere with the normal function of glands that produce juvenile hormones while chitin synthesis inhibitors, e.g. synthetic pesticides as benzoylureas, buprofezin and cyromazine, affect the ability of insects to produce new exoskeletons when molting. One famous juvenile hormone is juvabione which was found in the wood of balsam fir. Its effect was discovered by accident when paper towels made from this source were used to line insect-rearing containers resulting in a suppression of insect development (Varma and Dubey 1999). Analogues of insect juvenile hormones have been found including juvocimenes in *Ocimum basilicum* and juvabione in *Abies balsamea* (Balandrin et al. 1985).

4.4.2 Antifeedants and Attractants

Many natural plant chemicals deter insects from feeding (antifeedant effect), although none of them have been developed commercially so far. A considerable amount of literature documents the antifeedant potential of neem. Azadirachtin and limonoids such as limonin and nomilin originating from many different plant species in Meliaceae and Rutaceae, e.g. from *Citrus* fruits, have long been used successfully for insect control, especially in India. Azadirachtin has also some systemic properties as it also protects newly grown leaves of crop plants from feeding damage (Varma and Dubey 1999).

Certain essential oil constituents are effective attractants for some insects. Cinnamyl alcohol, 4-methoxy-cinnamaldehyde, cinnamaldehyde, geranylacetone and a-terpineol are attractants for adult corn rootworm beetles (*Diabrotica* spp.) (Hammack 1996). Geraniol and eugenol are used as lures in traps for the Japanese beetle *Popillia japonica* and methyl eugenol has been used to trap oriental fruit fly *Dacus dorsalis* (Vargas et al. 2000).

4.4.3 Chemosterilants

Oils showing chemosterilant activity may be utilized as botanical fumigants especially in the management of storage pests without running a big risk of selecting for physiological (resistant) races of the pest species. The compound β-asarone was extracted from rhizomes of *Acorus calamus*, it exhibits complete inhibition of ovarian development of different insects (Varma and Dubey 1999). *Quassia amara* (Surinam Wood), belonging to the family Simaroubaceae, is a tree species naturally distributed in several tropical countries. The male reproductive system, particularly spermatogenesis, sperm maturation and androgen biosynthesis, are highly sensitive to the metabolites of *Q. amara* which would be useful for insect pest control but may also affect male reproduction in non-target organisms. Therefore, their pharmacological effects on mammals should be worked out before recommendation to avoid any handling problems with such chemicals.

4.5 Safety Limit Profile of Essential Oils

Botanical antimicrobials have a very high level of safety for humans, animals, fish and other non-target organisms principally because they have been reported to act by very different modes of action than most organic chemical pesticides, which attack metabolic systems shared by both pest and non pest organisms (Dubey et al. 2012). Essential oils from aromatic and medicinal plants are potentially useful and safe as antimicrobial agents and their use as medicines has long been recognized (Kim et al. 1995). The antimicrobial products of different higher plant origin have shown comparatively higher limits LD_{50} than the upper limit required for acute toxicity tests by most of the pesticide regulatory agencies (Pavela 2007). The high LD_{50} value is the indication of favourable safety limit profile of the oils and hence these may be recommended as a safe preservative of food commodities (Isman 2006). These products may be recommended as safe antimicrobials in place of some organophosphate esters like tetraethylpyrophosphate, parathion, fonofos whose LD_{50} is 1–25 mg kg^{-1} (Coats 1994). The attraction of modern society towards ‘green consumarism’ desiring fewer synthetic ingredients in foods and recommendation of herbal products as ‘generally recognized as safe’ as food additives in the developed countries may lead scientific interest in plant based antimicrobials as food additive because of their high LD_{50} and favourable safety limit profile. Such products would be better alternatives of synthetic chemicals in eco-friendly management of plant pests. Because of plant origin such chemicals are renewable in nature and may be recommended as non-petrochemical preservatives (Tuley de Silva 1996; Smid and Goris 1999).

The most attractive aspect of using essential oils and/or their constituents as crop protectants (and in other contexts for pest management) is their favourable mammalian toxicity. The pesticidal plants can be continuously propagated year after year; hence, the use of plant materials for protection of stored food commodities would be a sustainable and biodegradable method without negative impact on the environment. Natural plant products will undoubtedly play a significant role in the future of microbes control in both industrialized and developing countries. Because of the need for new, safer pesticides, the bioactive products of higher plant origin especially essential oils may be exploited as eco-chemical biorational approach in integrated pest management programs. Botanical pesticides have golden chance for development and catch global market.

As food preservatives, volatile oils may have their greatest potential use. Essential oils and the components of many edible and medicinal plants are used in different a pharmaceutical preparation which minimizes questions regarding their safe use in food products (Holley and Patel 2005).

5 Future Prospects of Essential Oils

Although various essential oils have been screened for their antimicrobial activity against various pests but proper detailed studies viz. antifungal, aflatoxigenic and antioxidant activity, phytochemistry etc. have not been done with most of the oils. Therefore, there is urgent need to bioprospect the pesticidal property of different essential oils and detailed *in vitro* and *in vivo* investigations are required for their recommendation as antimicrobials for the control of pest infestations of food commodities and thereby enhancing shelf life of the commodities. Recently, research in essential (volatile) oils has received increased attention from both industrial and academic circles in this regard due to a growing interest in green consumerism (Table 1).

It has been established that the composition of essential oils varies significantly because of different species and chemotypes (Buttery et al. 1974; Tucker and Maciarello 1986; Tantaoui-Elaraki et al. 1993), geographical origin (Chalchat et al. 1993; Perry et al. 1999) season (Senatore 1996) and extraction procedure (Suhr and Nielsen 2003; Schaneberg and Khan 2002) and therefore their antimicrobial activities could also vary (Lawrence 1993; Shu and Lawrence 1997).

The use of essential oils in consumer goods is expected to increase in the future due to the rise of 'green consumerism', which stimulates the use and development of products derived from plants. This applies to the food and cosmetic sectors but also to medicinal products (Bassett 1990). There is a need to better understand how essential oil components and other natural antimicrobials interact with cells to cause antimicrobial

Table 1 Investigations on essential oils obtained from higher plants and their action against fungi and aflatoxin

Investigators	Essential oil	Test fungi	Investigations
Mishra and Dubey (1990)	*Prunus persica*	*Candida albicans*, *Aspergillus flavus*	100 % mycelial growth inhibition. Fungistatic in nature. Broad fungitoxic spectrum and non-phytotoxic in nature
Gundidza (1993)	*Artemisia* sp.	*Aspergillus* sp. *A. alternata*, *Penicilium citrium*, *Geotrichum candidum*	The oil exhibited significant antifungal activity
Mishra and Dubey (1994)	*Cymbopogon citrates*	*A. flavus*	Minimum inhibitory concentration (MIC) of *C. citratus* against *A. flavus* was 1,000 ppm. Oil showed fungitoxic nature of toxicity wide fungitoxic nature of toxicity, wide fungitoxic spectrum, non-phytotoxic, superiority over synthetic fungicides
Saxena and Mathela (1996)	*Nepeta leucophylla* and its compounds	*Sclerotium rolfsii*, *Macrophomina phaseolina*	Indodial beta-monoenal acetate was most effective against *S. rolfsii*. While acetinide highly active against *M. phaseolina*
Rana et al. (1997)	*Aegle marmelos*	*Fusarium udum*	Inhibited most resistant fungus *F. udum* 80 % at 400 ppm while 100 % inhibition found at 500 ppm in all other fungi
Dubey et al. (2000)	*Ocimum gratissimum*	Storage fungi	Oil of Indian chemotype exhibited antifungal properties
Nielson and Rios (2000)	*Brassica* sp. & its active principle Allyl isothiocynate (AITC)	*P. commune*, *P. roqueforte*, *A. flavus*, *Endomyces fibuligera*	Mustard oil showed strongest effect on fungus. MIC of AITC was 1.8–3.5 μg/ml. Weather AITC was fungistatic or fungicidal depends on its concentration and the conc. of spores.
Kobaisy et al. (2001)	*Hibiscus cannabinus*	*Colletotrichum fragarie*, *C. gloeosporiodes*, *C. accutatum*	Oil had antifungal properties
Jayaprakasha et al. (2001)	Turmeric oil	*A. flavus*, *A. parasiticus*, *F. moniliforme*, *P. digitatum*	Distilled fraction of turmeric oil was found to be more active against test fungi

(continued)

Table 1 (continued)

Investigators	Essential oil	Test fungi	Investigations
Varma and Dubey (2001)	*Mentha arvensis*, *Caesulia axillaris*	Storage fungi & insect *A. flavus*, *P. italicum*, *S. oryzae*, *T. castaneum*	Both oils inhibited fumigant activity to protect wheat sample from *A. flavus*, *S. oryzae* & *T. castaneum* at 1,300 and 600 ppm. Oils also controlled blue mould rot of oranges caused by *P. italicum*
Zeringue et al. (2001)	Neem oil	*Aspergillus* sp.	Reduced fungal radial growth and AFB_1 production
Kouokam et al. (2002)	*Scorodophloe szenkeri* and isolated sulphur compounds	Storage fungi	The oil completely inhibited the growth of all fungi except *A. flavus*
Juglal et al. (2002)	Clove oil	*A. parasiticus*, *F. moniliforme*	Clove oil (eugenol) successfully inhibited the growth of *A. parasiticus* and *F. moniliformae* can prevent the formation of fungal toxins in contaminated grain
Paranagama et al. (2003)	*Cymbopogon citratus*	*Aspergillus* sp.	Exhibited fungistatic, fungicidal and 100 % antiaflatoxigenic
Shahi et al. (2003)	*Cymbopogon flexuosus*	25 post-harvest fungal pathogens	Oil of *Cymbopogon flexuosus* showed potent bioactivity against dominant post-harvest fungal pathogens
Shin (2003)	*Cedrus atlantica*, *Styrax tonkinensis*, *Juniperus communis*, *Lavendula anguistifolia*, *Melaleuca alternifolia*, *Pelargonium graveolans*, *Postosteman patchouli*, *Rosmarinus officinalis*	*A. flavus*, *A. niger*	Most of the oils except *C. atlantica*, *J. communis* and *P. patchouli* significantly inhibited growth of *A. niger* at 0.78–12.5 mg/ml
Sridhar et al. (2003)	13 EOs	Food mould rot fungi	*Cymbopogon* exhibited significant activity
Motiejunaite and Peciulyte (2004)	*Pinus sylvestris*	*A. flavus*, *A. niger*, *A. parasiticus*, *A. fumigatus*, *A. oryzae*, *Urocladium oudemansii*	Pine oil active against *U. oudemansii* was most sensitive fungus to volatiles

(continued)

Table 1 (continued)

Investigators	Essential oil	Test fungi	Investigations
Rasooli and Abyaneh (2004)	*Thymus eriocalyx*, *T. xporlock*	*Aspergillus* sp.	Inhibition of fungal growth and aflatoxin production
Bankole et al. (2005)	*Cymbopogon citrates*	*Aspergillus* sp.	Completely inhibited aflatoxin (AFB_1) production and prevented the deterioration of melon seed up to 6 months
Ramagnoli et al. (2005)	*Tagetus patula*	*Botrytis cinera*, *Penicillium digitatum*	MIC 10 μl/ml and 1.25 μl/ml for these two fungus respectively
Kumar (2005)	*Mentha arvensis*	*Aspergillus* sp.	*Mentha* oil completely inhibited mycelial growth at 100 ppm and AFB_1 production at 50 ppm
Cardenas-Ortega et al. (2005)	*Chrysactinia mexicana*	*A. flavus*	MIC of oil has been found to be 1.25 mg/ml & piperitone (active principle) 0.6 mg/ml
Souza et al. (2007)	*Origanum vulgare*	Food spoilage yeasts	Effectiveness of *Origanum vulgare* L. essential oil to inhibit the growth of food spoiling yeasts
Dubey et al. (2007)	*Eupatorium cannabinum*	*Botryodiplodia theobromae* and *Colletotrichum gloeosporioides*	Evaluation of *Eupatorium* oil in enhancement of shelf life of mango fruits from fungal rotting
Singh et al. (2008a, b)	*Cinnamomum camphora*	*Aspergillus* sp.	Inhibition of fungal growth, aflatoxin production and elaboration
Srivastava et al. (2009)	*Artabotrys odoratissimus*	*Aspergillus* sp.	Inhibition of fungal growth, aflatoxin production and elaboration
Tatsadjieu et al. (2010)	*Ocimum gratissimum* *Lippia rugosa* *Xylopia aethiopica*	*Aspergillus flavus* and *Sitophilus zeamais* Motsch.	Comparative study of the simultaneous action of three essential oils
Srivastava et al. (2011)	*Cinnamomum tamala*	*Aspergillus* sp.	Inhibition of aflatoxin production from a toxigenic strain of *A. flavus*
Xing et al. (2012)	Clove oil	*Rhizopus nigricans*, *Aspergillus flavus and Penicillium citrinum*	The oil proved to be antifungal *in vitro* and in wounded fruit tests
Prakash et al. (2013)	*Cinnamomum glaucescens*	*Aspergillus flavus*	The oil acted against storage fungi, insect, aflatoxin secretion and as antioxidant

effects. If essential oils were to be required in much greater volumes than at present, bioengineering of their synthesis in plants could provide greater yields (McCaskill and Croteau 1999; Mahmoud and Croteau 2002). International standardisation of the composition of commercially available essential oils would be essential for reliable applications (Burt 2004; Carson and Riley 2001). Microencapsulation technology is recently prescribed to use the essential oils as antimicrobials for preservation of stored food commodities and in food industry for flavour stabilization. Recent researches have demonstrated future prospective as novel eco-friendly measures for the management of agricultural pests (Landolt et al. 2000; Moretti et al. 1998). Little is known about the physiological actions of essential oils on insects, but treatments with various essential oils or their monoterpene constituents cause symptoms that suggest a neurotoxic mode of action. It interrupts the functioning of a neuromodulator, octopamine, which results in total breakdown of the nervous system in insects. Therefore, the octopaminergic system of insects represents a bio-rational target for insect control. The lack of octopamine receptors in vertebrates likely accounts for the profound mammalian selectivity of essential oils as insecticides. Other studies on essential oils indicate inhibition of acetylcholinesterase enzyme activity as the major site of action. Ovicidal activity of essential oils is only apparent when the target nervous system begins to develop. Alternatively, changes in the permeability of the chorion and/or vitelline membrane may occur during embryogenesis and may facilitate the diffusion of essential oil vapours into older eggs so that vital physiological and biochemical processes are affected. These factors have been considered to play a dominant role in the susceptibility of eggs of many insect species to different ovicides. Furthermore, unlike conventional insecticides that are based on a single active ingredient, plant-derived Eos comprise an array of chemical compounds which act concertedly on both behavioural and physiological processes. Thus the chances of pests developing resistance to such substances are less likely.

6 Conclusions

The problems caused by synthetic pesticides and their residues have increased the need for effective biodegradable pesticides. Alternative strategies have included the search for new types of pesticides which are often effective against specific target species, are biodegradable into non-toxic products and are suitable for use in pest management programs. Because of the need for new, safe pesticides, essential oils of higher plant origin may gain more interest for an eco-chemical approach in integrated pest management programmes. More botanical pesticides are expected to come into development and reach global market in the near future. The use of essential oils is now emerging as one of the prime means to protect crops and their products and the environment from pesticide pollution.

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References

Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH (1985) Natural plant chemicals: sources of industrial and medicinal materials. Science 228:1154–1160

Bankole SA, Joda AO, Ashidi JS (2005) The use of powder and essential oil of *Cymbopogon citratus* against mould deterioration and aflatoxin contamination of "egusi" melon seeds. J Basic Microbiol 45(1):20–30

Barrata MT, Dorman HJD, Deans SG, Biondi D, Ruberto G (1998) Chemical composition, antimicrobial and antioxidative activity of laurel, sage, rosemary oregano and coriander essential oils. J Essent Oil Res 10:618–627

Bassett IB, Pannowitz DL, Barnetson RSC (1990) A comparative study of tea-tree oil versus benzoylperoxide in the treatment of acne. Med J Aus 153:455–458

Baysal O (1997) Determination of microorganisms decomposing essential oils of *Thymbra spicata* L. var. *spicata* and effect of these micro-organisms on some soil borne pathogens. M.Sc. thesis, Akdenniz University, Antalya (in Turkish)

Bazzoni E, Sanna-Passino G, Moretti MDL (2002) Essential oils and other control techniques against stored product insects. In: Majumdar DK, Govil JN, Singh VK (eds) Recent progress in medicinal plants, vol 8, Phytochemistry and pharmacology II. Sci Tech Publishing LLC, Houston, pp 313–342

Begon M, Harper JL, Townsend CR (1999) Ecology: individuals, populations and communities. Blackwell Scientific Publications, Oxford, pp 551–582

Brent KJ, Hollomon DW (1998) Fungicide resistance: the assessment of risk, Monograph, 2. FRAC, Global Crop Protection Federation, Brussels, pp 1–48

Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol 94:223–253

Buttery RG, Black DR, Guadagni DG, Ling LC, Connolly G, Teranishi R (1974) California bay oil. I. Constituents, odor properties. J Agric Food Chem 22:773–780

Caccioni DRL, Guizzardi M (1994) Inhibition of germination and growth of fruit and vegetable post-harvest pathogenic fungi by essential oil components. J Essent Oil Res 6:173–179

Calderone NW, Twilson W, Spivak M (1997) Plant extracts used for control of the parasitic mites *Varroa jacobsoni* (Acari: Varroidae) and *Acarapis wood* (Acari: Tarsonemidae) in colonies of *Apis mellifera* (Hymentoptera: Apidae). J Econ Entomol 90:1080–1086

Cardenas-Ortega NC, Zavala-Sanchez MA, Aguirre-Rivera JR, Perez-Gonzalez C, Perez-Gutierrez S (2005) Chemical composition and antifungal activity of essential oil of *Chrysactinia mexicana* gray. J Agric Food Chem 11:4347–4349

Carlton BC (1998) Development of genetically improved strains of *Bacillus thuringiensis* a biological pesticide. In: Biotechnology for crop protection. American Chemical Society, Washington, DC, pp 260–279

Carson CF, Riley TV (2001) Safety, efficacy and provenance of tea tree (*Melaleuca alternifolia*) oil. Contact Dermat 45:65–67

Chalchat JC, Garry RP, Michet A, Benjilali B, Chabart JL (1993) Essential oils of rosemary (*Rosmarius officinalis* L.). The chemical composition of oils of various origins (Morocco, Spain, France). J Essent Oil Res 5:613–618

Coats JR (1994) Risks from natural verses synthetic intsecticides. Annu Rev Entomol 39:489–515

Cutler HG, Cutler SJ (1999) Biological active natural products: agrochemicals. CRC Press, Boca Raton, p 299

Dasgupta S, Meisner C, Wheeler D, Xuyen K, Lam NT (2007) Pesticide poisoning of farm workers–implications of blood test results from Vietnam. Int J Hyg Environ Health 210:121–132

Davies WP (2000) Prospects for pest resistance to pesticides. In: Kadir AASA, Barlow HS (eds) Pest management and the environment in 2000. CAB International, Wallingford, pp 95–110

Deans SG, Ritchie G (1987) Antibacterial properties of plant essential oils. Int J Food Microbiol 5:165–180

Dichter GR (1987) Cost-effectiveness analysis of aflatoxin control programmes. A paper presented on the joint FAO/WHO/UNEP second international conference on mycotoxins. Bangkok, Thailand. http://www.fao.org/docrep/x5036e/x5036E1f.HTM

Diplock AT, Charleux JL, Crozier-Willi G, Kok FJ, Rice-Evans C, Roberfroid M, Stahl W, Vina-Ribes J (1998) Functional food science and defence against reactive oxidative species. Br J Nutr 80(1):S12–S77

Dubey NK, Bhargava KS, Dixit SN (1983) Protection of some stored food commodities from fungi by essential oils of *Ocimum canum* and *Citrus medica*. Int J Trop Plant Dis 1:177–179

Dubey NK, Tiwari TN, Mandin D, Andriamboavonjy H, Chaumont JP (2000) Antifungal properties of *Ocimum gratissimum* essential oil (ethyl cinnamate chemotype). Fitoter 71(5):567–569

Dubey RK, Kumar R, Jaya, Dubey NK (2007) Evaluation of *Eupatorium cannabinum* Linn. oil in enhancement of shelf life of mango fruits from fungal rotting. World J Microbiol Biotechnol 23:467–473

Dubey NK, Singh P, Prakash B, Mishra PK (2012) Plant chemicals in post harvest technology for management of fungal, mycotoxin and insect contamination of food commodities. In: Mérillon JM, Ramawat KG (eds) Plant defence: biological control, progress in biological control 12. doi:10.1007/978-94-007-1933-0_ Springer science + Buisness media B. V.

Elakovich SD (1988) Terpenoids as models for new agrochemicals. In: Cutler HG (ed) Biologically active natural products-potential use in agriculture, vol 380, American chemical society symposium. American Chemical Society, Washington, DC, pp 250–261

Feng W, Zheng X (2007) Essential oils to control *Alternaria alternata in vitro* and *in vivo*. Food Control 18:1126–1130

Ghannoum MA (1988) Studies on the anti candidal mode of action of *Allium sativum* (garlic). J Gen Microbiol 134:2917–2924

Gundidza M (1993) Antifungal activity of essential oil from Artemisia afra Jacq. Cent Afr J Med 39(7):140–142

Hammack L (1996) Corn volatiles as attractants for northern and western corn rootworm beetles (Coleoptera: Chrysomelidae: Diabrotica spp.). J Chem Ecol 22:1237–1253

Hedin PA, Hollingworth RM, Masler EP, Miyamoto J, Thompson GD (1997) Phytochemicals for pest control. American Chemical Society, Washington, DC, p 372

Holley AH, Patel H (2005) Improvement in shelf life and safety of perishable foods by plant essential oils and smoke antimicrobials. Int J Food Microbiol 22:273–292

Huang MT, Ferraro T, Ho CT (1994) Cancer chemoprevention by phytochemicals in fruits and vegetables. Am Chem Soc Symp Ser 546:2–15

Isman MB (2000) Plant essential oils for pest and disease management. Crop Prot 19:603–608

Isman MB (2006) Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu Rev Entomol 51:45–66

Isman MB, Akhtar Y (2007) Plant natural products as a source for developing environmentally acceptable insecticides. In: Isaac I, Ralf N, Rami Horowitz A (eds) Insecticides design using advanced technologies. Springer-Verlag, Berlin/Heidelberg, pp 235–248

Jayaprakasha GK, Negi PS, Anandharamakrishnan C, Sakariah KK (2001) Chemical composition of turmeric oil-a byproduct from turmeric oleoresin industry and its inhibitory activity against different fungi. Zeitschrift fur Naturforschung [C] 56(1–2):40–44

Juglal S, Govinden R, Odhav B (2002) Spice oils for the control of co-occurring mycotoxin-producing fungi. J Food Prot 65(4):683–687

Kim JM, Marshall MR, Cornell JA, Preston JF, Wei CI (1995) Antibacterial activity of carvacrol, citral and geraniol against *Salmonella typhimurium* in culture medium and on fish cubes. J Food Sci 60:1364–1368

Knobloch K, Pauli A, Iberl N, Weigand N, Weis HM (1989) Antibacterial and antifungal properties of essential oil components. J Essent Oil Res 1:119–128

Kobaisy M, Tellez MR, Webber CL, Dayan FE, Schrader KK, Wedge DE (2001) Phytotoxic and fungitoxic activity of the essential oil of kenaf (*Hibiscus cannabinus* L.) leaves and its composition. J Agric Food Chem 52(16):5004–5010

Koul O, Dhaliwal G (2001) Phytochemical biopesticides. Hardwood Academic, Amsterdam, p 223

Kouokam JC, Jahns T, Becker H (2002) Antimicrobial activity of the essential oil and some isolated sulfur-rich compounds from Scorodopholeus zenkeri. Planta Med 68(12):1082–1087
Kumar R (2005) Survey of plants for pesticidal activity with special reference to evaluation of some essential oils as botanical pesticides. Ph.D. thesis, Banaras Hindu University, Varanasi, India
Kumar R, Mishra AK, Dubey NK, Tripathi YB (2007) Evaluation of *Chenopodium ambrosioides* oil as a potential source of antifungal, antiaflatoxigenic and antioxidant activity. Int J Food Microbiol 115:59–164
Kumar A, Shukla R, Singh P, Prasad CS, Dubey NK (2008) Assessment of *Thymus vulgaris* L. essential oil as a safe botanical preservative against postharvest fungal infestation of food commodities. Innov Food Sci Emerg Technol 9(4):575–580
Landolt PJ, Hofstetter RW, Biddick LL (2000) Plant essential oils as arrestant and repellents for neonate larvae of the codling moth Lepidoptera: Tortricidae. Environ Entomol 28:954–960
Lawrence BM (1993) A planning scheme to evaluate new aromatic plants for the flavor and fragrance industries. In: Janick J, Simon JE (eds) New crops. Wiley, New York, pp 620–627
Lingk W (1991) Health risk evaluation of pesticide contamination in drinking water. Gesunde Pflanzen 43:21–25
Liu CH, Mishra AK, Tan RX, Tang C, Yang H, Shen YF (2006) Repellent and insecticidal activities of essential oils from *Artemisia princeps* and *Cinnamomum camphora* and their effect on seed germination of wheat and broad bean. Bioresour Technol 97:1969–1973
Lorini I, Collins PJ, Daglish GJ, Nayak MK, Pavic H (2007) Detection and characterization of strong resistance to phosphine in Brazilian *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). Pest Manag Sci 63:358–364
Madhavi DL, Salunkhe DK (1995) Toxicological aspects of food antioxidants. In: Madhavi DL, Despande SS, Salunkhe DK (eds) Food antioxidants. Marcel Dekker, New York, p 267
Mahmoud SS, Croteau RB (2002) Strategies for transgenic manipulation of monoterpene biosynthesis in plants. Trends Plant Sci 7(8):366–373
Marini-Bettolo GB (1977) Modern trends in the use of natural products for controlling pests and plant diseases. In: Marini-Bettolo GB (ed) Natural products and protection of plants. Elsevier Scientific Publishing Co, Oxford/New York, pp 1–24
McCaskill D, Croteau R (1999) Strategies for bioengineering the development and metabolism of glandular tissues in plants. Nat Biotechnol 17:31–36
Mishra AK, Dubey NK (1990) Fungitoxic properties of *Prunus persica* oil. Hindustan Antibiot Bull 32(3–4):91–93
Mishra AK, Dubey NK (1994) Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. Appl Environ Microbiol 60:1101–1105
Mishra AK, Kishore N, Dubey NK, Chansouria JPN (1992) An evaluation of the toxicity of the oils of *Cymbopogon citratus* and *Citrus medica* in rats. Phytother Res 6:279–281
Moretti MDL, Peana AT, Franceschini A, Carta C (1998) *In vivo* activity of *Salvia officinalis* oil against *Botrytis cinerea*. J Essent Oil Res 10:157–160
Motiejunaite O, Peciulyte D (2004) Fungicidal properties of *Pinus sylvestris* L. for improvement of air quality. Medicina (Kaunas) 40(8):787–794
Nielson PV, Rios R (2000) Inhibition of fungal growth on bread by volatile components from species and herbs, and the possible application in active packaging, with special emphasis on mustard essential oil. Int J Food Microbiol 60(2–3):219–229
Obeng-Ofori D, Reichmuth CH (1999) Plant oils as potential agents of monoterpenes for protection of stored grains against damage by stored product beetle pests. Int J Pest Manag 45(2):155–159
OHS (Occupational Health Services Inc.) (1991) MSDS for methyl parathion. Occupational Health Services, Inc, Secausus
Ormancey X, Sisalli S, Coutiere P (2007) Formulation of essential oils in functional perfumery. Parfum Cosmet Actual 157:30–40
Oxenham SK (2003) Classification of an *Ocimum basilicum* germplasm collection and examination of the antifungal effects of the essential oil of basil. Ph.D. thesis, Glasgow, UK, University of Glasgow

Pandey R (2003) Pesticides and sterility (India). Everyman's Sci 38(2):84–86

Paranagama PA, Abeysekara KH, Abeywickrama K, Nugaliyadde L (2003) Fungicidal and anti-aflatoxigenic effects of the essential oil of *Cymbopogon citratus* (DC.) Stapf. (lemongrass) against *Aspergillus flavus* link. Isolated from stored rice. Lett Appl Microbiol 37(1):86–90

Parmar BS, Devkumar C (1993) In botanical and biopesticides. West Vill Publishing House, New Delhi, p 197

Pavela R (2007) Possibilities of botanical insecticide exploitation in plant protection. Pest Technol 1:47–52

Perry NB, Anderson RE, Brennan NJ, Douglas MH, Heaney AJ, McGimpsey JA, Smallfield BM (1999) Essential oils from Dalmatian sage (*Salvia officinalis* L.): variations among individuals, plant parts, seasons, and sites. J Agric Food Chem 47:2048–2054

Pimentel D, Marklein A, Toth MA, Karpoff MN, Paul GS, McCormack R, Kyriazis J, Krueger T (2009) Food versus biofuels: environmental and economic costs. Human Ecol 37(1):1–12

Prakash B, Singh P, Yadav S, Singh SC, Dubey NK (2013) Safety profile assessment and efficacy of chemically characterized *Cinnamomum glaucescens* essential oil against storage fungi, insect, aflatoxin secretion and as antioxidant. Food Chem Toxicol 53:160–167

Ramagnoli C, Bruni R, Andreotti E, Rai MK, Vicentini CB, Mares D (2005) Chemical characterization and antifungal activity of essential oil of capitula from wild Indian *Tagetes patula* L. Protoplasma 225(1–2):57–65

Rana BK, Singh UP, Taneja V (1997) Antifungal activity and kinetics of inhibition by essential oil isolated from leaves of *Aegle marmelos*. J Ethnopharmacol 87(2–3):241–246

Rasooli I, Abyaneh MR (2004) Inhibitory effects of Thyme oils on growth and aflatoxin production by *A. parasiticus*. Food Control 15(6):479–483

Regnault-Roger C, Philogene BGR, Vincent D (2005) Biopesticides of plant origin. Lavoisier, Paris, p 313

Roy AK (2003) Mycological problems of crude herbal drugs-overview and challenges. Ind Phytopathol 56:1–13

Ruberto G, Baratta MT, Deans SG, Dorman HJ (2000) Antioxidant and antimicrobial activity of *Foeniculum vulgare* and *Crithmum maritimum* essential oils. Planta Med 66(8):687–693

Sarac A, Tunc I (1995) Toxicity of essential oil vapours to stored-product insects. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 102:429–434

Sawamura M (2000) Aroma and functional properties of Japaneseyuzu (*Citrus junos* Tanaka) essential oil. Aroma Res 1:14–19

Saxena J, Mathela CS (1996) Antifungal activity of new compounds from *Nepeta leucophylla* and *Nepeta clarkei*. Appl Environ Microbiol 62(2):702–704

Schaneberg BT, Khan IA (2002) Comparison of extraction methods for marker compounds in the essential oil of lemon grass by GC. J Agric Food Chem 50:1345–1349

Selvi AT, Joseph GS, Jayaprakasha GK (2003) Inhibition of growth and aflatoxin production in *Aspergillus flavus* by *Garcinia indica* extract and its antioxidant activity. Food Microbiol 20(4):455–460

Senatore F (1996) Influence of harvesting time on yield and composition of the essential oil of a thyme (*Thymus pulegioides* L.) growing wild in Campania (Southern Italy). J Agric Food Chem 44:1327–1332

Shahi SK, Patra M, Shukla AC, Dikshit A (2003) Use of essential oil as botanical-pesticide against post-harvest spoilage in Malus pumilo fruits. Biol Control 48:223–232

Sharma K, Meshram NM (2006) Bioactivity of essential oils from *Acorus calamus* Linn. and *Syzygium aromaticum* Linn. against *Sitophilus oryzae* Linn. in stored wheat. Biopest Int 2:144–152

Shin S (2003) Anti-Aspergillus activities of plant essential oils and their combination effects with ketoconazole or amphotericin B. Arch Pharm Res 5:389–393

Shu CK, Lawrence BM (1997) Reasons for the variation in composition of some commercial essential oils. In: Risch SJ, Ho CT (eds) Spices, flavor chemistry and antioxidant properties,

American chemical society symposium series. American Chemical Society, Washington, DC, pp 138–159
Singh P, Srivastava B, Kumar A, Dubey NK (2008a) Fungal contamination of raw materials of some herbal drugs and recommendation of *Cinnamomum camphora* oil as herbal fungitoxicant. Microbial Ecol 56:555–560
Singh P, Srivastava B, Kumar A, Kumar R, Dubey NK, Gupta R (2008b) Assessment of *Pelargonium graveolens* oil as plant based antimicrobial and aflatoxin suppressor in food preservation. J Sci Food Agric 88:2421–2425
Smid EJ, Gorris LGM (1999) Natural antimicrobials for food preservation. In: Rahman MS (ed) Handbook of food preservation. Marcel Dekker, New York, pp 285–308
Soliman KM, Badeaa RI (2002) Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food Chem Toxicol 40(11):1669–1675
Souza EL, Stamford TLM, Lima EO, Trajano VN (2007) Effectiveness of *Origanum vulgare* L. essential oil to inhibit the growth of food spoiling yeasts. Food Control 18:409–413
Sridhar SR, Rajagopal RV, Rajavel R, Massilamani S, Narsimhan S (2003) Antifungal activity of some essential oils. J Agric Food Chem 51(26):7596–7599
Srivastava B, Singh P, Shukla R, Dubey NK (2008) A novel combination of the essential oils of *Cinnamomum camphora* and *Alpinia galanga* in checking aflatoxin B_1 production by a toxigenic strain of *Aspergillus flavus*. World J Microbiol Biotechnol 24:693–697
Srivastava B, Singh P, Srivastava AK, Shukla R, Dubey NK (2009) Efficacy of *Artabotrys odoratissimus* oil as a novel plant based antimicrobial against storage fungi and aflatoxin B_1 secretion in food commodities. Int J Food Sci Technol 44:1909–1915
Srivastava B, Sagar A, Dubey NK (2011) Evaluation of *Cinnamomum tamala* oil and its phenylpropanoid eugenol for their antifungal and antiaflatoxigenic activity. Food Anal Methods 4:347–356
Suhr KI, Nielsen PV (2003) Antifungal activity of essential oils evaluated by two different application techniques against rye bread spoilage fungi. J Appl Microbiol 94(4):665–674
Tantaoui-Elaraki A, Lattaoui N, Errifi A (1993) Composition and antimicrobial activity of the essential oils of *Thymus broussonettii*, *T. zygis* and *T. satureioides*. J Essent Oil Res 5:45–53
Tatsadjieu NL, Yaouba A, Nukenine EN, Ngassoum MB, Mbofung CMF (2010) Comparative study of the simultaneous action of three essential oils on *Aspergillus flavus* and *Sitophilus zeamais* Motsch. Food Control 21:186–190
Tisch M, Schmezer P, Faulde M, Groh A, Maier H (2002) Genotoxicity studies on permethrin, DEET and diazinon in primary human mucosal cells. Eur Arch Otorhinolaryngol 259(3): 150–153
Tripathi P, Dubey NK (2004) Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruits and vegetables. Postharvest Biol Technol 32:235–245
Tripathi P, Dubey NK, Banerji R, Chansouria JPN (2004) Evaluation of some essential oils as botanical fungitoxicants in management of post-harvest rotting of *Citrus* fruits. World J Microbiol Biotechnol 20:317–321
Tucker AO, Maciarello MJ (1986) The essential oils of some rosemary cultivars. Flavor Fragr J 1:137–142
Tuley de Silva K (1996) A manual of the essential oil industry. United Nation Industrial Development Organization, Vienna
Unnikrishnan V, Nath BS (2002) Hazardous chemicals in foods. Ind J Dairy Biosci 11:155–158
USEPA (2006) Current methodologies and best practices in preparing port emission inventories-final report, vol 5. http://www.epa.gov/sectors/ports/bp_portemissionsfinal.pdf
Vargas RI, John DS, Kido MH, Ketter HM, Whitehand LC (2000) Methyl eugenol and cue-lure traps for suppression of male oriental fruit flies and melon flies (Diptera: Tephritidae) in Hawaii: effects of lure mixtures and weathering. J Econ Entomol 93(1):81–87

Varma J, Dubey NK (1999) Prospectives of botanical and microbial products as pesticides of tomorrow. Curr Sci 76:172–179

Varma J, Dubey NK (2001) Efficacy of essential oils of *Caesulia axillaries* and *Mentha arvensis* against some storage pests causing biodeterioration of food commodities. Int J Food Microbiol 68:207–210

Vaughan SF, Spencer GF (1991) Volatile monoterpenes inhibit potato tuber sprouting. Potato J 68:821–831

Wilson CL, Solar JM, Ghaouth AE, Wisniewski ME (1997) Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. Plant Dis 81(2):204–210

Woodhead SH, Leary AL, Rabatin SC (1990) Discovery, development, and registration of a biocontrol agent from an industrial perspective. Can J Plant Pathol 12:328–331

Xing Y, Xu Q, Li X, Che Z, Yun J (2012) Antifungal activities of clove oil against *Rhizopus nigricans*, *Aspergillus flavus* and *Penicillium citrinum* in vitro and in wounded fruit test. J Food Saf 32:84–93

Zeringue HJZ, Shih B, Bhatnagar D (2001) Effects of clarified neem oil on growth and aflatoxin B_1 formation in submerged and plate cultures of aflatoxigenic *Aspergillus* spp. Phytoparasitica 29(4):361–366

Organic Potato Farming Adoption in Iran

Seyed Alireza Ghadimi, Hossien Shabanali Fami, and Ali Asadi

Abstract Organic farming is an alternative system to conventional agriculture. Organic farming to produce enough food of high quality, while maintaining stability and sustainability in long term. In this regard organic farming replaces conventional agriculture. Potato is a major food after wheat, barley and rice in the world. Cultivation of organic potato is becoming a major goal of agricultural policy. But statistics shows that farmers are facing many challenges to expand area under cultivation of organic products, especially cultivation of organic potato. Thus the main objective of this research was to study adoption of organic potato cultivation in Iran by a survey. The Statistical population consisted of all potato planters in Friedan township. Two hundred farmers were selected based on the Cochran formula. Data was collected by questionnaires. The questionnaire reliability was confirmed by Cronbach's alpha of 0.77–0.91. SPSS statistical software has also been used for data analysis. The results showed that most potato planters have a high tendency to cultivate organic potato. Using factor analysis, deterrence variables that affect development of organic potato cultivation are grouped in eight general factors: economic, technical/farming, marketing, technical/informational, foundational, personal/cultural, communicative/educational and governmental/supportive. These factors control 76.23 % of the total variance.

Keywords Organic agriculture • Organic potato cultivation • Friedan township

1 Introduction

Nowadays, scientists, philosophers, politicians, and ecologists have protested against conventional agriculture in all over the world because evolutional procedure in agricultural section shows that agriculture after green revolution has attained considerable developments by using new technologies and reliance to natural and cheap resources in supplying foodstuffs. These successes have unfortunately brought

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prejudicial effects such as soil erosion, climate changes, water, soil and air pollution and have also decreased biodiversity. All efforts for maximizing crop yield in hectare results in calamity by irregular use of chemical inputs on industrial farming which puts whole life on earth in danger.

So that, totally in traditional and conventional agriculture, more than 300 types of dangerous and artificial chemical compound are used in order to control pests and insects and also maintain soil fertility which remaining of these materials after entering to body can cause numerous problems (Ghorbani et al. 2009a, b). Formal statistics on natural resources and environment in Iran reported by scientists are hopeless. In terms of magnitude of erosion and destruction of fertile land and natural resources Iran have the second rank in the world after Australia, which have soil destruction and erosion of 33 ton per hectare. One of the significant reasons is irregular consumptions of chemical fertilizers and pesticides in farming section (Kashani 2001). World health organization (WHO) has announced that Iran health level among other world countries is 123 in 2007 which its main reason is the lack of observance of optimum consumption principles of fertilizer and chemical pesticides and pests excretion hormones in farms and remaining their effects and compounds in farming crops (Chaychi 2009). Due to irregular use of fertilizers and chemical pesticides in production of agricultural crops, social and health costs are annually increasing for consumers of agricultural crops and government in Iran, and one of the problems of exporting Iranian agricultural products to international markets is their low quality especially high volume of chemical residues.

There is an urgent need for the development of agriculture techniques that are environmentally friendly, economically-socially productive and are required to be stable. The survival of agriculture in the world especially in Iran is to change the current system to a more sustainable system. Producing enough food for a growing population while preserving natural resources and environmental pollution and prevent further deterioration and provide food safety. In this context, organic farming as one of the best alternative farming systems for the production of healthy food without any chemicals is considered (Sharma 2005; Mahmudi et al. 2008; Sharifi Moghadam 2008; Malek Saeedi et al. 2010).

2 Organic Agriculture

Organic farming can be a solution for some of the world's food problems. Organic farming can minimize the negative economic effects of green revolution especially miss consideration of small farmers in green revolution. Also organic farming is decreasing social impact due to showing interest to production and consumer needs, and minimizing adverse environmental effects due to low consumption of chemicals and fertilizers. In organic farming complication of traditional agriculture which includes low production of farmers will be reduced (Abdullahi 2008). Positive effects of organic farming on environment and improvement of farmers' situation in terms of economic condition would be justified. With proper implementation of organic farming, food can be prepared as stable (Fuller et al. 2005; Bengtsson et al.

2005; Eyhorn et al. 2007; Gabriel and Tscharntke 2007). Organic agriculture is a system which improves ecological cycle and increasing soil activity and considers the minimum use of chemical inputs with purpose of health and producing quality crops in farm (Anonymous 2005). In different definitions, organic agriculture is called as environmental agriculture (Gosling et al. 2006) or dynamic environment (Lampkin 2002). It is emphasized in most of these definitions lack of use of pesticides and chemical fertilizers (Abdullahi 1998).

It is the stability of this type of agriculture as the most important reason which distinguished organic agriculture from other methods politically, scientifically and technologically and it places in opposite direction of current methods (Lampkin 1997) and the main reason causes organic agriculture differentiates from other stable agricultural methods, is the existence of collected standards which is applied for determining precise criterion, difference among organic agricultural system and other farming systems especially with marketing purposes (Dehghaniyan et al. 1996). In a sum up, on the basis of presented definitions, organic agriculture has two main characteristic:

- No use of heavy machinery, chemical inputs and transgenic plants.
- No damage to environment due to agricultural activities.

In recent years, organic agriculture has developed rapidly in all over the world and it has been accepted as one of the replacing systems for conventional agriculture by European Union and food and Agricultural organization (FAO), (Polat et al. 2008). So, this kind of agriculture has been performed in 120 countries in the world in 2006. According to last consideration (SOEL survey 2006) for the present time, more than 31 million hectares from world lands in 623,174 farms all over the world are running under organic management which is count about 0.7 % of whole agricultural lands in the world (Willer 2011) and according to world statistic (published statistic in report of the world of organic agriculture 2006) Australia with about 12 million hectares has the most organic lands in the world. China is placed is second rank with about 3/4 million hectares followed by Argentina with 2/8 million hectares (Willer and Yussefi 2006). Some of the factors that have caused organic farming to become a global approach and making rapid growth are its economic and ecological importance to the protection of soil and environment. It appears that production of organic crops in Iran is more economical and easier than other world regions due to dry environmental conditions and abundance of labor forces (Moradi et al. 2011).

3 Organic Potato Cultivation

Potato, among other agricultural crops has the more expanded distribution in the world after wheat, barley and rice and it is cultivated in more than 20 million hectares in 130 countries (Ranji and Ziyaee 2010). Potato is the main product in diet of the people of Iran and the rest of the world. Qualitative and quantitative improvements of potato production have been considered in previous years. Due to excessive use of chemical inputs especially for potato product, organic production of potato has been stressed in recent years. Organic potato production in the world is increasing every year. Most of the lands under potato cultivation in the world are located in Europe. Germany with 4,700 ha land under cultivation of organic potato has the most area under cultivation in the world and England and Denmark are in the next levels. The ratio of organic potato production to total production of potatoes, Denmark, Germany and Switzerland are ranked first to three respectively (Table 1) (Photo 1).

Iran ranked 11th in the world for production about 5 million tones potato annually. From 20 million hectares land under cultivation of this crop in the world, the land under cultivation of potato in Iran has estimated about 154,000 ha in farming year 2008–2009 (the office of statistic and information technology of agricultural

Table 1 Area under organic potato production, percentage of organic potato in total organic and total potato production

	Area under organic potato (ha)	% of organic potatoes in total potato production	% of organic potatoes in total organic production
Germany	4,700	1.58	3.36
United Kingdom	911	0.55	11.05
Denmark	755	2.10	1.95
Netherlands	749	0.59	15.14
France	579	0.35	1.61
Switzerland	500	0.74	11.45
Norway	125	0.74	11.96

Source: Tamml et al. (2004)

Photo 1 Organic potato

jihad organization 2008). According to statistic and existing information, potato is not produced organically in the country. Its cultivation is accompanied with high chemical inputs consumption. This issue alongside of deleterious effects of environment such as soil erosion, climate change and water pollution and … causes more economic damages annually. This is clearly seen in Freidan township, so due to lack of information of the negative consequences of excessive use of chemical fertilizers and pesticides, low level of education of farmers and mismanagement…chemical fertilizers and pesticide consumption is nearly three times in comparison to globally consumption per capita. This is in addition to soil erosion and environmental problems which causes many economic problems for potato farmers in the region. Due to low quality of potato in the city and uncontrolled chemical consumption, it lost their ability to export, and because of surplus of potato in the market it is lost as waste annually.

Due to above mentioned benefits and necessity to implement organic agricultural especially organic potato production in the country, it seems to be necessary to develop organic farming especially organic potato production. But because there are so many obstacles in organic farming, according to international statistics, (Willer 2011, Willer et al. 2008) the area under organic cultivation in Iran is very low. According to the latest statistics, Iran is only posses less than 0.02 % of cultivated organic certified products of the world (Tayefe Soltan Khahi 2011) and it ranked 105 in the world by 200 ha cultivated area under organic management (Abdullahi 2008).

For this purpose, recognition of obstacles and factors inhibiting development and adoption of organic agriculture and especially organic potato cultivation is inevitable. Thus the main purpose of this study is to discuss development obstacles and adoption of organic potato cultivation and to present suggestions for solving these obstacles. This study identifies barriers affecting the adoption of organic potato cultivation in the study area and also helps to increase the richness of the theoretical literature of organic potato cultivation in the country. Given the importance of potato crop and its crucial role in food basket and given the notion that organic farming is a new issue in the country and in the study area, therefore the results can help authorities and policy makers to do effective measures in development of organic agriculture especially development of organic potato cultivation.

Domestic and foreign scholars have conducted many studies on this subject in recent years that few of them are mentioned as follows:

Karimi et al. (2011) in a review of research on barriers to organic farming, have classified organic farming obstacles in three categories: Economical, cognitive-informational and attitudinal, that economic barriers was ranked first between barriers. Sharifi et al. (2010), in his study entitled barriers to conversion to organic farming, stated that the main obstacle to the adoption of organic farming involve "Production, natural, attitudinal-educational, infrastructure, institutional and economic" barriers. Abdullahi (2008), also in a study mentioned that the main problems and obstacles in the development of organic farming are the lack of certification centers and the lack of government support for organic farmers. Therefore, organic farmers in Iran do not make much income from their small lands. Markou and

Stylianides (2009), stated that lack of marketing and lack of government support are most important obstacles for development of organic farming.

Acs et al. (2005) believe that economic barriers is the first obstacle in the way of converting a conventional agro ecosystem to organic system. Wheeler (2005) in his research expressed the effect of social norms on adoption of organic farming and shows which there is a reverse relation between years of experience with adoption of organic agriculture. Angulo et al. (2003) concluded that consumers' uncertainty of organic products is the main obstacle to the acceptance of this type of product. Wynen (2002) find that one of the obstacles of the development of organic agriculture is the lack of proper market for sale, as some of the organic products have been sold as conventional product due to lack of organic market. For example, In Australia nearly one third of organic productions have been sold in common markets. Finally Gil et al. (2000), Richman and Dimitri (2000) showed that one of the development's obstacles in organic farming is decreasing consumption of organic crops due to high price of these types of crops in comparison with ordinary product.

4 Material and Methods

This study was conducted by descriptive survey methodology with cross-sectional method. Statistical population of research were potato farmers in Freidan township (N = 14,000). About 200 potato farmers were selected by Cochran formula. The main data gathering instrument of this research was questionnaire which has been formed of three parts: questions on demographic information of potato farmers, questions on measurement tendency of potato farmers to adoption of organic potato cultivation and questions on identification of adoption obstacles' of organic potato cultivation. Validity of the questionnaire was confirmed by a panel of expert of the professors of the department of Agricultural Extension and Education of the University of Tehran and its reliability was confirmed by Cronbach's alpha (0.77–0.91), SPSS statistical software has also been used for data analysis.

4.1 Study Region

Freidan Township with geographical coordinates 32.59 geographical latitude, and 50.25 eastern longitudes, is one of the townships of Isfahan province, which is located in 130 km west of province in mountainous region of Zagros Mountains and is one of the highest and coldest cities of Iran. The population of this township is about 85,000 which 75 % of the population are active in farming section (14,000 Farmer) and city's economy is based on agriculture and predominant crop is potato with average production about (180,000 ton) per annum is one of the main center of potato production in the country.

5 Results and Discussion

Demographic information of potato planters according to Table 2 showed that average age of potato planters is 46.58 which 80 % of their educational level is diploma and lower than diploma and only 20 % of them have university degree. The experience years of potato planters is about 15 years which shows the importance of potato cultivation in the region. Given that the average land area and the average number of land pieces and average of land size of potato growers are 3.6 ha, 4.79 patch and 0.63 ha respectively. As a result, agricultural land in the region is very small and scattered. This has become one of the challenges of agricultural development of the region. Given that potato is a prevailing cultivation of the region, average area under potato cultivation is 2.92 ha and average potato production per hectare is about 17 ton. This shows low yield of potato among potato planters. According to obtained information and also interview with potato planters, the reasons for low yield of potato were low soil quality, indiscriminate use of chemicals and fertilizers, continuous cultivation of potato and finally lack of rotation and fallow lands, so that (68.5 %) of potato planters mentioned that potatoes are grown consistently.

5.1 Fertilizers Consumption Rate

The allowance consumption rate of chemical fertilizer, (Nitrogen) which its local name is white fertilizer, is 120–150 kg per hectare. But according to information of Table 3 its consumption rate is more than allowable limit by potato planters in study region, so that its consumption average is about 404 kg per hectare which this rate is almost three times as much of allowable rate and also this case is true about Phosphor and Potassium consumption, as their consumption rate is three and two times as much of allowable rate respectively. These demonstrate the excessive use

Table 2 Demographic characteristics of potato planters

Independent variables	Mean	Standard deviation	Maximum	Minimum
Age	46.58	13.45	78	25
Years of experience	14.90	10.87	50	2
Total area under cultivation (ha)	3.60	7.06	32	0.5
Number of pieces of agricultural land	4.79	3.62	14	1
Average land size (ha)	0.63	2.63	9	0.4
Average potato cultivation (ha)	2.92	3.68	20	0.4
Average potato yield (ton)	17.56	9.95	45	5

Table 3 Frequency distribution of potato planters on the basis of fertilizer consumption

Fertilizers	Consumption standard	Consumption by potato planters			
		Mean	Standard deviation	Maximum	Minimum
Nitrogen(N)	120–150	404	148.13	750	100
Phosphor(P)	80–100	288	123.56	450	50
Potassium(K)	100–120	163	121.52	390	50

of chemical inputs for potato production, and the lack of organic cultivation of potato in the study area.

Notably, most of the potato growers have no knowledge about extra use of fertilizer by themselves, nevertheless they complained about quota reduction of fertilizer by government, and they feel that their production is largely dependent on the amount of chemical fertilizer. Approximately 70 % of potato growers believed that in the absence of chemical fertilizer and pesticide, their potato production will have more than 30 % decreases. Most of the potato growers expressed that their standard for deciding the use of fertilizer and pesticides are themselves and other farmers and only 25 % of potato growers expressed that they decide on the basis of expert's suggestion.

5.2 Potato Farmers' Tendency to Adopt Organic Potato

Prioritization component of measurement scale's tendency of potato growers to cultivate organic potato:

In order to determine the priority of the items that tend to measure potato growers willing to grow organic potatoes, the Pearson coefficient of variation was used. The results of this statistic are indicated in Table 4, items such as "agreement with potato cultivation organically" and "tendency to use mechanical methods instead of chemical for weed and pest control" allocated the first and second priority respectively. Average of potato growers' tendency to cultivate organic potato is 5.01 which indicates the high willingness of potato growers to cultivate in organic form.

According to Fig. 1 in order to classify potato growers' tendency to cultivate organic potato, scores of each item were added together and the overall tendency score was obtained. Then considering respondent score limit from 30 the highest score and 5 the lowest score, these were classified in three category as (Low, medium and high) to assess the respondents' tendency again.

According to Fig. 1 it is observed that most of potato growers have a high tendency to cultivate potato organically (%51) and about (%35.5) of potato growers have medium tendency and only (%13.5) of potato planters have low tendency.

Table 4 Potato growers' tendency to cultivate organic potato

Items	Mean (1–5)	SD	CV	Rank
To what extent, do you agree with organically grown potatoes	5.43	0.72	0.132	1
To what extent, you have tendency to use the mechanical methods for weeds and pests control instead of chemicals?	5.39	0.85	0.157	2
To what extent, you have tendency to use natural fertilizers (compost and animal waste and…) instead of chemicals?	4.95	1.01	0.204	3
To what extent, you have tendency to change your present agricultural method gradually and cultivate organically?	4.73	1.07	0.226	4
To what extent, you have tendency to use biological methods instead of pesticides and chemical pesticides?	4.59	1.18	0.257	5
Total mean	5.01			

Nothing: *1*, very little: *2*, a little: *3*, average: *4*, much: *5*, very much: *6*. *SD* (standard deviation), *CV* (coefficient of variation)

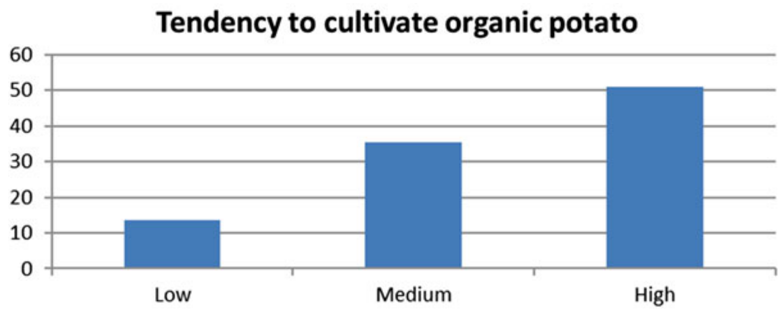

Fig. 1 Frequency distribution of potato growers based on tendency to cultivate organic potato. Low (score < 15), medium (15 < score < 20), high (20 < score)

5.3 *Ranking Items Related to Obstacles of Organic Potato Cultivation*

In Table 5, items have been prioritized to recognize the obstacles of organic potato cultivation, as perceived by potato growers on the basis of Coefficient of Variation statistic. As Table 6 shows, according to potato growers' point of view, first five priority of deterrence variables in cultivation and production of organic potato's adoption are consisted as: "fear of loss of production", "The inability to produce desirable if not using chemical fertilizers", "poverty and low economic situation of farmers and fear of risk", "Lack of introduction of applied methods for replacement and lack of use of fertilizer and chemical pesticides", and "Lack of farmers' knowledge of excessive use of fertilizer and chemical pesticides".

Table 5 Prioritizing obstacles of organic potato cultivation

Variable	Mean (1–5)	SD	CV	Rank
Fear of loss of production	4.42	0.81	0.183	1
The inability to produce desirable if not using chemical fertilizers	4.31	0.85	0.197	2
Poverty and low economic situation of farmers and fear of risk (productive, income – market)	4.26	0.89	0.208	3
Lack of introduction of applied method for replacement and lack of use of fertilizer and chemical prisons	4.24	0.90	0.212	4
Lack of farmers' knowledge of excessive use of fertilizer and chemical pesticides by them	4.22	0.91	0.215	5
Failing to distinguish between organic and non-organic products	4.11	0.90	0.226	6
The uncertainty of yield and access to appropriate market after production	4.01	0.95	0.238	7
Inability to control weeds and potato pests, if not using pesticides	3.93	0.95	0.241	8
Lack of farmers' knowledge of how to grow (inputs and required procedures)	3.99	0.99	0.250	9
Lack of convenient and adequate access to organic agricultural inputs	3.93	1.01	0.252	10
Existence of wrong attitude towards organic farming among farmers	3.92	0.99	0.254	11
Not giving subsidies to organic productions	3.88	1.00	0.257	12
The high cost of certification, especially for small farmers	4.02	1.08	0.261	13
Expensiveness of organic crops and unwillingness of people to pay more money	3.96	1.06	0.262	14
Lack of skilled workforce in the field of organic agriculture	4.02	1.06	0.263	15
Spirit of conservatism and fear of change among farmers	3.83	1.01	0.264	16
The low education level of farmers	3.64	0.97	0.266	17
Lack of farmers' communication with the outside environment and communication channels	3.82	1.03	0.269	18
Lack of adequate and proper market for organic crops	3.80	1.03	0.270	19
Not provide the necessary infrastructures to grow organically	3.92	1.06	0.271	20
Lack of public and farmers' awareness of the benefits of organic farming and damages of chemical fertilizers and pesticides	4.00	1.09	0.272	21
Lack of farmers trust to government and its programs	3.86	1.05	0.273	22
Lack of extension agents and experts in the field of organic agriculture	3.85	1.07	0.277	23
The absence of clear documented standards and criteria for organic production	3.91	1.09	0.278	24
Lack of government support from organic farmers	3.95	1.10	0.278	25
No insurance for lands that are cultivated organically	3.93	1.10	0.279	26
No related demonstration farm to teach farmers organic agriculture	3.82	1.08	0.282	27

(continued)

Table 5 (continued)

Variable	Mean (1–5)	SD	CV	Rank
Farmers' income was reduced in case of cultivating organically	3.62	1.03	0.284	28
Time consuming of producing organically	3.83	1.09	0.285	29
The transition from conventional to organic farming, takes time	3.69	1.06	0.287	30
No educational and extension classes about organic farming and organic potato	3.78	1.09	0.288	31
Old age of most farmers	3.69	1.07	0.289	32
The lack of institutional and consulting firms to train farmers in organic farming	3.80	1.10	0.289	33
The absence of specific enterprise certification for organic products and support of these products	3.67	1.07	0.290	34
Increased need for labor and ultimately increased wages	3.81	1.11	0.291	35
Existence of wrong traditional beliefs in the village	3.98	1.17	0.293	36
Consumer uncertainty of product of being organic crops	3.94	1.16	0.294	37
Farmers lack the technical knowledge about farming	3.91	1.16	0.296	38
Low soil quality and being forced to use too much fertilizers	3.86	1.15	0.297	39
Lack of farmers' access to information sources (such as book, internet and …)	3.88	1.18	0.304	40
Low consumer interest from organic product (lack of adequate market)	3.90	1.19	0.305	41
Increased risk (production – income – natural…)	3.59	1.11	0.309	42
Profitability of conventional agriculture	3.68	1.17	0.310	43
High cost of organic agriculture	3.62	1.13	0.312	44
Low farmers' interest to cultivate organically	3.75	1.19	0.313	45
This type of farming is not compatible with farmers' conditions especially small farmers	3.56	1.12	0.314	46

Nothing: *1*, very little: *2*, a little: *3*, average: *4*, much: *5*, very much: *6*. *SD* (standard deviation), *CV* (coefficient of variation)

Table 6 Eigen values and explained variance by each factor

Factors	Eigen values	% of variance	Cumulative
1	5.04	11.96	11.96
2	3.98	10.65	22.61
3	3.97	10.64	33.07
4	3.85	9.60	42.67
5	3.75	9.29	51.96
6	3.69	9.11	61.07
7	3.46	8.67	69.74
8	2.52	6.46	76.23

5.4 Result of Factor Analysis (EFA) of Barriers to Cultivation Organic Potato

Factor Analysis technique were used to classify barriers to cultivate organic potato. KMO value obtained is 0.763 and Bartlet test chi-square = 279.53 and its significant level was 1 % which shows suitability of data for factor analysis. Factor analysis listed 46 inhibitor variables for organic potato cultivation, and classified 36 variables in eight factors which it explain 76.23 % of variance. Eigen values and percentage of variance is presented in Table 6.

Positioning of related variable to obstacles of development and adoption of organic potato due to extracted factors, assuming the variables have loadings greater than 0.5, after the rotation by Verimax method and naming factors are presented in Table 7. Based on Table 7, economic obstacles explain 11.96 % of total variance and technical – farming obstacles explain 10.65 % of total variance and marketing obstacle explain 10.64 % of total variance are the most important obstacles of development and adoption of organic potato cultivation.

6 Conclusion

According to research findings in the case of (Freidan township) due to barriers that were classified in eight factors, potato is not grown organically and consumption of fertilizer and pesticides are about three times of world per capita allowance. However, given the high potential of study area for growing potato organically, most farms are small, also there is plenty of labors and high willingness among potato growers to grow organically. As a result it can be said that it is possible to grow potato organically in the study area. Feasibility of growing organic potato in the study area is subject to elimination of barriers to organic farming. As long as the barriers of organically grown potato have not been removed, we cannot hope that organically grown potato happens. Because of these obstacles are so effective and important given that potato planters have more tendencies to cultivate potato organically, but they do not grow potato organically and still use high amount of fertilizers and pesticides due to these barriers. Hence for removing barriers mentioned, and provide organically grown potato and also make it possible to cultivate in the study area, based on findings and inferential statistics and presence of researcher in the field and interview with potato growers, the following recommendations were made:

– As, it is observed in results of factor analysis, the most important factor which prevents from acceptance organically potato cultivation is economic obstacles and especially poverty and low economic situation of farmers in study region. Farmers have a few financial resources, because in study region there is continuous droughts and petty and livelihood farming and farmers do not have risk for consumption decrease of fertilizer and chemical pesticides and applying replacing and new organic methods. Which in this direction, it is proposed, self-confidence of other

Table 7 Characteristics of extracted factors resulting from factor analysis

Factor	Variable	Factor loading
Economic obstacles	Poverty, low economic condition of farmers and fear of risk (production – income – marketing)	0.804
	The high cost of certification especially for small farmers	0.752
	Increased need for labor and ultimately increased wages	0.653
	The transition from conventional to organic farming takes time	0.626
	No insurance for lands which are cultivated organically	0.613
Technical – farming obstacles	Lack of introduction of applied method for replacement and lack of use of fertilizer and chemical pesticides	0.811
	Fear of loss of potato production	0.805
	No capability in desirable potato production in case of not using chemical fertilizers	0.799
	Inability to control weeds and potato pests, if not using pesticides	0.744
	Lack of convenient and adequate access to organic agricultural inputs	0.714
	Time consuming of producing organically	0.693
Market obstacles	Consumer uncertainty of product, being organic	0.764
	The uncertainty of yield and access to appropriate market after production	0.643
	Expensiveness of organic products and unwillingness of people to pay more money	0.533
	Low consumer interest from organic crops (Lack of adequate market)	0.527
	No confidence of consumers from organic crops	0.502
Technical – information obstacles	Lack of farmers' technical knowledge of how to grow organically (inputs and required procedures)	0.758
	Lack of farmers' knowledge of excessive use of fertilizer and chemical pesticides	0.742
	Lack of extension agents and experts in the field of organic agriculture	0.632
	The lack of institutional and consulting firms to train farmers in organic farming	0.521
	No related demonstration farm to teach farmers organic agriculture	0.513
Infrastructural obstacles	Not provide the necessary infrastructures to grow organically	0.820
	The absence of specific enterprise certification for organic products and support these crops	0.685
	The absence of clear documented standards and criteria for organic production	0.572
Personal – cultural obstacles	Existence of wrong attitude towards organic agriculture among farmers	0.833
	A high age of majority of farmers	0.801
	Existence of wrong traditional belief in the village	0.695
	Old age of most farmers	0.508
	Spirit of conservatism and fear of change among farmers	0.502

(continued)

Table 7 (continued)

Factor	Variable	Factor loading
Communication – education obstacles	Lack of public and farmers' awareness of the benefits of organic farming and losses of chemical fertilizers and pesticides	0.713
	No educational and extension classes about organic farming and organic potato	0.686
	Lack of farmers' access to information resources (such as book, internet and …)	0.576
	Lack of farmers' communication with the outside environment and communication channels	0.555
Government – support obstacles	Lack of farmers trust to government and its programs	0.819
	Lack of government support from organic farmers	0.617
	Not giving subsidies to organic productions	0.513

farmers increase and their fear of risk and decrease in production will be decreased, at first to focus on educated and big owner farmers which have sufficient financial backing and their lands manage under organic cultivation management which these lands have training aspect and either simulative aspect for other farmers and in addition to this matter, it is necessary to cultivate lands under agricultural management and to be insured for decreasing productive and income risk.

– According to this case that organically potato production would cause average 20–25 % decrease, price increase causes providing income and economic explanation of its production which the essential condition is differentiation bet weed productive potato's organically and un organically by special labels of organic productions but according to research findings in study region, there is on organ for accomplishing this case. So, it is necessary to form an organization in local level for determining specified standards for organic productions and also certificate issuance and labels for recognition and differentiation of organic crops from other crops for encouraging farmers who plant organically. This affair, in addition to encourage and support of potato planters who plant organically, causes confidence of consumers in use of organic crops and increases demand.
– Changing conventional method of farming and use of organic agricultural methods and technologies for producing organic potato initially needs to financial capital and governmental supports. For example, one of suggested methods for fortifying soil and controlling pests and diseases and weeds of potato in organic agriculture is farming alternation and mixed cultivation and lands fallow which both of these crops for different cultivation process needs new tools and machineries. For this purpose, in order to buy above cases sufficient financial resources is needed, which according to farmers economic situation of study region, it is not possible to buy these tools. The results of this factor analysis emphasize this affair as potato planters of study region express one of acceptance obstacles of organic farming not supporting of government. Consequently, it is necessary that government and especially Agriculture Bank as main resources of agricultural

credits, pays facilities in form of long term loans and loans with low interest to potato planters.

- According to findings of research, the most significant way of soil fortifying and providing plant needs of potato planters in study region is chemical fertilizers consumption. As the main factor for determining, potato operation average is consumption rate of chemical fertilizers which most of farmers expressed not awareness of replacing methods and not sufficient replying of other methods as the reason of its great consumption. This affair is true about controlling pests and diseases and weeds of potato and also most of farmers mentioned that they cannot control pests and weeds in case of not consuming chemical pesticides and other methods can not be a good replacement for this case, so it is necessary which specialty training classes are constituted in relation to potato cultivation procedure organically and performance of agricultural methods and technologies in study region and these methods are trained to farmers in form of specialty and applicably courses. Because it can be hopeful when study region farmers decrease consumption of fertilizer and chemical pesticides and use methods and organic technologies which these methods can be proper replacement for supplying plant needs of potato and controlling pests and diseases and weeds and maintain production desirably.
- On the basis of factor analysis results, one of the most important acceptance of organic potato cultivation, have been expressed market obstacles and not existence of sufficient market for begin more expensive organic crops and not confidence of consumers from being organic (potato) crops which this obstacle is abundant in third world countries such as Iran, their people welfare level and economic conditions are not in desirable limit. So, it is necessary similar to experience of countries such as Australia, America and France, first, with 5–10 % increasing in prices, they began to present crops which makes more stimulation for consuming organic crops among consumers and establish fairs and markets for presenting organic crops permanently.
- It is observed problem of using more than usual, fertilizer and chemical pesticides for producing potato abundantly but the problem which makes the importance of this subject more than before and is significant and now has changed to one of the most significant obstacles of consumption decrease of chemical inputs and potato cultivation organically, not awareness of potato planters in regarding to great consumption of fertilizer chemical pesticides by them is in a manner when it is asked about the reason of their great consumption of chemical in puts, most of them are not informed about this subject and also they complain to government about quota decrease of fertilizer and chemical pesticides. For this purpose, considering training affair is as one of the most important steps of organic agricultural development and organic potato production and it is necessary was trained by communicative chemicals such as TV and … and specialty classes are disposed in this field for potato planters of study region.
- Agricultural promoters and experts have significant role in development of agricultural innovations such as organic farming if it occurs desirable change on agricultural system, this change can be possible only on the basis of recognition

and correct information and according to the results of factorial analysis, one of the most important technological – information obstacles of organic potato cultivation in study region is not existence of promoters and skillful experts in field of organic potato cultivation it is necessary, before organic agricultural development and propagation in a region, agricultural experts are explained and trained, because not its development in a region will be faced with failure and also causes pessimism of farmer in regarding to organic agriculture. So, it is suggested that celebrating trainings while on duty, seminars and congresses scientific and applicable skills of promoters and experts will be promoted.

- According to research findings and also presence in study region and interview with farmers, agricultural responsible of township foe organic agricultural development and potato cultivation organically before providing necessary substructures for organic farming and supplying its necessary inputs such as compost and animal fertilizers and other fertilizers and natural inputs for replacing with chemical inputs and also training replacing and new methods, only referring to decrease quota of fertilizer and chemical pesticides of farmers which this affair by considering not awareness of region farmers with potato cultivation organically and not acquaintance with application manner of replacing and new methods and also existence economic problems among farmers of study region causes disagreements among farmers in such a manner that they complained about decrease of this quota. It is suggested that quota decrease and presenting fertilizer and chemical pesticides with providing organic agricultural substructures and training replacing and new methods because other than this case will be faced with farmers disagreement and alteration their view in regarding to organic farming which this affair acceptance of organic farming even in future will encounter with difficulty.
- Personal characteristic in farming is as one of the most important effective factors on acceptance of agricultural innovations such as organic farming which it is proposed according to research findings for more success in development of organic potato cultivation, first it is focused on younger farmers and with high education who have positive observation to organic farming because this affair in addition to decrease costs of development and promotion cultivation of organic potato and increases its development speed and it is a type of demonstrative and training farm of other farmers which accept later and most of them are old and have low educations.
- Another reason of great consumption of chemical inputs in study region, is continuous cultivation of potato during last 50 years which causes intense falling in lands soil quality of region which this affair is abundant in case of great consumption of Nitrogen. So, it is necessary to change cultivation pattern of study region which most of time is just one crop and potato and new cultivation pattern which is accompanied with farming alternation and mixed cultivation and even lands fallow, is developed. In this direction, potato cultivation is accompanied with wheat; oats, alfalfa and sugar beet in form of farming alternation and potato cultivation with corn are ordered to mix cultivation.

– As it is observed in research finding, most of potato planters of study region know their potato production rate is dependent to consumption rate of chemical inputs, as about 70 % of potato planters expressed that not consumption of chemical inputs more that 30 % decreases potato production operation. This affair has changed to one of the most important acceptance obstacles of organic potato cultivation and consumption decrease of chemical inputs study region. For this purpose, it is necessary that farmers are trained by collected media and training classes which dependency to chemical inputs can be decreased by using organic agricultural technologies and methods and operation decrease is compensated by these methods.
– Given to this fact that about 70 % of persons job is agriculture and main source of their income is coming from agriculture, especially, from potato production, and most of the time, whole members of family are engage in agricultural activities, it can be concluded that agriculture and potato cultivation among people of study region is beyond the job and an economic activity but it is type of life method and livelihood which each kind of changes and deciding for changing common agricultural method and organic agricultural development in study region must be done by cooperation of region farmers. So, it is necessary to constitute a labor team includes of experts and specialists and presence of superior farmers and people representatives and even local leaders for increasing farmers cooperation and using knowledge and local forces.

References

Abdolahi S (1988) Agricultural exploitation systems of Iran. The Ministry of Jahad-e Agriculture, assistance of exploitation system affairs

Abdolahi S (2008) Consideration organic agricultural development in Iran. The Ministry of Jahad-e Agriculture, Agricultural Planning Economic & Rural Development Research Institute, Tehran

Acs S, Berentsen P, Wolf M, Huirne R (2005) Bio-economic modeling of arable farming system, comparison of conventional and organic farming systems in Netherlands. Business Economics Group, Department of Social Sciences, Wageningen University, Netherlands

Angulo A, Gil M, Tamburo L (2003) Food safety and consumers' willingness to pay for labeled beef in Spain. Paper presented at the 83rd EAAE seminar, China, 4–6 September. Available at: www.maich/eaae.gr

Anonymous (2005) The national organic standard board. Retrieved from: http://www.NOSB.al.htm

Bengtsson J, Ahnstrom J, Weibull A (2005) The effects of organic agriculture on biodiversity and abundance: a meta-analysis. J Appl Ecol 42:261–269

Chaychi B (2009) Organic farming, healthy soil, healthy plant, healthy human. J Anim Cult Ind 117:48–56

Dehghaniyan S, Kuchaki A, ColahiAhari A (1996) Ecological economy & organic agricultural economy. The Academic Center for Education, Culture and Research (ACECR), Mashhad

Eyhorn F, Ramakrishnan M, Mäder P (2007) The viability of cotton-based organic agriculture systems in India. Int J Agric Environ Sci 6(3):23–30

Fuller RJ, Norton RL, Feber RE, Johnson PJ, Chamberlain DE, Joys AC, Mathews F, Stuart RC, Townsend MC, Manley WJ, Wolfe MS, Macdonald DW, Firbank LG (2005) Benefits of organic agriculture to biodiversity vary among taxa. Biol Lett 1:431–434

Gabriel D, Tscharntke T (2007) Insect pollinated plants benefit from organic agriculture. Agric Ecosyst Environ 118:43–48

Ghorbani M, Koocheki A, Mahmoudi H (2009a) Estimation of virtual yield organic wheat: case study of Khorasan province. Environ Sci 6(3):23–30
Ghorbani R, Koocheki A, Jahan M, Nassiri M, Rezvani-Moghadam P (2009b) National organic standards for Iran: concepts, principles and aims of organic production and standards for agronomic and horticultural products. J Agroecol 1(1, Fall):129–142
Gil JM, Gracia A, Sanchez M (2000) Market segmentation and willingness to pay for organic products in Spain. Int Food Agribus Manag Rev 3:207–226
Gosling P, Hodge A, Goodlass G, Bending GD (2006) Arbuscular mycorrhizal fungi and organic farming. Agric Ecosyst Environ 113:17–35
IFOAM (2007) Organic agriculture worldwide: directory of IFOAM member organization and associates. IFOAM
Karimi A, Sedighi H, Babayi A (2011) Investigating experts' perception of barriers to organic farming. Iran J Agric Econ Dev Res 2:231–242
Kashani A (2001) Sustainable agriculture in Iran: concepts, methods & existed situation, jihad. 20:240–241, pp 5–8
Lampkin N (1997) Organic agriculture. Ferdowsi University of Mashad
Lampkin N (2002) Organic farming. Old Pond, Ipswich
Mahmudi H, Mahdavi damghani A, Liyaghat H (2008) Evaluation organic agriculture. The Academic Center for Education, Culture and Research (ACECR), Mashhad
Malek Saeedi H, Rezayi Moghadam K, Agili A (2010) Study knowledge of agricultural jihad experts in Fars province in field of organic agriculture. Iran Agric Ext Educ J 2:49–61
Markou M, Stylianides T (2009) A plan to place successfully organic products in the Cypriot market. J Dev Agric Econ 1(3):055–074
Moradi J, Heydari H, Azizi M, Yaghubi A (2011) Analysis organic agricultural place as agricultural stable development bed in view point of farmers (case study: Divandareh & Gharveh townships). Second national conference on sustainable rural development, Hamedan, Bu-Ali Sina University
Polat E, Demir H, Onus AN (2008) Comparison of some yield and quality criteria in organically and conventionally-grown lettuce. Afr J Biotechnol 7(9):1235–1239
Ranji H, Ziyaee M (2010) Pests, diseases & weeds of potato & their mixed management. The Academic Center for Education, Culture and Research (ACECR), Urmia
Richman N, Dimitri C (2000) Organic foods: niche ignallin venture into mainstream agricultural outlook, June–July, 11–14
Sharifi Moghadam M (2008) Guiding document of organic agricultural development in Iran. Agricultural Researches, Education & Extension Organization (AREEO)
Sharifi O, Sadati A, Rostami Ghobadi F, Sadati A, Mohamadi Y, Taher Tolou Del P (2010) Barriers to conversion to organic farming: a case study in Babol County in Iran. Afr J Agric Res 5(16):2260–2267. Available: http://www.academicjournals.org/AJAR
Sharma A (2005) A handbook of organic farming. Agrobios, Jodhpur
Statistic Office & Technology of Ministry of Jahad-e Agriculture (1999) Agricultural statistic of farming crops, farming year 1999–2009, The Ministry of Jahad-e Agriculture. www.maj.ir
Tamml et al (2004) Assessment of the socio-economic impact of late blight and state-of-the-art management in European organic potato production systems, research institute of organic agriculture FiBL, Switzerland. Available online at: http://www.organicprints.org/2936
Tayefe Sultan Khani A (2011) Organic agricultural role in establishing food security. J Train Res Food New Technol 13
Wheeler S (2005) Factors influencing agricultural professionals attitudes towards organic agriculture and biotechnology. Center for Regulation and Market Analysis, School of Commerce, University of South Australia. Available at: http://een.anu.edu.au
Willer H (2011) Organic agriculture worldwide key results from the global survey on organic agriculture. J Res Inst Org Agric. www.fibl.org
Willer H, Yussefi M (2006) The world of organic agriculture, statistics and emerging trends. International Federation of Organic Agriculture Movements (IFOAM)
Willer H, Yussefi-Mazle M, Sorensen M (2008) The world of organic agriculture; statistics and emerging trends. IFOAM/Fibl/Earthscan, London

Crop Plant Hormones and Environmental Stress

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Abstract Plant hormones play vital roles in the ability of plants to acclimatize to varying environments by mediating growth, development and nutrient allocation. Hormones move through specific pathways to regulatory sites where they respond to stress at awfully low concentration. All biological activities are directly or indirectly affected by both phytohormones. Here we review the role of hormones against abiotic tolerance in crop plants. The main findings are: (1) abscisic acid act as a mediator in plant responses to many stresses, including salt stress. (2) Stress modifies the level of indole acetic acid (IAA) thus reducing growth. (3) Functional analysis of cytokinin receptor mutants show that cytokinin receptors of Arabidopsis act as negative regulators in abscisic acid (ABA) signaling and in osmotic stress response. (4) The mechanisms by which gibberellic acid (GA) priming could induce salt tolerance in plants are not yet clear. Salinity perturbs the hormonal balance in plants. Under salt stress hormonal homeostasis might be the possible mechanism of GA3-induced plant salt tolerance. (5) A low level of salicylic acid and jasmonate is effective against abiotic stress by enhancing physiological processes and improving tolerance. (6) Role of brassinosteroids and triazole during environmental stress is emerging. (7) Ethylene is considered as a stress hormone; however, the role of ethylene in salt stress is equivocal. The present review focus on abscisic acid, indole acetic acid, cytokinins, gibberellic acid, salicylic acid, brassinosteroids, jasmonates, ethylene and triazole.

Keywords Abiotic stress • Plant growth regulators • Phytohormones

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1 Introduction

In response to different abiotic stresses plants adapt many strategies such as high salt tolerance, dehydration, cold, heat and excessive osmotic pressure which ultimately enhance the plant growth and productivity (Epstein et al. 1980; Yancey et al. 1982). Different mechanisms adapted by plants are extremely important for high production and several plants have evolved different mechanisms to overcome the adverse condition. These phenomena include change in morphological and developmental pattern as well as physiological and biochemical processes against several stresses (Bohnert et al. 1995). Adaptation to all these stresses is associated with metabolic adjustment that leads to the accumulation of several organic solutes like sugars, polyols, betaines and proline (Flowers et al. 1977; Greenway and Munns 1980). Sugars represent the major reserve in the seeds among these accumulating solutes (Bewlay and Black 1994) which synthesized maximally during germination and mobilized to various tissues like stem and internods (Smith 1967) in the form of sucrose, glucose and fructose, that are transportable readily to sites where they are required for growth (Mayer and Poljkoff-Mayber 1975) and osmotic regulations of cells are maintained (Garham et al. 1981). In different parts of plants accumulation of sugars is enhanced in response to the variety of environmental stresses (Macleod and Orquodale 1958; Escalada and Moss 1976; Garham et al. 1981; Prado et al. 2000).

During different abiotic stresses there are earlier reports on carbohydrate accumulation in the temperate grasses and cereals from the Gramineae family where during reproductive development long term carbohydrate storage occur (Archbold 1940; Meeir and Reid 1982).

Among various abiotic stresses, soil salinization is one of major stress that has got important throughout the world (Karen 2000; Liang et al. 2005). There are two main types of salinity (primary and secondary salinization). Primary salinization is natural phenomena in which soluble salts are accumulated through natural process due to presence of high salts contents in ground water. Secondary salinization occurs frequently as a result of over irrigation due to improper management of irrigation facilities, poor soil drainage condition and unstable quality of irrigation water (Yuan et al. 2007). Every year more and more lands become non-productive due to salt accumulation. Therefore, understanding the mechanisms of plant tolerance to salinity stress is important (Bartels and Sunkar 2005). Salinity is more important stress which can limit plant productivity (Hasegawa et al. 2000). On nearly 20 % of the cultivated area and half of the irrigated area of worldwide soil salinity is a major constraint limiting agricultural productivity (Zhu 2001). Inhibition of growth and development, reduction in photosynthesis, respiration and protein synthesis in sensitive species all these have been reported to cause by salt stress (Boyer 1982; Meloni et al. 2003; Pal et al. 2004). Excessive generation of reactive oxygen species such as superoxide anion, hydrogen peroxide and the hydroxyl radicals particularly in chloroplasts and mitochondria are important corollary of salinity stress in plants (Mittler 2002; Masood et al. 2006). Against the damaging effects of reactive oxygen species, plants used antioxidant enzymes like superoxide dismutase,

Table 1 Salt-affected soils of regional distribution, in million hectares. Total area affected around different region of the world by salinity is 3.1 % while sodic soil is of 3.4 %

Regions	Total area (Mha)	Saline soils		Sodic soil	
		Mha	%	Mha	%
Africa	1,899	39	2.0	34	1.8
Asia, the Pacific and Australia	3,107	195	6.3	249	8.0
Europe	2,011	7	0.3	73	3.6
Latin America	2,039	61	3.0	51	2.5
Near East	1,802	92	5.1	14	0.8
North America	1,924	5	0.2	15	0.8
Total	12,781	397	3.1	3.4	

Source: FAO Land and Plant Nutrition Management Service

ascorbate peroxidase and glutathione reductase for their protection (Asada 1992; Prochazkova and Wilhelmova 2007). Membrane disorganization, metabolic toxicity due to reactive oxygen species and attenuated nutrients are the factors, which initiate more catastrophic events in plants subjected to salinity stress (Frommer et al. 1999; Zhu 2000; Cost et al. 2005). According to a report published by Food and Agricultural Organization (FAO) and Plant Nutrition Management Service in 2005, that over 831 million hectares (8 %) were affected by salt including saline and sodic soil extended all over the continents including Africa, Asia, Australia and America (Table 1).

Different types of studies have been conducted to study the effect of salinity on plants (Duan et al. 2008). Investigations recently have more concerted on the salt tolerance mechanisms in plants (Dajic 2006; Munns and Tester 2008). Sharma et al. (2005) reported that phytohormones playing an important role in stress responses and adjustment. It is a concern that the exploitive consequence of salinity on seed germination and plant growth could be associated with a decline in endogenous phytohormones level (Zholkevich and Pustovoytova 1993; Jackson 1997; Debez et al. 2001). GomezCadenas et al. (1998) reported that salt stress resulted in an increased level of abscisic acid and ethylene. Abscisic acid has been found to alleviate the inhibitory effect of NaCl on photosynthesis, growth and translocation of assimilates (Popova et al. 1995). It has been reported that under salt stress, abscisic acid decreases the release of ethylene and leaf abscission possibly by reducing the toxic Cl^- ion's accumulation in citrus leaves (GomezCadenas et al. 2002). Abscisic acid concentration increases as a result of salinity and water stress (Shin Shinozaki and Yamaguchi-Shinozaki 1997). Abscisic acid is known to mediate signals in plant cells subjected to environmental stresses. These signals can bring about expression of stress related genes followed by synthesis of compatible osmolytes such as proline (Kavi Kishore et al. 2005). Early work have conjectured that endogenous content of plant hormones such as abscisic acid, auxin, cytokinins, zeatin and gibberellins changes in response to salt stress (Javid et al. 2011) (Fig. 1).

Fig. 1 Effect of drought condition on maize plants and kernel abortion. Drought severely affected the growth and yield of maize crop in field condition

Another major threat of abiotic stress to plants is high and low temperature stress. High temperature (heat) stress is considered to be one of the major environmental factors limiting crop growth and yield. This stress induces many physiological, biochemical and molecular changes that affect crop yield and quality (Shrivastava et al. 2012). The increase in atmospheric temperature causes detrimental effects on growth, yield, and quality of the rice crop by affecting its phenology, physiology, and yield components (Sheehy et al. 2005, Peng et al. 2004). Yields of rice have been estimated to be reduced by 41 % by the end of the twenty-first century (Ceccarelli et al. 2010). There is enough evidence that increasing night-time temperature has been the main cause of increases in global mean temperatures since the middle of the twentieth century, and is thus the main factor contributing to the yield decrease (Peng et al. 2004; Sheehy et al. 2005).

Here, we highlight the latest advances in our understanding of the role of hormones in ameliorating the adverse effect of abiotic stress in crop plants. We then discuss the recent progress in the engineering of hormone-associated genes aimed at improving crop stress tolerance.

2 Phytohormones

2.1 Abscisic Acid

Plants are frequently prone to different types of abiotic stresses such as drought, chilling, heat, or salinity stress. The phytohormone abscisic acid has been proposed to play an important role in stress responses and/or adaptation (Thomas and Eamus 1999; Sharma et al. 2005; Shaterian et al. 2005). Abscisic acid is an important isoprenoid plant hormone, which is produce in the plastidal 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway; unlike the structurally related sesquiterpenes, which are formed from the mevalonic acid-derived precursor farnesyl diphosphate, after cleavage of C_{40} carotenoids in MEP, the C_{15} backbone of abscisic acid is formed. It plays a significant role during many stages of the plant life cycle, including seed development and dormancy, and in plant responses to various environmental stresses.

The abscisic acid has been proposed to act as a mediator in plant responses to a range of stresses, including drought and salt stress. Abscisic acid is also the major internal signal enabling plants to survive adverse environmental conditions such as salt stress (Keskin et al., 2010). According to Zhang et al. (2006) reported that plant's exposure to salinity is known to make a proportional increase in abscisic acid concentration that is in the majority of conditions associated with leaf or soil water potential, considering that salt-induced endogenous abscisic acid is due to water deficit rather than to a specific effect of salt. This may not bear a resemblance to the prolonged increasing of endogenous abscisic acid levels that can occur in association with slowly increasing salinity stresses in nature or field situations (Etehadnia et al. 2008).

A signal is generated by abscisic acid during a plant's life cycle in order to control seed germination and developmental processes. Specifically, guard cells can target by the action of abscisic acid for stomatal closure induction but for modification towards severe water shortage it may also signal systemically. It is now well thought-out as a plant stress hormone because different stresses to induce abscisic acid synthesis (Mahajan and Tuteja 2005; Swamy and Smith 1999).

Abscisic acid, acts as an endognous messenger in the plant's water regulation status (Swamy and Smith 1999). Regulating water status in plant through guard cells and growth as well as by induction of genes that encode enzymes and other proteins involved in cellular dehydration tolerance abscisic acid plays a significant role (Luan 2002; Zhu 2002). Abscisic acid can act as a long-distance water stress signal in sensing incoming soil drying as early work showed (Davies and Zhang 1991). In drying soil, stress abscisic acid produced in dehydrated roots, then transported to the xylem and regulates stomatal opening and leaf growth in the shoots (Zhang et al. 1987; Zhang and Davies 1989, 1990a, b). This mechanism is modified by the ionic conditions and pH in the xylem (Trejo and Davies 1991; Wilkinson et al. 1998; Bacon et al. 1998). Concentration of abscisic acid increases in roots reported by Jia et al. (2002), when root continues their growth, suggests that these tissues may have different responses to the restricted abscisic acid concentration either in endogenous form, or when exogenously applied (Creelman et al. 1990).

Davies et al. (1994) reported that stress responses to the root and shoot tissues emerge to be coordinated by amounts of hormones increased moving in the xylem sap by 'root-to-shoot' communication. Though, a number of doubts still remain concerning the abscisic acid ability to act as a signal that mediates the effects of root-zone stress (Jia et al. 2002). Hartung et al. (2002) have reported that pH changes play a key role in the abscisic acid redistribution in leaf tissues and control the stomata at times when no significant changes in abscisic acid concentration are detected in the xylem.

Various transcription factors are known to regulate the abscisic acid -responsive gene expression (Mahajan and Tuteja 2005; Xiong et al. 2002). According to Wang et al. (2001) salinity up-regulated the generic stress hormone abscisic acid and induces genes involved in salt and osmotic alleviation. Regulations of AtNHX1 expression and tissue distribution by abscisic acid and salt stress have reported by Shi and Zhu (2002). Effects of abscisic acid on two genes expression *HVP1* and *HVP10* for vacuolar H^+-inorganic pyrophosphatase and one *HvVHA-A* for the catalytic subunit (subunit A) of vacuolar H^+-ATPase discussed by Fukuda and Tanaka (2006). By quantification of the transcript levels it was accomplished, to find out the hormones liable for adaptable expression of these genes in barley (*Hordeum vulgare* L.) in reaction to salt stress.

According to Keskin et al. (2010) in response to abscisic acid treatment in wheat, induction of these genes *MAPK4*-like, *TIP1* and *GLP1* were much faster. The evidence of this result could be possible for the role of these genes in the abscisic acid -induced pathways. Genes responsive to stress can be expressed either through an abscisic acid -dependent or abscisic acid -independent pathway (Chinnusamy et al. 2004). According to Zhang et al. (2006) abscisic acid -deficient and abscisic acid -insensitive mutants uses, have shown that water stress signaling may be understood in two major pathways: the abscisic acid -dependent and abscisic acid -independent gene expression pathways. There are two different routes of abscisic acid -dependent pathway: i.e. requiring new protein synthesis or not (Jensen et al. 1996; Shin Shinozaki and Yamaguchi-Shinozaki 1997; Bray 2002). In the route where there is no need of new protein synthesis, then in all abscisic acid -responsive genes the promoter domain has an abscisic acid -responsive element ABRE (PyACGTGGC where Py indicates a pyrimidine base, C or T). With corresponding bzip family of transcription factors such as EmBP-1 when ABRE is bound it can lead to abscisic acid -induced gene expression.

Recent development in field of plant molecular biology has shown that many group of genes played an important role in plant stress responses. However, not all of these genes are abscisic acid -related; it is believed that abscisic acid plays a central role. The mechanisms that up regulates abscisic acid biosynthesis genes by the abiotic stress are still need understand. Still we need to understand the functions of all the different types of abscisic acid -responsive genes. From this review, it observed that abscisic acid has been anticipated to act as a mediator in plant responses to a range of stresses, including salt stress. In nature environmental stress signals are complicated and the initial signaling processes control the whole cellular signaling cascades. While investigation should continue to study the expression of genes encoding known signaling components, more endeavors should be paying attention on revealing new signaling components and cascades and functions of signal components in stress tolerance.

2.2 Indole Acetic Acid

The first plant hormone that identified was indole acetic acid, although the biosynthetic pathway of IAA at the genetic level has remained unclear. For indole acetic acid biosynthesis two major pathways have been proposed: the tryptophan -independent and Trp-dependent pathways. In plants four pathways have been postulated in tryptophan -dependent indole acetic acid biosynthesis: (i) the indole-3-acetamide pathway; (ii) the indole-3-pyruvic acid pathway; (iii) the tryptamine pathway; and (iv) the indole-3-acetaldoxime pathway. However, many plant species may have unique strategies and modifications to optimize their metabolic pathways, plants would be projected to share evolutionarily conserved core mechanisms for auxin biosynthesis because in the plant life cycle indole acetic acid is a fundamental substance.

According to Aloni (2001) indole acetic acid is the major shoot signal which regulates all aspects of vascular differentiation in plants. From young shoot organs polar transport of indole acetic acid (Aloni 2004) downward via the cambium to the root tips (Aloni et al. 2006) induces and controls wood formation. The vascular tissues continuity along the plant axis is a result of the steady polar flow of indole acetic acid from leaves to roots (Aloni 1987). Indole acetic acid plays a key role on regulating plant growth. For example, it controls cell elongation, vascular tissue development and apical dominance (Wang et al. 2001). Indole acetic acid also responds to salinity in crop plants. Though, little information seems to be available on the relationship between auxin levels in plants and salinity stress and the role of auxin in mitigating salt stress. Under stress conditions, the variations in indole acetic acid content appeared to be similar to those of abscisic acid (Ribaut and Pilet 1991). According to Ribaut and Pilet (1994) the increased level of indole acetic acid has reportedly been correlated with reduced growth. Therefore, under stress conditions the reduction in plant growth could be result of altered hormonal balance. Hence, to counter the stress conditions their exogenous application provides an attractive approach.

An experiment conducted by Nilsen and Orcutt (1996) observed that exposure of rice plants to salinity stress there was a significant reduction in indole acetic acid levels in 5 days while in other experiment, salinity reduced indole acetic acid levels 75 % in tomato roots (Dunlap and Binzel 1996). According to Prakash and Prathapasenan (1990) concentration of indole acetic acid in rice leaves were significantly decreased when treated with NaCl. In this experiment, during the salinization period when GA_3 was applied it somewhat overcome the adverse effect of salinity on reducing indole acetic acid levels and from this result, it finds that hormone balances may influence by salinity and thus affecting plant growth and development. During an experiment conducted by Fahad and Bano (2012) salinity significantly affect the indole acetic acid levels in maize plants but when salicylic acid was applied it improves the level of indole acetic acid as compared to plants grown in saline area.

Transcriptions of a large number of genes are stimulated by auxin called primary auxin response genes. From different plant species, a large number of auxin-responsive genes have been identified and characterized, including soybean, *Arabidopsis* and rice (Hagen and Guilfoyle 2002). These responsive genes have been separated into three gene families: auxin/indoleacetic acid (*Aux/IAA*), *GH3* and small auxin-up RNA (*SAUR*) gene families (Guilfoyle et al. 1993). Auxin restricts the outgrowth of tiller buds in rice (*Oryza sativa* L.) by down regulating *OsIPT* expression and cytokinin biosynthesis in nodes (Liu et al. 2011). However, the novel genes identification involved in salt stress responses provides the basis for researchers to set further genetic engineering strategies to improve more stress tolerance cultivars (Zhu 2002).

In general, Auxin/indole-3-acetic acid or indole acetic acid proteins are short-lived, nuclear proteins, that play a vital role in regulating the expression of auxin response genes (Hagen and Guilfoyle 2002; Liscum and Reed 2002). In Arabidopsis (*Arabidopsis thaliana*) many of the 29 genes that encode these proteins are induced by auxin themselves (Abel et al. 1995). Auxin acts as a significant constituent engaged in defense responses via regulating the expression of a great number of genes and mediates crosstalk between abiotic and biotic stress responses. Although these annotations provide some hints, however biosynthetic pathway of indole acetic acid at the genetic level has remained unclear therefore In order to understand the real mechanism further researches should be conducted in future.

2.3 Cytokinins (CKs)

Cytokinins play a significant role during several plant growth and developmental processes, including cell division, chloroplast biogenesis, apical dominance, leaf senescence, vascular differentiation nutrient mobilization, shoot differentiation, anthocyanin production, and photomorphogenic development (Davies 2004). Against salinity and high temperature cytokinin help the plants to resist (Barciszewski et al. 2000). Responses of plant to cytokinins are often judged from their responses to exogenously applied cytokinins. However, at the time when we apply cytokinin and follow the plant response, it is important to consider that both natural and synthetic exogenous cytokinin can increase endogenous cytokinins contents by their uptake and by promotion of cytokinin biosynthesis (Pospíšilová et al. 2000). On the other side, they can increase cytokinin oxidase activity and CK degradation (Hare et al. 1997; Kaminek et al. 1997). Thus, the composition and concentration of cytokinin in the site of action might be quite different than in the site of application.

To increase plant tolerance to salt, seed priming with cytokinins was reported (Iqbal et al. 2006). According to Cowan et al. (1999) cytokinin application can reverse leaf and fruit abscission that are induced by abscisic acid or water stress, or in contrasts with abscisic acid inhibition of germination cytokinins release seed dormancy. During plant growth and development cytokinins are master regulators, and were recently shown to control plant adaptation to salt stress (Hadiarto and Tran 2011). Leaf senescence usually accelerates by water stress; while on the other hand, leaf senescence delay by cytokinins (Naqvi 1999). In water-stressed plants decreased content of cytokinins and accumulation of abscisic acid lead to strongly increased ABA/CKs ratio. In apoplastic solution of water-stressed cotton and sunflower this ratio was also increased, but the CK concentration was not significantly changed (Hartung et al. 1992; Masia et al. 1994). During several processes in plants cytokinins are often considered as abscisic acid antagonists may be the result of metabolic interactions and antagonists/synergists to auxins (Pospíšilová 2003). Main effects are promotion of stomatal opening and transpiration rate by cytokinins and inhibition by abscisic acid during water relations. Endogenous content of cytokinin mostly decreases under water stress which amplifies the response of shoot to increasing concentration of abscisic acid. Cytokinin contributes at least in part, a common biosynthetic origin with abscisic acid.

In shoots and roots of barley cultivars the concentrations of Z, ZR, iP, and iPA decreased significantly after exposure to increased concentration of NaCl (Kuiper et al. 1990). Kirkham et al. (1974) observed that kinetin application to bean plants in salinity stress make worse its effects. During stress salt-sensitive variety of barley addition of benzyl adenine inhibited its growth, but in a salt-tolerant variety it overcame the decline in growth rate, shoot/root ratio and internal CK content (Kuiper et al. 1990). Chakrabarti and Mukherji (2003a, b) reported that Kinetin acts as a direct free radical scavenger or it may also involve in the mechanism of antioxidative that are related to the safety of purine breakdown. In stress responses a possible involvement of genes is often inferred from changes in the transcript abundance in response to a given stress trigger. In stress-response assays functional analyses of cytokinin receptor mutants exhibited that all three cytokinin receptors of Arabidopsis act as negative regulators in abscisic acid signaling and in the osmotic stress responses. Cytokinin dependence of this activity was demonstrated for *CRE1/AHK4* (Tran et al. 2007). Expression of a great number of stress-induced genes regulates by plant hormones, including CKs (Itai 1999; Naqvi 1999). Merchan et al. (2007) reported that cytokinin receptor genes of other species are regulated by changes in the osmotic conditions as well, demonstrating that in the osmotic stress response their function might be common although mechanistically not well understood. Despite their established role in plant/crop development, information as to how cytokinins are reduced under stressful conditions is meager. The reduction in the cytokinin level raises the question whether the hormones' biological activity was minimized by enhancing temporary storage or whether it was metabolized so further research work will be required in the future.

2.4 Gibberellic Acid

Gibberellins constitute a group of tetracyclic diterpenes which have positive effect on seed germination, leaf expansion, stem elongation, flower and trichome initiation, flower and fruit development (Yamaguchi 2008). Through their influence on photosynthetic enzymes gibberellins are known to improve the photosynthetic efficiency of plants, leaf-area index, light interception, enhanced use efficiency of

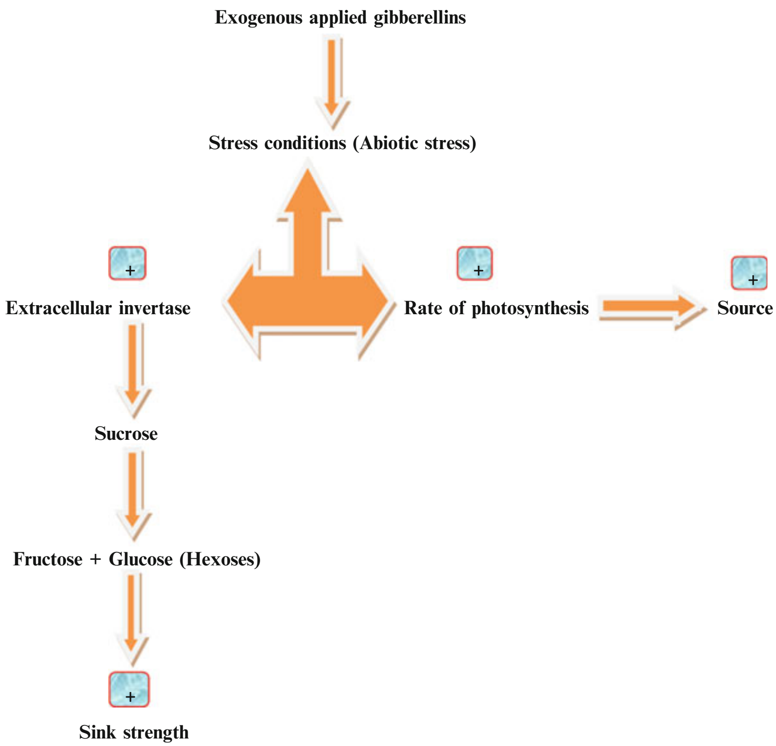

Fig. 2 Gibberellins action on the source–sink relation under stress condition. Both gibberellins and stress regulated extracellular invertase, and invertase that act on sucrose to form hexose, which increases strength of the sink. Under stress condition the increase in photosynthetic rate by gibberellins as a result increases source potential

nutrients and plays an important role in modulating diverse processes throughout plant development (Khan et al. 2007). The integrated mechanisms induced by gibberellic acid enhance the source potential and redistribution of photosynthates increases sink strength (Khan et al. 2007). In the process of germination GA_3 is found to play an important part (Ritchie and Gilroy 1998) through a multiple regulatory mechanism. Gibberellins have been shown to promote many aspects of plant growth and development including germination, and seed development (Sun and Gubler 2004).

Different types of hormones are used to improve plant growth under stress condition. Among them, the main focuses of some plant scientists have been gibberellins (Basalah and Mohammad 1999; Hisamatsu et al. 2000) (Fig. 2).

Regulation of plant responses to the external environment, Gibberellic acid is known to be importantly concerned (Chakrabarti and Mukherji 2003a, b). It is important plant growth bio-regulator that has increased the salt tolerance of many crop plants (Haroun et al. 1991; Hoque and Haque 2002). Gibberellic acid has also been shown to alleviate the effects of salt stress on water use efficiency (Aldesuquy and Ibrahim 2001). It is significant that against various stresses, one group of compounds protects plants and for this reason, the triazoles have been referred to as plant "multi-protectants" (Fletcher and Hofstra 1985). It has been verified that the morphological and stress protective effects of triazoles are inverted by GA_3 (Gilley and Fletcher 2007), thereby indicating an intimate relationship -between GAs and plant stress protection.

When plants are exposed to both biotic (McConn et al. 1997) and abiotic stresses (Lehmann et al. 1995) gibberellic acid accumulates rapidly. The concentration of cytokinin and gibberellic acid (GA_3) were decreased while abscisic acid increased in salt-stressed plants (Mizrahi et al. 1971; Boucaud and Ungar 1976) led to the suggestion that changes in water relations, and membrane permeability induces by salt stress (Ilan 1971; Karmoker and Van Steveninck 1979). Maggio et al. (2010) reported that in tomato plant GA_3 application decreased stomatal resistance and improved efficiency of plant water use at low salinity. Kumar and Singh (1996) reported that gibberellic acid treatment increased growth and grain yield of wheat plant under saline condition. To return metabolic activities to their normal levels exogenous application of growth hormones may be useful (Iqbal et al. 2011). Under abiotic stress at a certain concentration GA_3 has been shown to be beneficial for the physiology and metabolism of many plants, since it may provide a mechanism to regulate the metabolic process as a function of sugar signaling and antioxidative enzymes (Iqbal et al. 2011).

Improvement in grain yield in wheat plants was assigned to the GA_3-priming-induced modulation of ions uptake and partitioning (within shoots and roots) and hormones homeostasis under saline conditions however, this hormones homeostasis mechanism is still unclear (Iqbal and Ashraf 2010). Gibberellic acid is very useful for rice growth under saline condition (Prakash and Prathapasenan 1990). The GA_3 are known to alleviate salinity effect in some halophytic seeds (Li et al. 2005) while it was ineffective in other halophytes like *Suaeda fruticosa* and *Haloxylon recurvum* (Khan and Gul 2006), *Sarcobatus vermiculatus*, *Ceratoides lanata* and *H. glomeratus* (Khan and Gul 2006). Radi et al. (2006) state that pre-soaking wheat (Sakha 92) seeds in GA3 increased the germination potential especially at moderate salinization levels.

Under stress conditions reduced plant growth could result from an altered hormonal balance and application of phytohormone provides an attractive approach to cope with stress (Vettakkorumakankav 1999). Between gibberellic acid levels and the acquisition of stress protection in barley an intimate relationship has been suggested to exist (Vettakkorumakankav 1999). Dwarfism in *Arabidopsis* and over expression of Dwarf and Delayed Flowering 1 (DDF1) causes a reduction in GA_4 content (Magome et al. 2004). There is a correlation among the survival of salt toxicity and the function of DELLA proteins (Achard et al. 2006). These results suggest that the salt-inducible DDF1 gene is involved in growth responses under high salinity conditions in part through altering GA levels and improves seed germination (Kaya et al. 2009). Auxin (IAA) promotes GA biosynthesis has recently been discovered in different species (Wolbang et al. 2004). On the other hand, catabolism of abscisic acid enhances by gibberellic acid application (Gonai et al. 2004). However, it is still yet not known the way by which GA_3-priming could induce salt tolerance in plants. The hormonal balance in plants perturbs by salinity. Therefore, under salt stress hormonal homeostasis might be the possible mechanism of GA_3-induced plant salt tolerance (Iqbal and Ashraf 2010). In this study we have verified that GAs have a role in stress protection of plants. This study displays that modulation of GAs is an attractive approach for conferring stress protection. We have also recommended possible means to modulate GA levels in plants by either classical or molecular genetics as well as by the use of newer plant growth retardants with lower persistence in the soil. The integration of these concepts could form the basis for novel crop protection strategies with global implications.

2.5 Salicylic Acid

Under harsh stress conditions, the antioxidant capacity may not be enough in order to minimize the destructive effects of oxidative damage. For our better understanding of how plants respond to environmental stresses, synthesis of signal molecules in plants is an important step (Asim et al. 2011). Therefore, in plants several such signal molecules have been identified such as jasmonic acid, ethylene and salicylic acid.

Salicylic acid is a phenolic compound produced by common plant. Compounds can function as a growth regulator in this group (Aberg 1981). It is colorless crystalline organic acid. It functions as plant hormones and is widely used in organic synthesis. In addition, salicylic acid could be included in the category of Phytohormones (Raskin 1992). According to Cutt and Klessing (1992) exogenous application of salicylic acid may influence a range of diverse processes in plants, including seed germination, stomatal closure (Larque-Saavedra 1979), ion uptake and transport (Harper and Balke 1981), membrane permeability (Barkosky and Einhelling 1993), photosynthetic and growth rate (Khan et al. 2003). According to Lamb and Dixon (1997) SA received a great deal of attention related to its role in plant disease resistance.

Salicylic acid also known as an important signal molecule for modulating plant responses to environment stress (Senaratna et al. 2000). Most genes respond positive to acute salicylic acid treatment are related to stress and signaling pathways which eventually led to cell death (Jumali et al. 2011). This consists of genes encoding chaperone, heat shock proteins, antioxidants and genes that take part in the biosynthesis of secondary metabolite, such as sinapyl alcohol dehydrogenase, cinnamyl alcohol dehydrogenase and Cytochrome P450 (Jumali et al. 2011). According to Wang et al. (2001) acidic pathogen-related genes induce by salicylic acid and inhibit basic PR genes, while jasmonic acid has antagonistic reaction. There are also antagonistic interactions present between salicylic acid and jasmonic acid, which affect PR protein gene's expression in tomato (Thaler et al. 2002). It is now clear that salicylic acid provides protection against a number of abiotic stresses such as heat stress in mustard seedlings (Dat et al. 1998), chilling damage in different plants (Kang and saltveit 2002; Tasgin et al. 2003), heavy-metal stress in barley seedlings (Metwally et al. 2003), drought stress on wheat plants (Singh and Usha 2003) and salt stress in wheat (El-Tayeb 2005).

Major role of salicylic acid in plant is thought to be the regulation of responses to biotic stresses; however, a large body of literature now suggests that salicylic acid is also involved in responses to several abiotic stresses such as ultraviolet light, drought, salt, chilling and heat (Sharma et al. 1996). Dela-Rosa and Maiti (1995) found an inhibition in the chlorophyll biosynthesis in sorghum plants because of salt stress. Increasing effects of salicylic acid on photosynthetic capacity could be attributed to its stimulatory effects on rubisco activity and pigment contents. Salicylic acid treated plants exhibited higher values of pigment concentration than those of control or salinity-treated samples. Soybean plants treatment with salicylic acid, increased pigments content as well as the rate of photosynthesis (Zhao et al. 1995).

Sinha et al. (1993) pointed out that chlorophyll and carotenoid contents of maize leaves were increased upon treatment with salicylic acid. Maize plants subjected to NaCl salinity treatment, showed a progressive increased in their soluble sugar content with the increase in salinity level, while an opposite trend was obtained with respect to polysaccharide concentration. salicylic acid treatments might also be assumed to inhibit polysaccharide- hydrolyzing enzyme system on one hand and accelerate the incorporation of soluble sugar into polysaccharides. In contrast, Sharma and Lakhhvir (1988) found that a foliar spray of salicylic acid to raya (*Brassica Juncea* L.) plants resulted in decreasing their soluble sugar levels. In another study, salicylic acid treatment of salt stressed maize was found to stimulate their salt tolerance viva accelerating their photosynthesis performance and carbohydrate's metabolism (Khodary 2004). According to Fahad and Bano (2012) observed that salicylic acid treatment to maize plants increased the level of indole acetic acid while on other hand decreased the abscisic acid contents. Similarly under stress condition salicylic acid treatments showed an increase in SOD, POD and Ascorbate peroxidase activities of maize as compared to salt treatment while decreased the catalase activity. In control treatment, it did not have any significant effect (Fahad and Bano 2012) (Fig. 3).

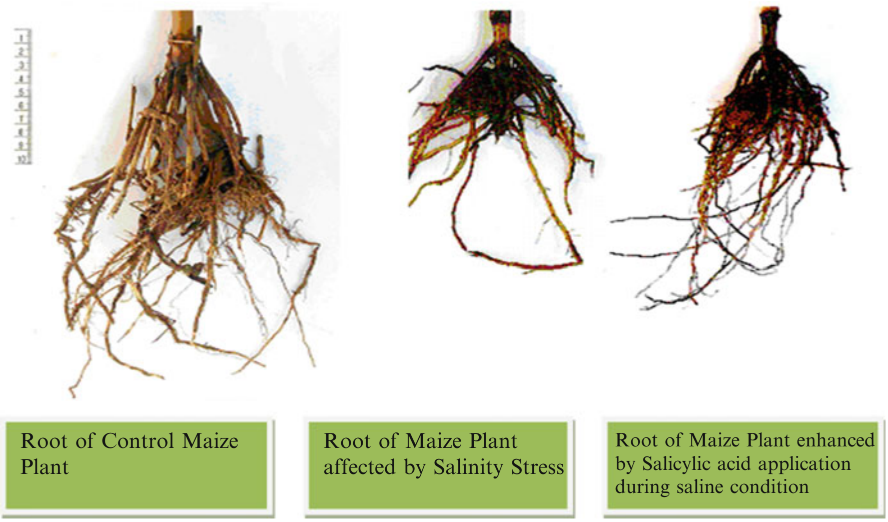

Fig. 3 Effect of salicylic acid on root length of maize under salinity stress. Salicylic acid application improves the growth of maize root under saline condition as compared to stress conditions

In future use of this plant hormone will attract a great attention as a management tool for providing tolerance to our agricultural crops against the aforesaid constrains as a result aiding to accelerate our potential crop yield. However, a lot of work still needed to explain the exact pathway of salicylic acid biosynthesis, whether major or minor, important regulatory point of its biosynthesis, mode of action and other key and mutual regulatory roles performed by salicylic acid that have remained elusive up to date.

2.6 Brassinosteroids

Brassinosteroids are plant growth regulators that are novel group which regulate growth and development of plants. Until 1979, it was not confirmed that plants possess the ability to synthesize a variety of steroids, which play an important function as hormone (Khripach et al. 2000). About 36 years ago research on brassinosteroids began when Mitchell et al. (1970) first reported that stem elongation and cell division in plants can be promoted by used an organic extract. From *Brassica napus* pollen, brassinosteroids were first extracted, that are the growth promoter steroid hormones (Clouse and Sasse 1998). In view of some reports, it is evident that it improves growth and yield of some plants as a foliar spray application e.g. tomato (Vardhini and Rao 2001), and *Arachis hypogaea* (Vardhini and Rao 1998). Arabidopsis with CPD gene encodes a cytochrome P450 protein (CYP90) that shares similarity of sequence with mammalian steroid hydroxylases (Majid et al. 2011). According to Krishna (2003) feeding experiments with different brassinosteroids indicate that CYP90 acts at the C-23 hydroxylation step in the biosynthetic pathway of brassinosteroids.

Exogenous application of brassinosteroids can also alleviate the adverse effects of various environmental stresses and produces resistance in plants against these stresses e.g., salt stress (Hathout 1996; Vardhini and Rao 1997), heat stress (Zhu et al. 1998), drought stress (Li and Van Staden 1998), and chilling stress (Wilen et al. 1995). It stimulates cell elongation when it acts along with auxins (Katsumi 1991; Sasse 1991). Brassinosteroid ameliorated the adverse effects of salt stress on seed germination and growth when it exogenously applied (Anuradha and Rao 2001), and root elongation (Amzallag and Goloubinoff 2003). Similarly, under saline conditions exogenous application of brassinosteroids were also effective in seed germination and growth of rice plants (Anuradha and Rao 2001). In the regulation of ion uptake brassinosteroids are known to play a vital role (Khripach et al. 2000). Accumulation of heavy metals and radioactive elements were also reduced by the used of brassinosteroids (Khripach et al. 1996). In view of a number of studies, it is evident that exogenous application of brassinosteroids induces abiotic stress tolerance in plants, e.g. exogenous application of brassinosteroids increased salinity tolerance in rice (Anuradha and Rao 2001), tomato (Prakash et al. 1999) and chickpea (Ali et al. 2007). The present results clearly demonstrate that to decrease the damaging effects of salinity on germination of seedling growth and plant yield, brassinosteroids plays a very vital role. There are many other areas where further research should be productive e.g. the sites, pathways and enzymology of their biosynthesis, source-sink relationships, developmental and stress physiology, interactions with micro organisms, fungi and animals, and the realization of applications. As well, a most important and critical assignment is the integration of knowledge of the effects and vital roles of brassinosteroids into our general models of plant growth and development.

2.7 Jasmonates

Methyl jasmonate and its free-acid, jasmonic acid, jointly referred to as jasmonates, are vital cellular regulators involved in diverse plant developmental processes, such as seed germination (Nojavan-Asghari and Ishizawa 1998), callus growth (Ueda and Kato 1982), primary root growth (Staswick et al. 1992), flowering (Albrechtová and Ullmann 1994), formation of gum and bulb (Saniewski et al. 1998), and senescence (Ueda and Kato 1980). Biosynthesis of Jasmonic acid occurs in leaves and there is evidence of a similar pathway in roots. As well, cellular organelles such as chloroplasts and peroxisomes are considered to be the main sites of Jasmonic acid biosynthesis (Cheong and Choi 2003).

In addition jasmonates activate plant defense responses to insect wounding, attack by various pathogens, and environmental stresses, such as drought, low temperature, and salinity (Creelman and Mullet 1995; Wasternack and Parthier 1997; Seo et al. 2001; Rakwal et al. 2002). A role for Jasmonic acid in plant response to water deficit has been suggested because this stress induces the expression of several genes that also respond to Jasmonic acid (Mason and Mullet 1990). Such as, expression of the genes encoding the soybean vegetative, storage protein acid phosphatases was enhanced in plants subjected to water deficit and in plants treated with Jasmonic acid (Mason and Mullet 1990). VSPα and VSPβ accumulate in soybean vacuoles and to a smaller amount in cell walls. Based on their abundance, localization, and accumulation in depodded plants the acid phosphatases were identified as vegetative storage proteins (Staswick 1994). According to Creelman et al. (1992) that due to the accumulation of Jasmonic acid, the VSP mRNA levels increase in wounded tissue. The vegetative storage protein acid phosphatases B promoter analysis revealed the presence of a DNA domain that mediates responses to Jasmonic acid (Mason et al. 1993). In addition, a second DNA domain was found to mediate responses to sugars (positive) (Mason et al. 1993), auxin (negative) (Dewald et al. 1994), and phosphate (negative) (Sadka et al. 1994). One question the present study addresses is whether Jasmonic acid increases in plant tissues exposed to water deficit.

Likewise, jasmonates exogenous application to plants induced stress-related or pathogenesis-related genes (Moons et al. 1997; Mei et al. 2006). Thus, in response to a biotic stress, the role(s) of jasmonate has been well documented; however, a very little information is known about its involvement in response to abiotic stress. Jasmonate levels were enhanced in soybean (*Glycine max*; Creelman and Mullet 1995) and *Pinus pinaster* (Pedranzani et al. 2007) upon plant introduction to drought and in tomato (*Solanum lycopersicum*; Pedranzani et al. 2003) and *Iris hexagona* (Wang et al. 2001) upon exposure to high salinity. In rice leaves and roots, both drought and high salinity increased levels of jasmonate, resulting in the induction of stress-related PR and jasmonates biosynthetic genes (Moons et al. 1997; Kiribuchi et al. 2005; Tani et al. 2008). These abiotic stress-induced increases in jasmonate levels were studied only in vegetative tissues. Whether drought conditions augment jasmonate levels in reproductive organs remains to be determined.

Kang et al. (2005) reported that the jasmonates concentrations in salt-sensitive cultivar plants were littler than in salt-tolerant cultivar plants. Also, methyl jasmonate levels in rice roots enhanced significantly in 200 mM NaCl (Moons et al. 1997). Therefore, in salt-tolerant plants high levels of abscisic acid accumulated after salt treatments can be an effective protection against high salinity. Though, there seems to be little knowledge available about how salinity affects endogenous jasmonate levels in plants. Kang et al. (2005) reported that with post-application of exogenously applied JA can ameliorate salt-stressed rice seedlings, particularly the salt-sensitive rather than the salt-tolerant cultivar. In addition, by exogenous jasmonates application sodium concentration dramatically decreased. After salt treatment exogenous application of jasmonates may change the endogenous hormones balance, such as abscisic acid, which provides a significant hint for understanding the protection mechanisms against salt stress (Kang et al. 2005). These results undoubtedly show that exogenous jasmonates may be engaged in the defense not only during wounding and pathogen stress, but also during salt and drought stress. In summary exogenously applied jasmonates may be involved in the defence not only during wounding and pathogen stress, but also during salt and water stress. After salt treatment in different research work exogenous jasmonates application may change the balance of endogenous plants hormones, which provides an important clue for understanding the protection mechanisms against salt stress.

2.8 Ethylene

Ethylene is a gaseous hormone that regulates plant growth and development. According to Adams and Yang (1979) ethylene is produced from methionine via *S*-adenosyl-L-methionine (AdoMet) and the cyclic non-protein amino acid 1-aminocyclopropane-1-carboxylic acid (ACC). The enzymes catalyzing the conversion of AdoMet to and of ACC to ethylene are ACC synthase and ACC oxidase, respectively (Kende 1993). ACC synthase produces, besides ACC, 50-methylthioadenosine, which is used for the synthesis of new methionine via a modified methionine cycle (Miyazaki and Yang 1987). This salvage pathway preserves the methylthio group through every revolution of the cycle at the cost of one

ATP molecule. Thus high rates of ethylene biosynthesis can be maintained even when the pool of free methionine is small.

Based on the mutant analysis of triple responses of etiolated seedlings treated with ethylene, a signal transduction pathway of ethylene has been put forward in Arabidopsis (*Arabidopsis thaliana*) that involves ethylene receptors, CTR1, EIN2, and EIN3, and other components (Bleecker and Kende 2000; Wang et al. 2002; Chang and Bleecker 2004; Guo and Ecker 2004; Chen et al. 2005). In Arabidopsis five receptor genes have been found, and based on structural features they are categorized into two subfamilies. Subfamily I includes ETR1 and ERS1 (Chang et al. 1993; Hua et al. 1995). Subfamily II includes ETR2, EIN4, and ERS2 (Hua and Meyerowitz 1998; Sakai et al. 1998). All these receptors of ethylene can bind ethylene, and ETR1 has His kinase activity (Schaller and Bleecker 1995; Gamble et al. 1998; Hall et al. 2000; O'Malley et al. 2005). Other ethylene receptors of Arabidopsis had Ser kinase activity (Moussatche and Klee 2004). Accorded to Morgan and Drew (1997) ethylene has long been regarded as a stress hormone. However, in abiotic stress responses the roles of the ethylene signaling remain an open question.

Ethylene signaling is significant in regulating plant growth and stress responses, and ethylene functions through its receptors. Though it is usually believed that ethylene signaling functions in multiple stress responses, it is not clear that under salt stress what specific roles the receptors can play. An experiment conducted by Wan-Hong et al. (2007) In which they transformed a tobacco type II ethylene receptor homolog gene *NTHK1* into Arabidopsis and observed that the resulting transgenic plants, with *NTHK1* mRNA and protein expression, were salt sensitive as can be seen from high electrolyte leakage, and decreased root growth under salt stress. Because in the transgenic plants over expression of *NTHK1* seems to represent gain of function of ethylene receptor, so from this study we conjecture that the receptor function would lead to the salt-sensitive responses. The high electrolyte leakage can be entirely or partly suppressed by 1-aminocyclopropane-1-carboxylic acid treatment, recommending that a negative effect exerts by ethylene on ethylene receptors. This fact also suggests that for plant salt tolerance ethylene may be required.

Ethylene has been considered as a stress hormone and is induced by many stresses (Abeles et al. 1992; Morgan and Drew 1997). However, in salt stress its role is equivocal. El-Iklil et al. (2000) has reported that lesser ethylene production was related with salt tolerance. On the other side, higher production of ethylene has been regarded as an indicator for salt tolerance in rice (Khan et al. 1987). According to Pierik et al. (2006) ethylene has long been known as a growth inhibitor, but it can also promote growth. Achard et al. (2006) suggest that in Arabidopsis, ethylene signaling promotes salt tolerance. An experiment conducted by Wan-Hong et al. (2007) suggests that ethylene receptor function leads to salt sensitivity and ACC appears to suppress this salt sensitivity, implying that for salt tolerance ethylene signaling is needed.

Response of plant to salt stress may depend on the balance and/or interaction between receptor and ethylene. When receptor signaling is prevalent, the plant is susceptible to salt stress but shows large rosette and late flowering. When ethylene signaling is prevalent, the plant is tolerant to salt stress but has small rosette and early flowering. Between these two extreme situations the plant needs to make adjustment. At various levels fine-tuning may make plants in an active homeostasis, and then under stress condition the plants can survive better and have relatively normal growth. Overall, Plants have evolved various specific mechanisms to adapt themselves to the changing environment. Ethylene signaling is one of these pathways that plants have adopted for regulation of salt stress responses. However, many enigmas inside in the ethylene signaling pathway remain to be illuminated.

2.9 Triazoles

Triazoles are a group of compounds that have been developed to be utilized as either fungicides or plant growth regulators; although in different degrees they have both properties (Fletcher et al. 2000). It protects plants from stress related injuries of drought, heat, chilling, ozone and SO_2 (Fletcher and Hofstra 1985, Asare-Boamah and Fletcher 1986). In NaCl stressed plants of radish (Panneerselvam et al. 1997) and pigeon pea (Karikalan et al. 1999) triazoles augmented the plant dry mass. At 10–30 mg ml″ level, it also enhanced the shoot fresh weight and yield of peas. At 30 mg ml′ level, a maximum increase of 50 % was recorded, which reduced on increasing triazole dose to 40 mg ml″. Generally, TRlAD (common name of trizole) enhanced the photosynthesis rate (Fletcher et al. 2000) which was attributed to enhanced chlorophyll content (Davis et al. 1988, Sankhla et al. 1997) and more well packed chloroplasts within a smaller leaf area (Khalil 1995). TRlAD addition to radish raised the net photosynthetic rate and intercellular CO_2 concentration in spite of being stressed by NaCl (Panneerselvam et al. 1997). Such an advantage is observed in drought prone wheat plants too (Sairam et al. 1989).

According to Blum (1988) that high temperature can generate several physiological and biological effects in plants, including denaturation of protein, enzyme inactivation, membrane damage, altered metabolic rate, reduced chloroplast and subdued biochemical activity. One of the initial report of triazole induced protection from heat stress demonstrated that TRIAD protected bean plants from high temperature stress and stopped electrolyte leakage (Asare-Boamah and Fletcher 1986).

Under water stress conditions, triazole application in wheat crop also stimulated the nitrate reductase activity (Sairam et al. 1989) and protease activity under salt-stress in radish (Muthukumarasamy and Panneerselvam 1997a), peanuts (Muthukumarasamy and Panneerselvam 1997b) and cowpea (Gopi et al. 1999). Antioxidant enzymes activities like peroxidase, superoxide dismutase and polyphenoloxidase were also enhanced by TRlAD in pigeon pea suffering from salt-stress (Karikalan et al. 1999). Though there is considerable evidence about the triazoles effect on plants, but the information about the triazoles role in alleviate salt stress in crops is not so much (Fig. 4).

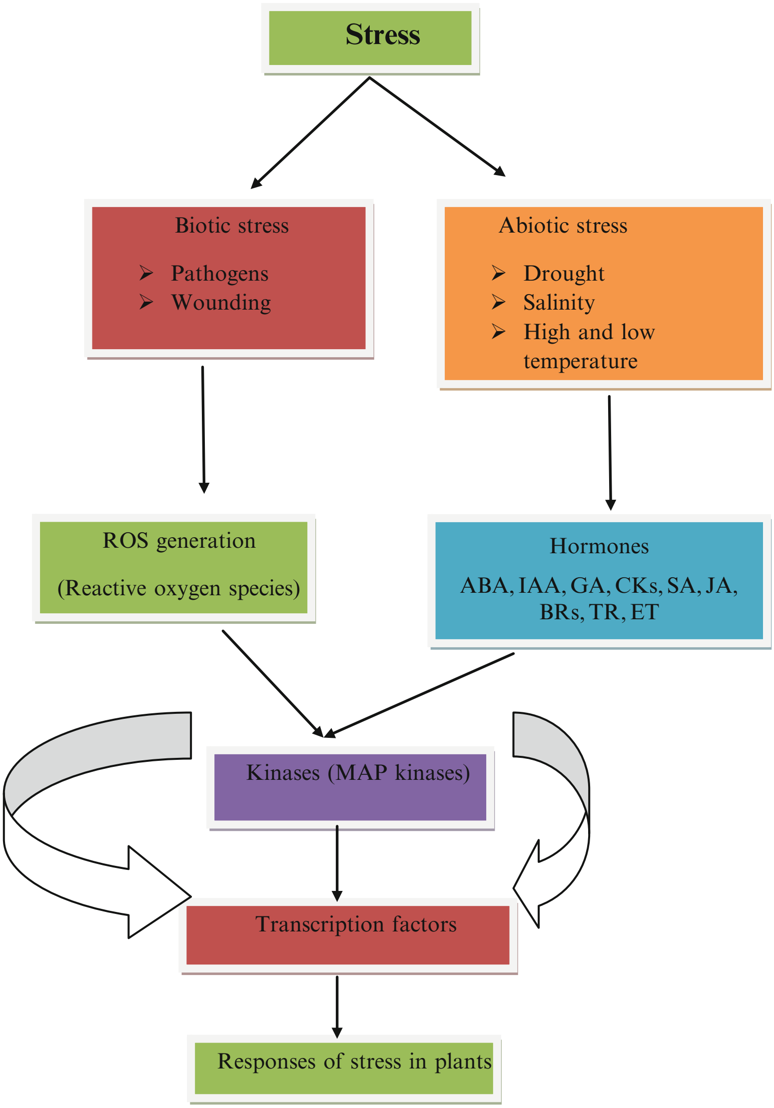

Fig. 4 Generalized model of the plant stress signalling networks. *ABA* abscisic acid, *BRs* brassinosteroids, *ET* ethylene, *JA* jasmonic acid, *SA* salicylic acid, *TR* trizole

3 Conclusion

Agriculture is the oldest economic sector in the world, and it is more reliant on fertile soils and a stable climate than any other type of trade. At the same time, it has been facing a large number of problems including biotic and abiotic stresses. We have made great progress in understanding the plant responses to abiotic stress. Responses of plants to abiotic stress are dynamic and complex. During the past few years the molecular mechanisms regulating hormone synthesis, signaling, and action have been illuminated, and the roles of plant hormones for responses to changing environments have been exhibited. These results will make possible the modification of hormone biosynthetic pathways for the transgenic plants generation with augmented abiotic stress tolerance. Controlling dose/response ratio of hormone remains an uphill task, since the hormone levels attained should be moderate in order to sustain a balance between the positive effects of plant hormones on different abiotic stress tolerance and the negative effects on growth and development. The use of conditional promoters driving gene expression at specific developmental stages, in specific tissues/organs and/or in response to specific environmental cues circumvents this problem and will make possible the generation of transgenic crops able to grow under various abiotic stresses with minimal yield losses. To get a comprehensive understanding of plant responses to abiotic stress in future, more extensive work should be done on the mechanisms of up regulation of ABA biosynthesis genes by the abiotic stress, biosynthetic pathway of IAA at the genetic level, hormones homeostasis of GA_3 and exact pathway of salicylic acid biosynthesis. Current progress of our review is exemplified by the identification and validation of several significant genes that enhanced crops tolerance to stress in the field.

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References

Abel S, Nguyen MD, Theologis A (1995) The PS-IAA4/5-like family of early auxin-inducible mRNAs in *Arabidopsis thaliana*. J Mol Biol 251:533–549

Abeles FB, Morgan PW, Saltveit ME Jr (1992) Ethylene in plant biology, 2nd edn. Academic, San Diego

Aberg B (1981) Plant growth regulators XLI. Mono substituted benzoic acid. Swed J Agric Res 11:93–105

Achard P, Cheng H, Grauwe LD et al (2006) Integration of plant responses to environmentally activated phytohormonal signals. Science 311:91–94

Adams DO, Yang SF (1979) Ethylene biosynthesis: identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proc Natl Acad Sci U S A 76:170–174

Albrechtová JPT, Ullmann J (1994) Methyl jasmonate inhibits growth and flowering in *Chenopodium rubrum*. Biol Plant 36:317–319

Aldesuquy HS, Ibrahim AH (2001) Interactive effect of seawater and growth bio-regulators on water relations, absicisic acid concentration and yield of wheat plants. J Agron Crop Sci 187: 185–193

Ali B, Hayat S, Ahmad A (2007) 28-Homobrassinolide ameliorates the saline stress in chickpea (*Cicer arietinum* L.). Environ Exp Bot 59:217–223

Aloni R (1987) Differentiation of vascular tissues. Annu Rev Plant Physiol 38:179–204

Aloni R (2001) Foliar and axial aspects of vascular differentiation – hypotheses and evidence. J Plant Growth Regul 20:22–34
Aloni R (2004) The induction of vascular tissue by auxin. In: Davies PJ (ed) Plant hormones: biosynthesis, signal transduction, action. Kluwer Academic Publishers, Dordrecht, pp 471–492
Aloni R, Aloni E, Langhans M, Ullrich CI (2006) Role of cytokinin and auxin in shaping root architecture: regulating vascular differentiation, lateral root initiation, root apical dominance and root gravitropism. Ann Bot 97:883–893
Amzallag GN, Goloubinoff P (2003) An Hsp90 inhibitor, geldanamycin, as a brassinosteroid antagonist: evidence from salt-exposed roots of *Vigna radiata*. Plant Biol 5:143–150
Anuradha S, Rao SSR (2001) Effect of brassinosteroids on salinity stress induced inhibition of seed germination and seedling growth of rice (*Oryza sativa* L.). Plant Growth Regul 33:151–153
Archbold HK (1940) Fructosans in the monocotyledons. Rev New Phytol 39:185–219
Asada K (1992) Ascorbate peroxidase-a hydrogen peroxide scavenging enzyme in plants. Physiol Plant 85:235–241
Asare-Boamah NK, Fletcher RA (1986) Protection of bean seedlings against heat and chilling injury by triadimefon. Physiol Plant 67(3):353–358
Asim M, Day S, Rezaei F, Hajyzadeh M, Mahmud TS, Ozcan S (2011) *In vitro* shoot regeneration from preconditioned explants of chickpea (*Cicer arietinum* L.) cv. Gokce. Afr J Biotechnol 10(11):2020–2023
Bacon MA, Wilkinson S, Davies WJ (1998) pH-regulated leaf cell expansion in droughted plants is abscisic acid dependent. Plant Physiol 118:1507–1515
Barciszewski J, Siboska G, Rattan SIS, Clark BFC (2000) Occurrence, biosynthesis and properties of kinetin (N6-furfuryladenine). Plant Growth Regul 32:257–265
Barkosky RR, Einhelling FA (1993) Effect of salicylic acid on plant water relationship. J Chem Ecol 19:237–247
Bartels D, Sunkar R (2005) Drought and salt tolerance in plants. Crit Rev Plant Sci 24:23–58
Basalah MO, Mohammad S (1999) Effect of salinity and plant growth regulators on seed germination of *Medicago sativa* L. Pak J Biol Sci 2:651–653
Bewlay JD, Black M (1994) Seeds: physiology of developmental and germination, 2nd edn. Plenum Press, New York
Bleecker AB, Kende H (2000) Ethylene: a gaseous signal molecule in plants. Annu Rev Cell Dev Biol 16:1–18
Blum A (1988) Plant breeding for stress environments. CRC Press, Florida, p 223
Bohnert HJ, Nelson DE, Jonsen RG (1995) Adaptations to environmental stresses. Plant Cell 7:1099–1111
Boucaud J, Ungar IA (1976) Hormonal control of germination under saline conditions of three halophyte taxa in genus Suaeda. Physiol Plant 36:197–200
Boyer JS (1982) Plant productivity and environment. Science 218:443–448
Bray EA (2002) Abscisic acid regulation of gene expression during water-deficient stress in the era of *Arabidopsis* genome. Plant Cell Environ 25:153–161
Ceccarelli S, Grando S, Maatougui M, Michael M, Slash M, Haghparast R, Rahmanian M, TaherI A, AL-Yassin A, Benbelkacem A, Labdi M, Mimoun H, Nachit M (2010) Plant breeding and climate changes. J Agric Sci Camb 148:627–637
Chakrabarti N, Mukherji S (2003a) Alleviation of NaCl stress by pretreatment with phytohormones in *Vigna radiata*. Biol Plant 46:589–594
Chakrabarti N, Mukherji S (2003b) Effect of phytohormone pretreatment on nitrogen metabolism in *Vigna radiata* under salt stress. Biol Plant 46:63–66
Chang C, Bleecker AB (2004) Ethylene biology. More than a gas. Plant Physiol 136:2895–2899
Chang C, Kwok SF, Bleecker AB, Meyerowitz EM (1993) Arabidopsis ethylene response gene *ETR1*: similarity of product to two-component regulators. Science 262:539–544
Chen YF, Etheridge N, Schaller E (2005) Ethylene signal transduction. Ann Bot (Lond) 95:901–915
Cheong JJ, Choi YD (2003) Methyl jasmonate as a vital substance in plants. Trends Genet 19:409–413

Chinnusamy V, Schumaker K, Zhu JK (2004) Molecular genetics perspectives on cross-talk and specificity in abiotic stress signaling in plants. J Exp Bot 55:225–236

Clouse SD, Sasse JM (1998) Brassinosteroids: essential regulators of plant growth and development. Annu Rev Plant Physiol Plant Mol Biol 49:427–451

Cost P, Neto A, Bezerra M, Prisco T, Filho E (2005) Antioxidant- enzymatic system of two *Sorghum* genotypes differing in salt tolerance. Braz J Plant Physiol 17:139–147

Cowan AK, Cairns ALP, Bartels-Rahm B (1999) Regulation of abscisic acid metabolism: towards a metabolic basis for abscisic acid-cytokinin antagonism. J Exp Bot 50:595–603

Creelman RA, Mullet JE (1995) Jasmonic acid distribution and action in plants: regulation during development and response to biotic and abiotic stress. Proc Natl Acad Sci U S A 92:4114–4119

Creelman RA, Mason HS, Bensen RJ, Boyer JS, Mullet JE (1990) Water deficit and abscisic acid cause differential inhibition of shoot versus root growth in soybean seedlings. Plant Physiol 92:205–214

Creelman RA, Tierney ML, Mullet JE (1992) Jasmonic acid/methyl jasmonate accumulate in wounded soybean hypocotyls and modulate wound gene expression. Proc Natl Acad Sci U S A 89(11):4938–4941

Cutt JR, Klessing DF (1992) Salicylic acid in plants: a changing perspective. Pharm Technol 16:25–34

Dajic Z (2006) Salt stress. In: Madhava Rao KV, Raghavendra AS, Janardhan Reddy K (eds) Physiology and molecular biology of salt tolerance in plant. Springer, Dordrecht, pp 41–99

Dat JF, Foyer CH, Scott IM (1998) Changes in salicylic acid and antioxidants during induced thermotolerance in mustard seedlings. Plant Physiol 118:1455–1461

Davies PJ (2004) Plant hormones: biosynthesis, signal transduction, action. Kluwer Academic Publishers, Dordrecht

Davies WJ, Zhang J (1991) Root signals and the regulation of growth and development of plants in drying soil. Annu Rev Plant Physiol Plant Mol Biol 42:55–76

Davies WJ, Tardieu F, Trejo CL (1994) How do chemical signals work in plants that grow in drying soil? Plant Physiol 104:309–314

Davis T, Steffens G, Sankhla N (1988) Triazole plant growth regulators. Hortic Rev 10:63–105

Debez A, Chaibi W, Bouzid S (2001) Effect du NaCl et de regulatoeurs de croissance sur la germination d' *Atriplex halimus* L. Cah Agric 10:135–138

Dela-Rosa IM, Maiti RK (1995) Biochemical mechanism in glossy sorghum lines for resistance to salinity stress. J Plant Physiol 146:515–519

DeWald DB, Sadka A, Mullet JE (1994) Sucrose modulation of soybean Vsp gene expression is inhibited by auxin. Plant Physiol 104(2):439–444

Duan J, Li J, Guo S, Kang Y (2008) Exogenous spermidine affects polyamine metabolism in salinity-stressed *Cucumis sativus* roots and enhances short-term salinity tolerance. J Plant Physiol 165:1620–1635

Dunlap JR, Binzel ML (1996) NaCl reduces indole-3-acetic acid levels in the roots of tomato plants independent of stress-induced abscisic acid. Plant Physiol 112:379–384

EI-Tayeb MA (2005) Response of barley grains to the interactive effect of salinity and salicylic acid. Plant Growth Regul 45:215–224

El-Iklil Y, Karrou M, Benichou M (2000) Salt stress effect on epinasty in relation to ethylene production and water relations in tomato. Agronomie 20:399–406

Epstein E, Rush JD, Kingsbury RW, Kelley DB, Cinnigham GA, Wrono AF (1980) Saline culture of crops: a genetic approach. Science 210:399–404

Escalada JA, Moss DN (1976) Changes in non-structural carbohydrate fractions of developing spring wheat kernels. Crop Sci 16:627–631

Etehadnia M, Waterer DR, Tanino KK (2008) The method of ABA application affects salt stress responses in resistant and sensitive potato lines. J Plant Growth Regul 27:331–341

Fahad S, Bano A (2012) Effect of salicylic acid on physiological and biochemical characterization of maize grown in saline area. Pak J Bot 44(4):1433–1438

Fletcher RA, Hofstra G (1985) Triadimefon a plant multi-protectant. Plant Cell Physiol 26(4): 775–780

Fletcher RA, Gill A, Davis TD, Sankhla N (2000) Triazoles as plant growth regulators and stress protectants. Hortic Rev 24:55–138

Flowers JP, Trake F, Yeo AR (1977) The mechanisms of salt tolerance in halophytes. Annu Rev Plant Physiol 28:89–121

Frommer WB, Ludewig U, Rentsch D (1999) Taking transgenic plants with a pinch of salt. Science 285:1222–1223

Fukuda A, Tanaka Y (2006) Effects of ABA, auxin and gibberellin on the expression of genes for vacuolar H^+-inorganic pyrophosphatase, H^+-ATPase subunit A, and Na^+/H^+ antiporter in barley. Plant Physiol Biochem 44:351–358

Gamble RL, Coonfield ML, Schaller GE (1998) Histidine kinase activity of the ETR1 ethylene receptor from *Arabidopsis*. Proc Natl Acad Sci U S A 95:7825–7829

Garham J, Hughes LY, Wynjanes RG (1981) Low molecular weight carbohydrates in some salt stressed plants. Physiol Plant 53:27–33

Gilley A, Flecher RA (2007) Gibberellin antagonizes paclobutrazole induced stress protection in wheat seedlings. J Plant Physiol 103(1–2):200–207

GomezCadenas A, Tadeo FR, PrimoMillo E, Talon M (1998) Involvement of abscisic acid and ethylene in the responses of citrus seedlings to salt shock. Plant Physiol 103:475–484

GomezCadenas A, Arbona V, Jacas J, PrimoMillo E, Talon M (2002) Abscisic acid reduces leaf abscission and increases salt tolerance in citrus plants. J Plant Growth Regul 21:234–240

Gonai T, Kawahara S, Tougou M, Satoh S, Hashiba T, Hirai N, Kawaide H, Kamiya Y, Yoshioka T (2004) Abscisic acid in the thermoinhibition of lettuce seed germination and enhancement of its catabolism by gibberellin. J Exp Bot 55:111–118

Gopi R, Sujatha BM, Rajan SN, Karkalan L, Pannerselvam R (1999) Effect of triadimefon in the sodium chloride stressed cowpea (*Vigna unguiculata* L.) seedlings. Indian J Agric Sci 69:743–745

Greenway H, Munns R (1980) Mechanism of salt tolerance in nonhalophytes. Annu Rev Plant Physiol 31:149–190

Guilfoyle TJ, Hagen G, Li Y, Ulmasov T, Liu Z, Strabala T, Gee MA (1993) Auxin-regulated transcription. Aust J Plant Physiol 20:489–502

Guo H, Ecker JR (2004) The ethylene signaling pathway: new insights. Curr Opin Plant Biol 7:40–49

Hadiarto T, Tran LS (2011) Progress studies of drought-responsive genes in rice. Plant Cell Rep 30:297–310

Hagen G, Guilfoyle T (2002) Auxin-responsive gene expression: genes, promoters and regulatory factors. Plant Mol Biol 49:373–385

Hall AE, Findell JL, Schaller GE, Sisler EC, Bleecker AB (2000) Ethylene perception by the ERS1 protein in Arabidopsis. Plant Physiol 123:1449–1457

Hare PD, Cress WA, van Staden J (1997) The involvement of cytokinins in plant responses to environmental stress. Plant Growth Regul 23:79–103

Haroun SA, Badawy AH, Shukry WM (1991) Auxin induced modification of *Zea mays* and *Lupinus termis* seedlings exposed to water stress imposed by polyethylene glycol (PEG 6000). Sci J 18:335

Harper JP, Balke NE (1981) Characterization of the inhibition of K^+ absorption in oat roots by salicylic acid. Plant Physiol 68:1349–1353

Hartung W, Weiler EW, Radin JW (1992) Auxin and cytokinins in the apoplastic solution of dehydrated cotton leaves. J Plant Physiol 140:324–332

Hartung W, Sauter A, Hose E (2002) Abscisic acid in the xylem: where does it come from, where does it go to? J Exp Bot 53:27–32

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. Annu Rev Plant Physiol Plant Mol Biol 51:463–499

Hathout TA (1996) Salinity stress and its counteraction by the growth regulator (Brassinolide) in wheat plants (*Triticum aestivum* L. cultivar Giza 157). Egypt J Physiol Sci 20:127–152

Hisamatsu T, Koshioka M, Kubota S, Fujime Y, King RW, Mander LN (2000) The role of gibberellin in the control of growth and flowering in *Matthiola incana*. Physiol Plant 109:97–105

Hoque M, Haque S (2002) Effects of GA3 and its mode of application on morphology and yield parameters of mungbean (*Vigna radiate* L.). Pak J Biol Sci 5:281–283

Hua J, Meyerowitz EM (1998) Ethylene responses are negatively regulated by a receptor gene family in Arabidopsis thaliana. Cell 94:261–271

Hua J, Chang C, Sun Q, Meyerowitz EM (1995) Ethylene insensitivity conferred by Arabidopsis *ERS* gene. Science 269:1712–1714

Ilan I (1971) Evidence for hormonal regulation of the selectivity of ion uptake by plant cells. Physiol Plant 25:230–233

Iqbal M, Ashraf M (2010) Gibberellic acid mediated induction of salt tolerance in wheat plants: growth, ionic partitioning, photosynthesis, yield and hormonal homeostasis. Environ Exp Bot 86:76–85. doi:10.1016/j.envexpbot.2010.06.002

Iqbal M, Ashraf M, Jamil A (2006) Seed enhancement with cytokinins: changes in growth and grain yield in salt stressed wheat plants. Plant Growth Regul 50:29–39

Iqbal N, Nazar R, Iqbal MRK, Masood A, Nafees AK (2011) Role of gibberellins in regulation of source sink relations under optimal and limiting environmental conditions. Curr Sci 100(7): 998–1007

Itai C (1999) Role of phytohormones in plant responses to stresses. In: Lerner HR (ed) Plant responses to environmental stress. From phytohormones to genome reorganization. Marcel Dekker, New York/Basel, pp 287–301

Jackson M (1997) Hormones from roots as signals for the shoots of stressed plants. Elsevier Trends J 2:22–28

Javid MG, Sorooshzadeh A, Moradi F, Sanavy SAMM, Allahdadi I (2011) The role of phytohormones in alleviating salt stress in crop plants. Aust J Crop Sci 5:726–734

Jensen AB, Bush PB, Figueras M, Alba MM, Peracchia G, Messeguer R, Goday A, Pages M (1996) Drought signal transduction in plants. Plant Growth Regul 20:105–110

Jia W, Wang Y, Zhang S, Zhang J (2002) Salt stress-induced ABA accumulation is more sensitively triggered in roots than in shoots. J Exp Bot 53:2201–2206

Jumali SS, Said IM, Ismail I, Zainal Z (2011) Genes induced by high concentration of salicylic acid in *Mitragyna speciosa*. Aust J Crop Sci 5:296–303

Kaminek M, Motyka V, Vankova R (1997) Regulation of cytokinin content in plant cells. Physiol Plant 101:689–700

Kang HM, Saltveit ME (2002) Chilling tolerance of maize, cucumber and rice seedlings leaves and roots are differentially affected by salicylic acid. Physiol Plant 115:571–576

Kang DJ, Seo YJ, Lee JD, Ishii R, Kim KU, Shin DH, Park SK, Jang SW, Lee IJ (2005) Jasmonic acid differentially affects growth, ion uptake and abscisic acid concentration in salt-tolerant and salt-sensitive rice cultivars. J Agron Crop Sci 191:273–282

Karen R (2000) Salinity. In: Sumner ME (ed) Handbook of soil science. CRC Press, Boca Raton, pp G3–G25

Karikalan L, Rajan SN, Gopi R, Sujatha BM, Pannersevalam R (1999) Induction of salt tolerance by triadimefon in pigeon pea (*Cajanus cajan* L.) Mill sp. Indian J Exp Biol 37:825–829

Karmoker JL, Van Steveninck FM (1979) The effect of abscisic acid on the uptake and distribution of ions in intact seedlings of *Phaseolus vulgaris* cv. Redland Pioneer. Physiol Plant 45: 453–459

Katsumi M (1991) Chapter 21: physiological modes of brassinolide action in cucumber hypocotyls growth. In: Cutler HG, Yokota T, Adam G (eds) Brassinosteroids chemistry, bioactivity and applications, ACS symposium series, 474. American Chemical Society, Washington, DC

Kavi Kishore PB, Sangam S, Amrutha RN, Laxmi PS, Naidu KR, Rao KRSS, Rao S, Reddy KJ, Theriappan P, Sreenivasulu N (2005) Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. Curr Sci 88:424–438

Kaya C, Tuna AL, Yoka I (2009) The role of plant hormones in plants under salinity stress. In: Ashraf M, Ozturk M, Arthur HR (eds) Salinity and water stress: improving crop efficiency. Springer, Dordrecht

Kende H (1993) Ethylene biosynthesis. Annu Rev Plant Physiol Plant Mol Biol 44:283–307

Keskin BC, Sarikaya AT, Yuksel B, Memon AR (2010) Abscisic acid regulated gene expression in bread wheat. Aust J Crop Sci 4:617–625
Khalil IA (1995) Chlorophyll and carotenoid contents in cereals as affected by growth retardants of triazole series. Cereal Res Commun 23:183–189
Khan MA, Gul B (2006) Halophyte seed germination. In: Eco-physiology of high salinity tolerant plants. Springer, Dordrecht, pp 11–30
Khan AA, Akbar M, Seshu DV (1987) Ethylene as an indicator of salt tolerance in rice. Crop Sci 27:1242–1248
Khan W, Prithiviraj B, Smith D (2003) Photosynthetic response of corn and soybean to foliar application of salicylates. J Plant Physiol 160:485–492
Khan NA, Singh S, Nazar R, Lone PM (2007) The source–sink relationship in mustard. Asian Aust J Plant Sci Biotechnol 1:10–18
Khodary SEA (2004) Effect of salicylic acid on growth, photosynthesis and carbohydrate metabolism in salt stressed maize plants. Int J Agric Biol 6:5–8
Khripach V, Zhabinskii VN, Lakhvich FA (1996) Prospects for practical use of brassinosteroids a new class of phytohormones. Seliskokhozyaistvennaya Biologia 1:3–11
Khripach V, Zhabinskii VN, deGroot AE (2000) Twenty years of brassinosteroids: steroidal plant hormones warrant better crops for the XXI century. Ann Bot 86:441–447
Kiribuchi K, Jikumaru Y, Kaku H, Minami E, Hasegawa M, Kodama O, Seto H, Okada K, Nojiri H, Yamane H (2005) Involvement of the basic helix-loop-helix transcription factor RERJ1 in wounding and drought stress responses in rice plants. Biosci Biotechnol Biochem 69:1042–1044
Kirkham MB, Gardner WR, Gerloff GC (1974) Internal water status of kinetin-treated, salt-stressed plants. Plant Physiol 53:241–243
Krishna P (2003) Brassinosteroid-mediated stress responses. J Plant Growth Regul 22:289–297
Kuiper D, Schuit J, Kuiper PJC (1990) Actual cytokinin concentrations in plant tissue as an indicator for salt resistance in cereals. Plant Soil 123:243–250
Kumar B, Singh B (1996) Effect of plant hormones on growth and yield of wheat irrigated with saline water. Ann Agric Res 17:209–212
Lamb C, Dixon RA (1997) The oxidative burst in plant disease resistance. Annu Rev Plant Physiol Plant Mol Biol 48:251–275
Larque-Saavedra A (1979) Stomatal closure in response to acetylsalicylic acid treatment. Z Pflannzen Physiol 93:371–375
Lehmann J, Atzorn R, Bruckner C, Reinbothe S, Leopold J, Wasternack C, Parthier B (1995) Accumulation of jasmonate, abscisic acid, specific transcripts and proteins in osmotically stressed barley leaf segments. Planta 197:156–162
Li L, Van Staden J (1998) Effects of plant growth regulators on the antioxidant system in seedlings of two maize cultivars subjected to water stress. Plant Growth Regul 25:81–87
Li W, Liu X, Khan MA, Yamaguchi S (2005) The effect of plant growth regulators, nitric oxide, nitrate, nitrite and light on the germination of dimorphic seed of *Suaeda sala* under saline conditions. J Plant Res 118:207–214
Liang YC, Zhang WQ, Chen J, Ding R (2005) Effect of silicon on H^+-ATPase and H^+-PPase activity, fatty acid composition and fluidity of tonoplast vesicles from roots of salt-stressed barley (*Hordeum vulgare* L.). J Environ Exp Bot 53:29–37
Liscum E, Reed J (2002) Genetics of Aux/IAA and ARF action in plant growth and development. Plant Mol Biol 49:387–400
Liu Y, Xu J, Ding Y, Wang Q, Li G, Wang S (2011) Auxin inhibits the outgrowth of tiller buds in rice (*Oryza sativa* L.) by downregulating *OsIPT* expression and cytokinin biosynthesis in nodes. Aust J Crop Sci 5:169–174
Luan S (2002) Signalling drought in guard cells. Plant Cell Environ 25:229–237
Macleod AM, Orquodale MC (1958) Water soluble carbohydrates of seeds of the gramineae. New Phytol 57:168–182
Maggio A, Barbieri G, Raimondi G, De Pascale S (2010) Contrasting effects of GA3 treatments on tomato plants exposed to increasing salinity. J Plant Growth Regul 29:63–72

Magome H, Yamaguchi S, Hanada A, Kamiya Y, Odadoi K (2004) Dwarf and delayed-flowering 1, a novel Arabidopsis mutant deficient in gibberellins biosynthesis because of over expression of a putative AP2 transcription factor. Plant J 37:720–729

Mahajan S, Tuteja N (2005) Cold, salinity and drought stresses: an overview. Arch Biochem Biophys 444:139–158

Majid GJ, Ali S, Foad M, Seyed AMMS, Iraj A (2011) The role of phytohormones in alleviating salt stress in crop plants: a review. Aust J Crop Sci 5(6):726–734

Masia A, Pitacco A, Braggio L, Giulivo C (1994) Hormonal responses to partial drying of the root system of *Helianthus annuus*. J Exp Bot 45:69–76

Mason HS, Mullet JE (1990) Expression of two soybean vegetative storage protein genes during development and in response to water deficit, wounding, and jasmonic acid. Plant Cell 2:569–579

Mason HS, DeWald DB, Mullet JE (1993) Identification of a methyl jasmonate-responsive domain in the soybean vspB promoter. Plant Cell 5:241–251

Masood A, Shah NA, Zeeshan M, Abraham G (2006) Differential response of antioxidant enzymes to salinity stress in two varieties of Azolla (*Azolla pinnata* and *Azolla filiculoides*). Environ Exp Bot 58:216–222

Mayer AM, Poljkoff-Mayber A (1975) The germination of seeds, 2nd edn. Pergamon Press, New York, pp 167–193

McConn M, Creelman RA, Bell F, Mullet JE, Browse J (1997) Jasmonate is essential for insect defense in *Arabidopsis*. Proc Natl Acad Sci U S A 94:5473–5477

Meeir H, Reid JSG (1982) Reserve polysaccharides other than starch in higher plants. In: Loewus FA, Tanner W (eds) Encyclopedia of plant physiol, vol 13a, New series. Springer, Berlin, pp 418–471

Mei C, Qi M, Sheng G, Yang Y (2006) Inducible overexpression of a rice allene oxide synthase gene increases the endogenous jasmonic acid level, *PR* gene expression, and host resistance to fungal infection. Mol Plant Microbe Interact 19:1127–1137

Meloni DA, Oliva MA, Martinez CA, Cambraia J (2003) Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environ Exp Bot 49:69–76

Merchan F, de Lorenzo L, González-Rizzo S, Niebel A, Megías M, Frugier F, Sousa C, Crespi M (2007) Analysis of regulatory pathways involved in the reacquisition of root growth after salt stress in *Medicago truncatula*. Plant J 51:1–17

Metwally A, Finkmemeier I, Georgi M, Dietz KJ (2003) Salicylic acid alleviates the cadmium toxicity in barley seedlings. Plant Physiol 132:272–281

Mitchell JW, Mandava NB, Worley JF, Plimmer JR, Smith MV (1970) Brassins: a new family of plant hormones from rape pollen. Nature 225:1065–1066

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7:405–410

Miyazaki JH, Yang SF (1987) The methionine salvage pathway in relation to ethylene and polyamine biosynthesis. Physiol Plant 69:366–370

Mizrahi Y, Blumonfeld A, Bittner S, Richmond AE (1971) Abscisic acid and cytokinin content of leaves in relation to salinity and relative humidity. Plant Physiol 48:752–755

Moons A, Prinsen E, Bauw G, Montagu MV (1997) Antagonistic effects of abscisic acid and jasmonates on salt stress-inducible transcripts in rice roots. Plant Cell 9:2243–2259

Morgan PW, Drew MC (1997) Ethylene and plant responses to stress. Physiol Plant 100:620–630

Moussatche P, Klee HJ (2004) Autophosphorylation activity of the Arabidopsis ethylene receptor multigene family. J Biol Chem 279:48734–48741

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681

Muthukumarasamy M, Panneerselvam R (1997a) Triazole induced protein metabolism in the salt stressed *Raphanus sativus* seedlings. J Indian Bot Soc 76:39–42

Muthukumarasamy M, Panneerselvam R (1997b) Amelioration of NaCl stress by triadimefon in peanut seedlings. Plant Growth Regul 22:157–162

Naqvi S (1999) Plant hormones and stress phenomena. In: Pessarakli M (ed) Handbook of plant and crop physiology. Marcel Dekker, New York, pp 645–660

Nilsen ET, Orcutt DM (1996) The physiology of plants under stress-abiotic factors. Wiley, New York, pp 118–130

Nojavan-Asghari M, Ishizawa K (1998) Inhibitory effects of methyl jasmonate on the germination and ethylene production in cocklebur seeds. J Plant Growth Regul 17:13–18

O'Malley RC, Rodriguez FI, Esch JJ, Binder BM, O'Donnell P, Klee HJ, Bleecker AB (2005) Ethylene-binding activity, gene expression levels, and receptor system output for ethylene receptor family members from Arabidopsis and tomato. Plant J 41:651–659

Pal M, Singh DK, Rao LS, Singh KP (2004) Photosynthetic characteristics and activity of antioxidant enzymes in salinity tolerant and sensitive rice cultivars. Indian J Plant Physiol 9:407–412

Panneerselvam R, Muthukumarasamy M, Karikalan L (1997) Triadimefon enhance growth and net photosynthetic rate in Nacl stressed plant of *Raphanus stivus* L. Photosyntheica 34(4):605–609

Pedranzani H, Racagni G, Alemano S, Miersch O, Ramírez I, Peña-Cortés H, Taleisnik E, Machado-Domenech E, Abdala G (2003) Salt tolerant tomato plants show increased levels of jasmonic acid. Plant Growth Regul 4(1):149–158

Pedranzani H, Sierra-de-Grado R, Vigliocco A, Miersch O, Abdala G (2007) Cold and water stresses produce changes in endogenous jasmonates in two populations of *Pinus pinaster* Ait. Plant Growth Regul 52:111–116

Peng S, Huang J, Sheehy JE, Laza RC, Visperas RM, Zhong X, Centeno CS, Khush GS, Cassman KG (2004) Rice yields decline with higher night temperature from global warming. Proc Natl Acad Sci U S A 101(27):9971–9975

Pierik R, Tholen D, Poorter H, Visser EJW, Voesenek LACJ (2006) The Janus face of ethylene: growth inhibition and stimulation. Trends Plant Sci 11:176–183

Popova LP, Stoinova ZG, Maslenkova LT (1995) Involvement of abscisic acid in photosynthetic process in *Hordeum vulgare* L during salinity stress. J Plant Growth Regul 14:211–218

Pospíšilová J (2003) Interaction of cytokinins and abscisic acid during regulation of stomatal opening in bean leaves. Photosynthetica 41:49–56

Pospíšilová J, Synková H, Rulcová J (2000) Cytokinins and water stress. Biol Plant 43:321–328

Prado FE, Boero C, Gallarodo M, Gonzalez JA (2000) Effect of NaCl on germination, growth and soluble sugar content in *Chenopodium quinoa* willd seeds. Bot Bull Acad Sin 41:27–34

Prakash L, Prathapasenan G (1990) NaCl and gibberellic acid induced changes in the content of auxin, the activity of cellulose and pectin lyase during leaf growth in rice (*Oryza sativ*a). Ann Bot 365:251–257

Prakash M, Kannan K, Kumar JS, Veeramani BB, Ganeshan J (1999) Effect of brassinosteroids on germination and seedling behaviour of tomato. Annu Plant Physiol 13(2):178–180

Prochazkova D, Wilhelmova N (2007) Leaf senescence and activities of the antioxidant enzymes. Biol Plant 51:401–406

Radi AF, Shaddad MAK, El-Enany AE, Omran FM (2006) Interactive effects of plant hormones (GA3 or ABA) and salinity ongrowth and some metabolites of wheat seedlings. Dev Plant Soil Sci Plant Nutr 92:436–437

Rakwal R, Tamogami S, Agrawal GK, Iwahashi H (2002) Octadecanoid signaling component "burst" in rice (*Oryza sativa* L.) seedling leaves upon wounding by cut and treatment with fungal elicitor chitosan. Biochem Biophys Res Commun 295:1041–1045

Raskin I (1992) Role of salicylic acid in plants. Annu Rev Plant Physiol Plant Mol Biol 43:439–463

Ribaut JM, Pilet PE (1991) Effect of water stress on growth, osmotic potential and abscisic acid content of maize roots. Physiol Plant 81:156–162

Ribaut JM, Pilet PE (1994) Water stress and indole-3ylacetic acid content of maize roots. Planta 193:502–507

Ritchie S, Gilroy S (1998) Gibberellins, regulating genes and germination. New Phytol 140:363–383

Sadka A, DeWald DB, May GD, Park WD, Mullet JE (1994) Phosphate modulates transcription of soybean *VspB* and other sugar-inducible genes. Plant Cell 6:737–749

Sairam RK, Deshmukh PS, Shukla DS (1989) Effect of ABA and CCC on water stress induced carbon dioxide and nitrate assimilation processes in tolerant and susceptible wheat genotypes. Ann Plant Physiol 3:98–107

Sakai H, Hua J, Chen QG, Chang C, Medrano LJ, Bleecker AB, Meyerowitz EM (1998) *ETR2* is an *ETR1*-like gene involved in ethylene signaling in Arabidopsis. Proc Natl Acad Sci U S A 95:5812–5817

Saniewski M, Miyamoto K, Ueda J (1998) Methyl jasmonate induces gums and stimulates anthocyanin accumulation in peach shoots. J Plant Growth Regul 17:121–124

Sankhla D, Sankhla N, Upadhyaya A, Kachhwaha S, Mali S, Dinesh R, Joshi S (1997) Effect of uniconazole on growth and activities of active oxygen processing enzymes in Ziziphus seedlings. Proc Plant Growth Regul Soc Am 24:208–212

Sasse JM (1991) The case for brassinosteroids as endogenous plant hormones. In: Cutler HG, Yokota T, Adam G (eds) Brassinosteroids: chemistry, bioactivity and applications. American Chemical Society, Washington, DC, pp 158–166

Schaller GE, Bleecker AB (1995) Ethylene-binding sites generated in yeast expressing the Arabidopsis ETR1 gene. Science 270:1809–1811

Senaratna T, Touchell D, Bumm E, Dixon K (2000) Acetylsalicylic acid (Aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. Plant Growth Regul 30:157–161

Seo HS, Song JT, Cheong JJ, Lee YH, Lee YW, Hwang I, Lee JS, Choi YD (2001) Jasmonic acid carboxyl methyltransferase: a key enzyme for jasmonate-regulated plant response. Proc Natl Acad Sci U S A 98:4788–4793

Sharma R, Lakhvir S (1988) Effect of phenolic compounds on some biochemical parameters during seed development in raya *Brassica juncea L.* J Plant Sci Res 4:69–72

Sharma YK, Leon J, Raskin I, Davis KR (1996) Ozone-induced resistances in *Arabidopsis thaliana*—the role of salicylic acid in the accumulation of defense-related transcripts and induced resistance. Proc Natl Acad Sci U S A 93:5099–5104

Sharma N, Abrams SR, Waterer DR (2005) Uptake, movement, activity, and persistence of an abscisic acid analog (80 acetylene ABA methyl ester) in marigold and tomato. J Plant Growth Regul 24:28–35

Shaterian J, Georges F, Hussain A, Waterer D, De Jong H, Tanino KK (2005) Root to shoot communication and abscisic acid in calreticulin (*CR*) gene expression and salt-stress tolerance in grafted diploid potato clones. Environ Exp Bot 53:323–332

Sheehy JE, Elmido A, Centeno G, Pablico P (2005) Searching for new plant for climate change. J Agric Meteorol 60:463–468

Shi HZ, Zhu JK (2002) Regulation of expression of the vacuolar Na^+/H^+ antiporter gene *AtNHX1* by salt stress and abscisic acid. Plant Mol Biol 50:543–550

Shin Shinozaki K, Yamaguchi-Shinozaki K (1997) Gene expression and signal transduction in water-stress response. Plant Physiol 115:327–334

Shrivastava P, Saxena RR, Xalxo MS, Verulkar SB (2012) Effect of high temperature at different growth stages on rice yield and grain quality traits. J Rice Res 5(1 and 2):29–42

Singh B, Usha K (2003) Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. Plant Growth Regul 3:137–141

Sinha SK, Srivastava SH, Tripathi RD (1993) Influence of some growth regulators and cations on inhibition of chlorophyll biosynthesis by lead in maize. Bull Environ Contam Toxicol 51:241–246

Smith D (1967) Carbohydrates in grasses. II. Sugar and fructosan composition of the stem bases of bromegrass and timothy at several growth stages and in different plant partsat anthesis. Crop Sci 7:62–67

Staswick PE (1994) Storage proteins of vegetative *plant* tissues. Annu Rev Plant Physiol Plant Mol Biol 45:303–322

Staswick PE, Su W, Howell SH (1992) Methyl jasmonate inhibition of root growth and induction of a leaf protein are decreased in an *Arabidopsis thaliana* mutant. Proc Natl Acad Sci U S A 89:6837–6840

Sun TP, Gubler F (2004) Molecular mechanism of gibberellin signaling in plants. Annu Rev Plant Physiol Plant Mol Biol 55:197–223

Swamy PM, Smith B (1999) Role of abscisic acid in plant stress tolerance. Curr Sci 76:1220–1227

Tani T, Sobajima H, Okada K, Chujo T, Arimura S, Tsutsumi N, Nishimura M, Seto H, Nojiri H, Yamane H (2008) Identification of the *OsOPR7* gene encoding 12-oxophytodienoate reductase involved in the biosynthesis of jasmonic acid in rice. Planta 227:517–526

Tasgin E, Atici O, Nalbantoglu B (2003) Effects of salicylic acid and cold on freezing tolerance in winter wheat leaves. Plant Growth Regul 41:231–236

Thaler JS, Fidantsef AL, Bostock RM (2002) Antagonism between jasmonate- and salicylate-mediated induced plant resistance: effects of concentration and timing of elicitation on defense-related proteins, herbivore, and pathogen performance in tomato. J Chem Ecol 28:1131–1159

Thomas DS, Eamus D (1999) The influence of predawn leaf water potential on stomatal responses to atmospheric water content at constant Ci and on stem hydraulic conductance and foliar ABA concentrations. J Exp Bot 50:243–251

Tran LSP, Urao T, Qin F, Maruyama K, Kakimoto T, Shinozaki K, Yamaguchi-Shinozaki K (2007) Functional analysis of AHK1/ATHK1 and cytokinin receptor histidine kinases in response to abscisic acid, drought, and salt stress in Arabidopsis. Proc Natl Acad Sci U S A 104: 20623–20628

Trejo CL, Davies WJ (1991) Drought-induced closure of *Phaseolus vulgaris* L. stomata precedes leaf water deficit and any increase in xylem ABA concentration. J Exp Bot 42:1507–1516

Ueda J, Kato J (1980) Isolation and identification of a senescence-promoting substance from wormwood (*Artemisia absinthium* L.). Plant Physiol 66:246–249

Ueda J, Kato J (1982) Inhibition of cytokinin-induced plant growth by jasmonic acid and its methyl ester. Physiol Plant 54:249–252

Vardhini BV, Rao SSR (1997) Effect of brassinosteroids on salinity induced growth inhibition of ground nut seedlings. Indian J Plant Physiol 2(2):156–157

Vardhini BV, Rao SSR (1998) Effect of brassinosteroids on growth, metabolic content and yield of *Arachis hypogaea*. Phytochemistry 48:927–930

Vardhini BV, Rao SSR (2001) Effect of brassinosteroids on growth and yield of tomato (*Lycopersicon esculentum* Mill.) under field conditions. Indian J Plant Physiol 6:326–328

Vettakkorumakankav NA (1999) Crucial role for gibberellin in stress protecting of plants. Plant Cell Physiol 40:542–548

Wan-Hong C, Jun L, Xin-Jian H, Rui-Ling M, Hua-Lin Z, Shou-Yi C, Jin-Song Z (2007) Modulation of ethylene responses affects plant salt-stress responses. Plant Physiol 143(2):707–719

Wang Y, Mopper S, Hasentein KH (2001) Effects of salinity on endogenous ABA, IAA, JA, and SA in *Iris hexagona*. J Chem Ecol 27:327–342

Wang KLC, Li H, Ecker JR (2002) Ethylene biosynthesis and signaling networks. Plant Cell 14 Suppl:S131–S151

Wasternack C, Parthier B (1997) Jasmonate-signalled plant gene expression. Trends Plant Sci 2(8):302–307

Wilen RW, Sacco M, Gusta LW, Krishna P (1995) Effects of 24-epibrassinolide on freezing and thermo tolerance of brome grass (*Bromus inermis*) cell cultures. Physiol Plant 95:195–202

Wilkinson S, Corlett JE, Oger L, Davies WJ (1998) Effects of xylem pH on transpiration from wild-type and flacca tomato leaves. Avital role for abscisic acid in preventing excessive water loss even from wellwatered plants. Plant Physiol 117:703–710

Wolbang CM, Chandler PM, Smith JJ, Ross JJ (2004) Auxin from the developing inflorescence is required for the biosynthesis of active gibberellins in barley stems. Plant Physiol 134:769–776

Xiong L, Schumaker K, Zhu JK (2002) Cell signaling during cold, drought and salt stress. Plant Cell 14:165–183

Yamaguchi S (2008) Gibberellin metabolism and its regulation. Annu Rev Plant Biol 59:225–251

Yancey PH, Clark ME, Hand SC, Bowlis RD, Somero GN (1982) Living with water stress: evolution of osmolyte system. Science 217:1214–1222

Yuan BC, Li ZZ, Liu H, Gao M, Zhang YY (2007) Microbial biomass and activity in salt affected soils under arid conditions. Appl Soil Ecol 35:319–328. doi:10.1016/j.apsoil.2006.07.004

Zhang J, Davies WJ (1989) Abscisic acid produced in dehydrating roots may enable the plant to measure the water status of the soil. Plant Cell Environ 12:73–81

Zhang J, Davies WJ (1990a) Changes in the concentration of ABA in xylem sap as a function of changing soil water status will account for changes in leaf conductance. Plant Cell Environ 13:277–285

Zhang J, Davies WJ (1990b) Does ABA in the xylem controls the rate of leaf growth in soil-dried maize and sunflower plants. J Exp Bot 41:1125–1132

Zhang J, Schurr U, Davies WJ (1987) Control of stomatal behaviour by abscisic acid which apparently originates in roots. J Exp Bot 38:1174–1181

Zhang J, Jia W, Yang J, Ismail AM (2006) Role of ABA in integrating plant responses to drought and salt stresses. Field Crop Res 97:111–119

Zhao HJ, Lin XW, Shi HZ, Chang SM (1995) The regulating effect of phenolic compounds on the physiological characteristics and yield of soybeans. Acta Agron Sin 21:351–355

Zholkevich VN, Pustovoytova TN (1993) The role of *Cucumis sativum* L. leaves and content of phytohormones under soil drought. Russ J Plant Physiol 40:676–680

Zhu JK (2000) Genetic analysis of plant salt tolerance using *Arabidopsis*. Plant Physiol 124:941–948

Zhu JK (2001) Plant salt tolerance. Trends Plant Sci 6:66–71

Zhu JK (2002) Salt and drought stress signal transduction in plants. Annu Rev Plant Physiol Plant Mol Biol 53:247–273

Zhu BC, Su J, Cham MC, Verma DPS, Fan YL, Wu R (1998) Over expression of pyrroline-5-carboxylate synthetase gene and analysis of tolerance to water stress and salt stress in transgenic rice. Plant Sci 139:41–48

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