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Eric Lichtfouse
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Cereals

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Chapter 1

Drought and Salt Stress in Cereals

Linda Mondini and Mario Augusto Pagnotta

Abstract Abiotic stresses, such as salinity, drought, extreme temperatures, chemical toxicity and oxidative stress represent a grave threat to agriculture dramatically affecting the crop production around the world. Climate changes are projected to have a significant impact on temperature and precipitation profiles increasing the incidence and severity of climate changes-related stresses and reducing in particular the productivity of rain-fed crops. In fact, drought and salinity stresses determine the primary cause of worldwide crop loss. Plant adaptation to environmental stresses is based on the activation of molecular networks involved in stress perception, signal transduction, and expression of specific stress-related genes and metabolites. Plants respond to the stresses in part by modulating gene expression in order to restore cellular homeostasis, detoxifying the toxins present into the cells and through the recovery of growth.

In present chapter the physiological and biochemical aspects of plant response to water stresses are reviewed together with the new frontiers studies on the genetic tools on stress tolerance. The recent exploitation of next generation resources applied to the functional genomics combined with a gradual increasing in transformation frequencies for many grasses, is supporting the study and the manipulation of abiotic stresses in grasses, notably increasing the plant tolerance. Mutational analysis and microarrays have led to the identification of numerous candidate genes involved in a series of stresses comprising drought, salt, freezing, and heat. The variability found in the genetic traits related with abiotic stress tolerance has permitted to identify and mapping several candidate genes and has confirmed the importance of wild relatives to identify the traits that domestication has canceled in the selected lines. The recent knowledge on candidate genes organization has led to the identification of promising allelic variants that, through Marker Assisted Selection (MAS), can be easily transferred into the susceptible commercial lines. Thence, the advent and development of molecular markers in quantitative genetics have greatly facilitated the study of complex quantitatively inherited traits by the construction of high density genome linkage maps for crops such as wheat. The

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identification of Quantitative Trait Loci (QTLs) ruling the genetic variability of the traits controlling such tolerance and the consequent manipulation to use in MAS is of crucial importance. The knowledge of the number and effects of QTLs can help breeders to understand the genetic control of these traits and to design more efficient selection strategies for improvement. To date, the modern commercial cultivars, able to survive to severe abiotic stresses regimes performing a good level of productivity, are the result of this activity.

Keywords Abiotic stresses • Ion homeostasis • Signal transduction • Transcription factors • Drought • Salinity • Cereals

Abbreviations

ABA	Abscisic acid
ALDH	Aldehyde Dehydrogenase
HSPs	Heat Shock proteins
LEA	Late Embryogenesis Abundant
MAS	Marker Assisted Selection
NILs	Near Isogenic Lines
NSCs	Non Selective Cation Channels
QTLs	Quantitative Traits Loci
RILs	Recombinant Inbred Lines
ROS	Reactive Oxygen Species
TFBSs	Transcription Factors Binding Sites.
TFs	Transcription Factors

1.1 Introduction

Despite a global scarcity of water resources and the increased salinization of soils and water, abiotic stresses are, at present, the main limiting factors in plant growth and crop cultivation and are bound to soon become even more severe as desertification covers most of the world's terrestrial area. Drought and salinity are actually widespread in many regions around the world and are expected to increase rapidly reaching the salinization of more than 50 % of the arable lands by the 2050 (Fig. 1.1). Moreover, the frequency and duration of dry spells and heat waves are likely to increase, especially in the dryland areas. Extremes, variability, and rates of change are all key features in addressing vulnerability and adaptation to climate changes. The combination of larger human population pressure, which is increasing at a rate of about 1.3 % per year, and climate uncertainty will expose millions of people to severe water shortages and this is expected to have a proportionally high impact on social, economic, and ecological aspects.

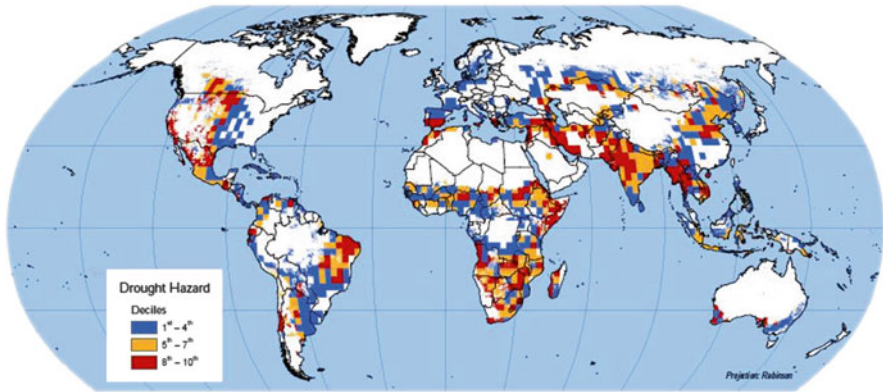


Fig. 1.1 Global drought hazard distribution. Drought periods were defined using an index known as the Weighted Anomaly of Standardized precipitation (WASP). The WASP index assesses the precipitation deficit or surplus over a three-month running average for the 21-year period from 1980–2000. Findings show that about 38 % of the world’s land area has some level of drought exposure



Fig. 1.2 Wheat cultivation and depleted soil under drought conditions

In particular, abiotic stresses to which currently plants are exposed, include a decreased availability of water, extreme peaks of temperature including freezing, decreased availability of essential nutrients from the soil, poisoning of the plant due to the build-up of toxic ions during the stress, excessive irradiation, and increased hardness of the soil that limits root penetration (Fig. 1.2). Most abiotic stresses are

interconnected by the fact that their disruptive effect on plant *equilibrium* determining cellular damages, are due to the alteration and interference with the plant water regime. In particular, this effect occurs in conjunction with water scarcity or altered ion content with a consequent water loss for osmosis caused by salinity or for dehydration due to the formation of extracellular ice during freezing stress. It must be considered that water is required as a medium for biochemical activities and in particular for plant cells, water creates the turgor pressure which represents the driving force for cell expansion and growth. Therefore, through the maintenance of a positive turgor pressure, cells can regulate the osmotic potential in order to balance their water content. Weighty changes in water potentials in the soil can carry osmotic stress to plant compromising normal cellular activities so much to cause plant death.

Abiotic stresses determine, in plants, a series of morphological, biochemical, physiological and genetic changes causing a critical decreasing in growth and productivity. Moreover, oxidative stress frequently consequent to high temperature, salinity, and drought stresses often can cause denaturation of functional and structural proteins (Smirnov 1998). Thence, these stresses even if diverse, can activate parallel cell signaling pathways (Shinozaki and Yamaguchi-Shinozaki 2000; Zhu 2002) and cellular responses comprising the up-regulation of anti-oxidants, the production of stress proteins, and the accumulation of solutes (Vierling and Kimpel 1992; Cushman and Bohnert 2000). The plant response to abiotic stresses involves so a complex of genes and mediator regulated by biochemical and genetic interlinked mechanisms. These genes include three major categories distinguished for their function: (i) genes involved in transcriptional control and signaling cascade; (ii) genes involved in the protection of membranes and proteins and finally (iii) genes involved in water and ion uptake and transport. In this perspective, the strategy for enhance abiotic stress tolerance in plants aims to manipulate genes involved in the protection and maintaining of the functions and the structures of cellular compounds. Nevertheless, on the contrary to the genes involved in resistance to biotic stresses which are constituted by monogenic traits, the gene network that controls the tolerance to abiotic stresses, is very complex and thence very difficult to understand and engineer.

Higher plants, have still developed different but interlinked stratagems to survive and to get over the stress. On the contrary, these stratagems are not equally present in the majority of crops. In addition, for crops, the traditional breeding programs involving the transfer of desirable genetic traits from wild relatives, are ineffective due to the objective difficulty to manipulate and breeding for tolerance traits. In particular for cereals, most of the abiotic stress tolerance traits are localized in landraces and related wild species, nevertheless most of these traits have been not yet identified in grass. In fact, although wheat, rye and barley possess a good level of abiotic stress tolerance respect to maize and rice, information about genes and mechanisms involved are very scarce or sometimes lacking making impossible the improvement of commercial lines for the stress tolerance traits. So the future challenge of abiotic stress research consists in to plug the gap between the knowledge and the transfer of related traits into promising material.

1.2 Strategies for Stress Tolerance: Drought Stress

1.2.1 Water Potential

One of the components that cause the occurrence of a stress condition is the decrease in the availability of water in soil. This reduced water availability are quantified as a reduction in water potential (ψ_w , Kramer and Boyer 1995), defined as the chemical potential of water divided for the partial molar volume. In this perspective, the free energy of water with the cell turgor are expressed in units of pressure and as a direct consequence of the water movement in the plant/soil system. A reduction of the ψ_w , increases the difficulty for the plant to take up water touching off a cascade of responses that help the plant to avoid the water loss. The avoidance strategies can include changes in water fluxes starting from the plant *in toto* up to the cellular level and consist in: i) the implementation of stratagems for the continuing water up taking at reduced ψ_w and ii) the capability of plant to tolerate a decreased tissue water content (Fig. 1.3).

1.2.2 Tolerance of Low ψ_w

The first response of plants to a reduced ψ_w is to avoid it. Tissue ψ_w and water content are constantly maintained by the plant at the *equilibrium* condition and when a decrease in water supply occurs, these are retained close to the unstressed condition

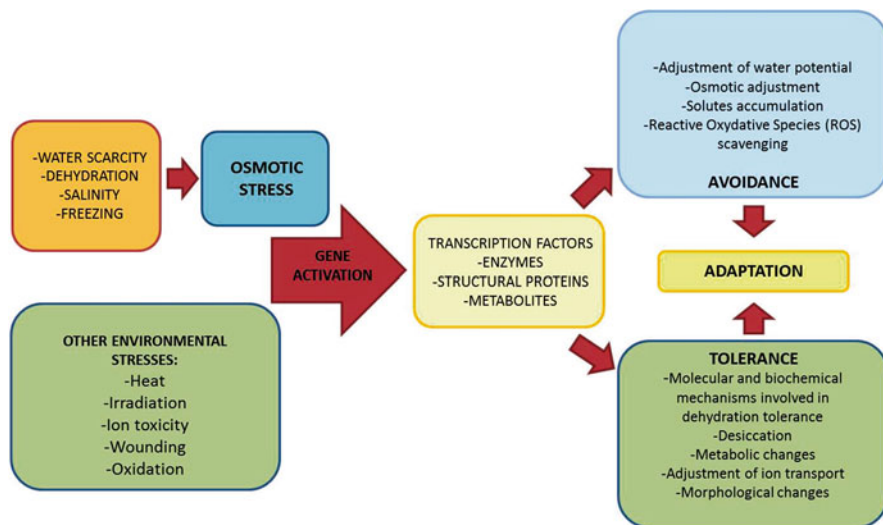


Fig. 1.3 Chart of the different typology of abiotic stresses activating different metabolic pathways which confer adaptation either to avoidance or tolerance strategies

for the time being, by limiting the water loss through a fast stomatal closure and increasing water uptake. Long term effects comprise the increase of water capability and water permeability in tissues, the expansion of cuticle volume and thickness and, finally the alteration of growth ranking of sprouts and roots. In particular the modification of growth ranking of sprouts and roots permits to increase the sprout/root ratio that represents the main strategy of drought avoidance. Nevertheless, these mechanisms of low ψ_w avoidance are useful and efficient only for limited time periods, due to a reduction of photosynthesis process for the scarcity of CO_2 caused by the stomatal closure. If long term drought stress occurs plants must resort to further mechanisms.

1.2.3 Dehydration Avoidance

The dehydration avoidance is a stratagem consisting in the adjustment made by the plant of ψ_w as a consequence of a reduced level of ψ_w in the soil. This modification consists in a decrease of ψ_w of the plant to avoid the water loss from roots through the accumulation of solutes and cell wall reinforcement. The movement of water into or out the plant, strictly depends on ψ_w gradient created between the cell and the environment. Thence, a plant, extracting water from the soil, must possess a ψ_w lower than that. However the ψ_w of a cell provided of a cell wall, includes two different components: the osmotic potential (ψ_s) and the turgor pressure (ψ_p) and is represented from this equation: $\psi_w = \psi_s + \psi_p$. The maintenance of equilibrium between the two components is crucial, in fact at a given ψ_w to a higher level of ψ_p due to accumulation of solutes inside the cell must correspond a lower level of ψ_s . The accumulation of solutes as a consequence of a reduced ψ_w is known as “osmotic adjustment” (Zhang et al. 1999). It is crucial for a correct cell functioning, that the accumulated solutes should not affect the cellular function. Low ψ_w is due to freezing or salinity or other type of abiotic stresses. Generally, plants tend to contrast low ψ_w accumulating compatible solutes unaffacting their metabolism and having also protective function (Yancey et al. 1982). Another protective mechanisms includes the maintenance of cell turgor adjusting osmotic pressure into the cell. There are several specialized osmolytes such as proline, mannitol, glycine betaine, and ectoine, synthesized by the cell in order to readjust the osmotic potential. Among these, proline has received much attention due to the fact that its accumulation has been observed in several plant species upon exposure to abiotic stresses (Delauney and Verma 1993). Recently, genetic and transgenic studies have confirmed the capability of proline to increase the abiotic stress tolerance in plants (Xin and Browse 1998; Hong et al. 2000; Ronde et al. 2000). The possibility of plants to accumulate compatible solutes is crucial to adverse drought stress in field (Morgan 1984, 1991). For this reason the genetic research aims to engineer transgenic plants to increase the synthesis of compatible solutes and consequently, the tolerance to abiotic stresses (Bohnert and Shen 1999). Nevertheless, several works have

demonstrated that the increase of proline production, under abiotic stresses, is not enough considerable for overall osmotic adjustment (Zhu 2001) suggesting that probably the osmolytes work for osmotic adjustment, mainly scavenging reactive oxygen species and so maintaining the natural status of macromolecules (Smirnoff and Cumbes 1989; Hong et al. 2000). Moreover, damaged proteins are immediately eliminated by the cell to prevent their aggregation. The elimination allows the directly degradation of damaged proteins up to the destruction of the entire cell through apoptosis. Both of these events have been identified in plant cells under abiotic stresses (Katsuhara 1997). Considering that proteolytic systems occur through an ubiquitin-mediated degradation into the proteasomes, the expression of genes encoding ubiquitin-related genes has been deeply studied (Ciechanover et al. 2000). In confirmation, it has been demonstrated that under abiotic stresses condition and in particular under drought stress, the expression of these genes is consistently enhanced proportionally to the protein degradation requirement (Ingram and Bartels 1996) (Fig. 1.4). Finally, the cell wall itself, thanks to its deformability, works to plant's advantage promoting the dehydration avoidance.

1.2.4 Dehydration Tolerance

When the exposure to abiotic stresses becomes severe and continuous, the ψ_w starts to decrease until for plants it is impossible to avoid dehydration. At this stage, becomes crucial to carry out all the molecular and biochemical mechanisms involved in dehydration tolerance. Some plants are able to escape to dehydration through “desiccation” recurring to a fully air-dried state (Olive et al. 2000; Vicré et al. 2004). During this phase, the plant is in a dormant state where the metabolism is temporarily reduced as happens in seed dormancy. Nevertheless, most plants tends to carry out tolerance mechanisms exhibiting a wide range of responses at the molecular, cellular and whole-plant levels (Greenway and Munns 1980; Bohnert and Shen 1999; Hasegawa et al. 2000). These mechanisms, tending to mainly protect the cellular structures from the dehydration damages and controlling the level of reactive oxygen species (ROS), involve a series of changes. These includes: (i) morphological and developmental changes; (ii) adjustment in ion transport (uptake, extrusion and sequestration of ions) and (iii) metabolic changes (synthesis of compatible solutes and carbon metabolism) (Figs. 1.4 and 1.5). The different typologies of responses to the stress are activated by the primary osmotic stress signals instead others can be triggered by secondary stresses through a chain reaction sparked by the primary signals. The secondary signals comprise phytohormones (e.g. Ethylene, abscisic acid ABA), reactive oxygen species (ROS) and intracellular second messengers (e.g. phospholipids). Some of them are not restricted in the sites where the primary signal occurs but can move to other parts of the whole plant contributing to the network and organization of total plant response (Fig. 1.5).

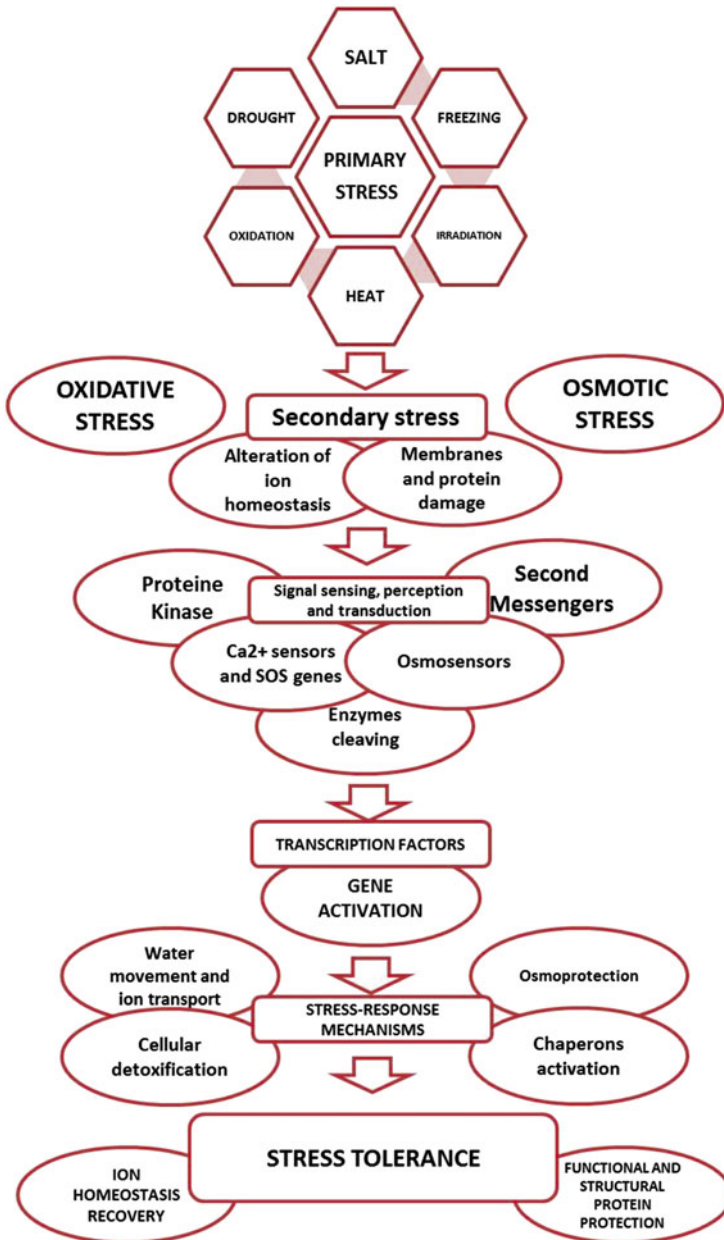


Fig. 1.4 Cascade of biochemical, physiological and genetic mechanisms and interacting factors occurring under abiotic stress

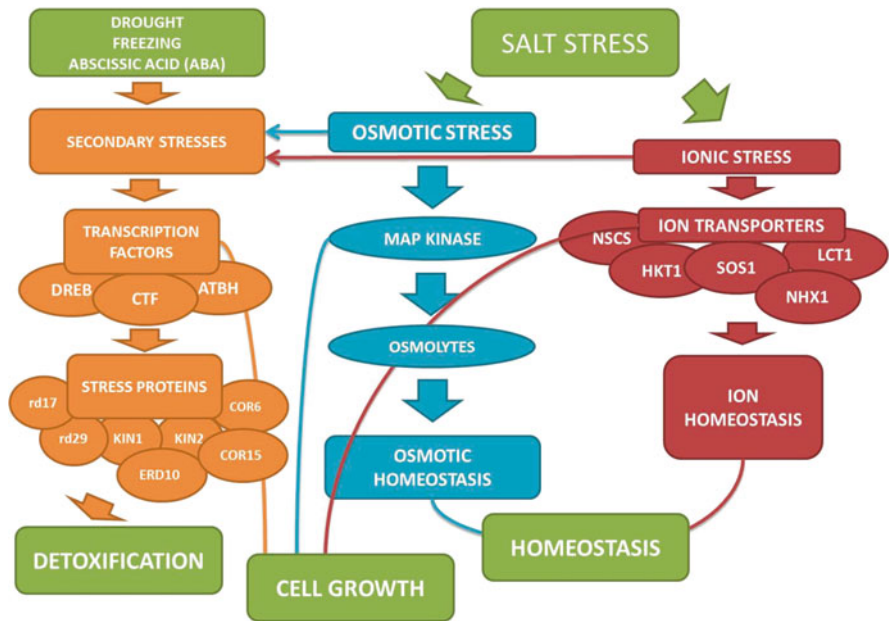


Fig. 1.5 The three different components of plant tolerance to salinity stress (detoxification, cell growth control and homeostasis)

1.2.5 Cell Protection

Plant cells, to avoid damages, make use of systems assigned to protect protein structures. Among these, Heat-Shock Proteins (Hsps), molecular chaperones and Late Embryogenesis Abundant (LEA) proteins are mostly involved in cells protection (Wang and Luthe 2003, Wang et al. 2004, Goyal et al. 2005). The protection function comprises the control of folding and conformation of both functional (such as enzymes) and structural proteins (i.e. proteins constituting cell membrane and complexes). Some functional genomics and proteomics studies have confirmed an increasing in Hsps expression occurring under stress conditions (Sun et al. 2002; Wang and Luthe 2003; Sung and Guy 2003). Crop plants that grow under drought condition are particularly subjected to other different stresses caused by high temperatures, soil resistance to root penetration, excessive irradiance and low water potential. These stresses can produce protein denaturation and dysfunction. Despite the presence of photo-protective mechanisms able to dissipate excess light energy, the persistent exposure to extreme excitation energy can cause photo-reduction of oxygen molecules generating highly toxic reactive oxygen species (ROS). ROS, comprising super-oxides and peroxides, are usually produced by normal cellular activity occurring during photorespiration and beta oxidation of fatty acids and so their level increases under abiotic and biotic stress conditions. These dangerous

molecules, causing chemical damage to DNA and proteins, bring to grave or even fatal effects on cellular metabolism. Reactive species can be neutralized by antioxidants and ROS scavengers and improving this capacity through manipulation, can represent an important goal. ROS elimination is mainly performed by antioxidant such as carotenoids, glutathione, ascorbic acid, and thioredoxin and by ROS scavenger enzymes such as catalase, superoxide dismutase, glutathione peroxidase. Bowler et al. (1992) demonstrated that under drought conditions the production of these components increases dramatically with a consequent higher scavenger activity enhancing plant drought tolerance. Aldehyde dehydrogenase (ALDH), known as one of the most effective ROS scavenger, catalyzes the oxidation of toxic aldehydes accumulated from the side reactions among ROS, lipids and proteins (Kirch et al. 2004). Transgenic plants overexpressing aldehyde dehydrogenase showed improved tolerance to dehydration, heavy metals, hydrogen peroxide and salt stresses (Sunkar et al. 2003). Moreover, as previously described, there are several osmolytes that can protect the cell maintaining the turgor pressure. Among these, proline and some sugars can protect the proteins covering their surfaces by the solutes in the medium and reducing so their unfolding (Hoekstra et al. 2001). LEA proteins and dehydrins seem to work when the desiccation rate becomes more severe, substituting the water through the constitution of hydrogen bonds for polar amino acid residues on the protein surface and thence decreasing the rate of unfolding (Crowe et al. 1997).

1.3 Salt Stress

High concentrations of salts in the soil, particularly NaCl, can cause ion imbalance and hyperosmotic stress in the plant. The consequence of these events is a secondary stress consisting in the oxidative damage previously described. Damages due to the salt presence can be prevented re-establishing homeostatic conditions and resuming the growth also at a reduced rate. The homeostasis re-establishment in stressful plants requires both ionic and osmotic homeostasis recovering. Ionic homeostasis is maintained by several ion transporters that work avoiding the accumulation to high levels of Na⁺ into the cytosol, in the organelles and in the vacuole. Na⁺ inhibits many enzymes, thence the prevention of its accumulation is fundamental. The transport of Na⁺ into the cell is usually mediated by non-selective cation channels (NSCs), although other mediators have been recently identified such as LCT1 and HKT1 transporters which were originally identified as K transporters. Some works on the identification of mutations in the genes for HKT1 and LCT1 have demonstrated an increased salt tolerance in plants (Amtmann et al. 2001; Mondini et al. 2011). Nevertheless, the main aim of the studies focusing on salt tolerance consists in the identification of all the transporters involved in Na⁺ entry into the plant. A consequent targeted mutagenization of these transporters could stop the Na⁺ entry and increase the salt tolerance. Recently, it was reported in *Arabidopsis* that plants overexpressing AtNHX1 or SOS1 have significantly increased salt tolerance. In

Arabidopsis, ion homeostasis is mediated mainly by the SOS signal pathway, which consists of three main components. SOS1 encodes a plasma membrane Na^+/H^+ antiporter that plays a critical role in sodium extrusion and in controlling long-distance Na^+ transport from the root to shoot (Shi et al. 2002). SOS2 encodes a Ser/Thr protein kinase (Liu et al. 2000) and finally SOS3 encodes an EF-hand Ca^{2+} -binding protein that functions as a calcium sensor for salt tolerance (Liu and Zhu 1998). AtNHX1 is the first studied plant vacuolar protein that can mediate Na^+ transport into vacuoles (Apse et al. 1999). Overexpression of SOS1 or AtNHX1 has been reported to deeply improve the plant salt tolerance (Apse et al. 1999; Zhang and Blumwald 2001; Zhang et al. 2001; Shi et al. 2003). This kind of transporter is very important due to its capability to store and remove from the cytosol the Na^+ in excess making the vacuolar compartmentation of Na^+ an efficient method to sequester Na^+ . Moreover, it must be considered that plants, unlike animals, fungi and algal cells, do not contain Na^+/K^+ ATP-ases for Na^+ efflux, anyway the same function of antiporter activity has been identified in plasma membrane-enriched membrane vesicles and in particular in the product of SOS1 gene (Blumwald et al. 2000; Shi et al. 2000). Another component of salt tolerance is the growth regulation. Salt stress inhibits growth, thence the slowing down of growth represents an important adaptive stratagem for plant survival allowing a more efficient resources exploitation. Salt stress acts directly on cell growth (i) reducing the photosynthesis efficiency caused by the stomatal closure and thence by the decrease of carbon dioxide uptake, (ii) inhibiting cell division and finally (iii) reducing the cell expansion. Several studies have been confirmed the connection between salt stress and growth rate decreasing (Holmström et al. 1996; Liu et al. 1998; Soderman et al. 2000). Considering that currently the overexpression of transgenes does not compromise or alter the plant cell growth, the overexpression of genes, known to be involved in salt tolerance, causes a slow growth of transgenic plants. Among these, DREB1 (Dehydration Responsive Binding elements-1) (Riechmann and Meyerowitz 1998), CBF1 (CRT/DRE binding factor 1) (Zhang et al. 2011) and ATBH7 (lipocalin-like protein) (Soderman et al. 2000) are cold and drought stresses-inducible genes and are not expressed in normal growth conditions. DREB genes family plays an important role in salt, drought and freezing stress tolerance even if their gene products or downstream target molecules act with different and sometimes contrasting actions which contribute to hinder the comprehension of their action mechanisms (Shen et al. 2003; Egawa et al. 2006; Mondini et al. 2012).

1.4 Stress Networks

The response of a plant to “environmental injury” and in particular to abiotic stresses aggression comprises multiple, complex and connected networks (Fig. 1.5). Stress tolerance is realized by the plant with the contemporary action of physical and molecular changes activated through a cascade of reactions by the stress itself (Fig. 1.4). The first limit to unravel the mechanism of action of plant response

consists in the difficulty to assign to a specific stress typology the corresponding response. In fact, different stresses can jointly act through the same network and with the same cascade of reactions, for example drought, salt, and freezing stresses can all determine a decrease in cellular osmotic potential going to activate the same cellular response. At the same time, also abiotic stresses and nutrient deficiency can act with the same mechanisms, for instance drought stress and boron toxicity are strictly related in cereals causing both a decreasing in root development. Moreover, stresses causing an osmotic unbalancing can act producing two different type of responses, following two different ways operating through abscisic acid ABA-dependent and ABA-independent via. In addition, this primary response can activate a second ionic response where ion transporters confine ions in excess into specific cellular compartments.

1.4.1 Signaling Pathway

The starting perception of the stress immediately activates a downstream signal transduction pathway acting through the simultaneous induction and repression of different genes with the consequent activation of key enzymes. This cascade of reactions comprises molecular and biochemical changes which represent the entire network of abiotic stress responses. The transduction of the signal immediately generates an exchange of information within and between the cells and the induction of both regulatory and functional sets of genes (Ingram and Bartels 1996; Ramanjulu and Bartels 2002; Bartels and Sunkar 2005). Very little is known about the early events in the signaling pathway (Urao et al. 1999; Ueguchi et al. 2001; Wohlbach et al. 2008). Nevertheless, at large it can be distinguished into abscisic acid (ABA) dependent and ABA independent (Shinozaki and Yamaguchi-Shinozaki 1997, 2000). Among the key genes, transcription factors belonging to the class of DRE-binding protein (DREB), C-repeat-binding factor (CBF), ABA-binding factor (ABF), MYC, and MYB (Abe et al. 1997; Bartels and Sunkar 2005; Sakuma et al. 2006), including the identification of the stress-responsive cis-elements ABA-responsive element (ABRE) and dehydration responsive element (DRE; Yamaguchi-Shinozaki and Shinozaki 1994; Ramanjulu and Bartels 2002; Bartels and Sunkar 2005) play a key role. The presence of these different classes of elements in the promoters of drought, salt and freezing signal transduction suggests that these probably represent a point of convergence among the different pathways that permit a coordinated response to different stresses (Fig. 1.4). In this perspective, also the oxidative stress pathway seems to be connected with Ca^{2+} mediated response. In fact, among the early events in abiotic stress perception, the downstream signaling includes genes and molecules acting as secondary messengers, in particular the elevation of cytoplasmic Ca^{2+} levels, reveals the role of Ca^{2+} as secondary messenger (Bartels and Sunkar 2005). The elevation of Ca^{2+} concentration leads to the activation of signal transduction pathway involving Ca^{2+} -regulated protein phosphatases (Knight and Knight 2001). In particular, Urao et al. (1999) have demonstrated that

when an osmotic stress occurs, the perception of the stress involves a plasma membrane histidine-kinase.

On this base, in transgenic plants, the engineering of the tolerance through the up regulation of transcription factors or other regulatory elements acting in the signal-transduction pathway represents a powerful and promising approach (Cushman and Bohnert 2000; Umezawa et al. 2004). Studying the different pathways, an important factor must be considered: the simultaneous exposure of a plant to different abiotic stresses results in a contemporary co-activation of different pathways acting with synergic or antagonistic mechanisms. Nevertheless, a dissection of the dedicated pathway specific for the particular stress is very difficult. The cross-talk among different pathways can occur at different levels: (i) integrations of networks of calcium-ROS signaling (Bowler and Fluhr 2000; Mittler et al. 2004); (ii) integrations of transcription factors-MAP Kinases (Cardinale et al. 2002; Xiong and Yang 2003); (iii) integrations among hormones (ABA, ethylene, jasmonic acid, etc.) (Anderson et al. 2004); (iv) integrations of receptors and signaling complexes (Casal 2002). For instance, Suzuki et al. (2006) and Kotak et al. (2007) have confirmed the involvement of factors other than classical heat-stress responsive genes in thermo-tolerance. In fact both plant hormones such as ethylene and reactive oxygen species also contribute to heat-stress signaling. The expression of co-activator MBF1c in *Arabidopsis* enhances at the same time, the tolerance to heat and osmotic stresses activating or disturbing the ethylene response signal transduction pathway.

1.5 Transcription Factors

Transcription factors are modular proteins that often contain separate domains that participate in DNA binding, protein/protein interactions, and transcriptional activation or repression. Transcription factors work largely by interacting with the basal transcriptional machinery and/or chromatin modifying proteins, thereby altering the rate of gene transcription (Latchman 1998; Lee and Young 2000). Transcription factors physically interact with genomic cis-regulatory DNA elements, referred to as transcription factor-binding sites (TFBSs), through a specific DNA-binding domain. Numerous types of DNA-binding domains have been identified in plant genomes that contain a large number of transcription factors such as *Arabidopsis* that contains more than 1,500 transcription factors covering a portion of about 5.9% of its genome (Riechmann et al. 2000). The most important transcription factor families are represented by MYB, AP2/EREBP, ZIP and WRKY elements (Table 1.1 and Fig. 1.6). These are large and multi-gene families where different members belonging to the same gene family respond to different stress typology. At the same time, it has been observed that different stresses can induce the expression of the same genes, as demonstrated by similar expression profiles indicating that some stress responsive genes can share the same transcription factors (Seki et al. 2001; Chen et al. 2002, Kreps et al. 2002). C-repeat binding factors (CBF) and dehydration responsive binding elements (DREB) specifically recognize CRT and DRE

Table 1.1 Main Transcription factors involved in plant abiotic stress tolerance

Gene family	Functional domain	ABA induced	Gene	Stress susceptible	Species investigated
DREB/ CBF	AP2/EREBP	No	AtDREB1A	Cold	<i>Arabidopsis thaliana</i>
		No	AtDREB2A	Drought, salinity	<i>Arabidopsis thaliana</i>
		No	AtDREB12C	Cold, salt, Mannitol	<i>Arabidopsis thaliana</i>
		No	OsDREB1A	Salt, Cold, wounding	<i>Oryza sativa</i>
		No	OsDREB1B	Cold	<i>Oryza sativa</i>
		Yes	OsDREB1C	Salt, drought, cold, wounding	<i>Oryza sativa</i>
		No	OsDREB1D	–	<i>Oryza sativa</i>
		No	OsDREB1F	Salt, drought, cold	<i>Oryza sativa</i>
		No	OsDREB1G	–	<i>Oryza sativa</i>
		No	OsDREB2A	Salt, drought, cold	<i>Oryza sativa</i>
		No	OsDREB2B	Cold, heat	<i>Oryza sativa</i>
		No	OsDREB2C	–	<i>Oryza sativa</i>
		No	OsDREB2E	–	<i>Oryza sativa</i>
		Yes	HvDRF1	Salt, drought	<i>Hordeum vulgare</i>
		No	ZmDREB2A	Salt, drought, heat, cold	<i>Zea mays</i>
		No	PgDREB2A	Salt, drought, cold	<i>Pennisetum glaucum</i>
		No	SbDREB2	Drought	<i>Sorghum bicolor</i>
		No	SiDREB2	Salt, drought	<i>Setaria italica</i>
		No	CaDREB-LP1	Wounding, salt, drought	<i>Capsicum annum</i>
		No	TaDREB1	Drought, cold	<i>Triticum aestivum</i>
		Yes	TaDREB2A	Salt, drought, cold	<i>Triticum aestivum</i>
		No	TaDREB3	–	<i>Triticum aestivum</i>
		No	TaDREB4	–	<i>Triticum aestivum</i>
		No	TaDREB5	–	<i>Triticum aestivum</i>
		No	GmDREBa	Salt, drought, cold	<i>Glycine max</i>
		Yes	GmDREBb	Salt, drought, cold	<i>Glycine max</i>
		No	GmDREBc	Salt, drought	<i>Glycine max</i>
		No	GmDREB2	Salt, drought	<i>Glycine max</i>
		No	CBF1	Cold	<i>Arabidopsis thaliana</i>
		No	CBF2	Cold	<i>Arabidopsis thaliana</i>
		No	CBF3	Cold	<i>Arabidopsis thaliana</i>
		Yes	CBF4	Drought	<i>Arabidopsis thaliana</i>
WRKY	WRKY	–	TaWRKY1	Drought	<i>Triticum aestivum</i>
		–	Os WRKY45	–	<i>Oryza sativa</i>
		–	Gm WRKY21	–	<i>Glycine max</i>
		–	Gm WRKY54	–	<i>Glycine max</i>
		–	Gm WRKY13	–	<i>Glycine max</i>
		–	Nb WRKY	–	<i>Nicotiana benthamiana</i>

(continued)

Table 1.1 (continued)

Gene family	Functional domain	ABA induced	Gene	Stress susceptible	Species investigated
bZIP	ABREs	Yes	ABF1	Cold	<i>Arabidopsis thaliana</i>
		Yes	ABF2	Drought, salt	<i>Arabidopsis thaliana</i>
		Yes	ABF3	Salt	<i>Arabidopsis thaliana</i>
		Yes	ABF4	Drought, salt, cold	<i>Arabidopsis thaliana</i>
		Yes	OsAREB1	Heat, drought	<i>Oryza sativa</i>
		Yes	Wlip19	Drought, cold	<i>Triticum aestivum</i>
		Yes	OsbZIP23	PEG, salt, drought	<i>Oryza sativa</i>
		Yes	GmbZIP44	Salt, drought	<i>Glycine max</i>
		Yes	GmbZIP62	Drought, cold, salt	<i>Glycine max</i>
		Yes	GmbZIP78	Salt, drought	<i>Glycine max</i>
		Yes	GmbZIP132	Salt, drought, cold	<i>Glycine max</i>
		Yes	OsABI5	Salt	<i>Oryza sativa</i>
MYB	MYB	Yes	MYB15	Salt, cold, drought	<i>Arabidopsis thaliana</i>
		–	OsMYB3R-2	Salt, cold, drought	<i>Oryza sativa</i>
		Yes	AtMYB2	Salt, drought	<i>Arabidopsis thaliana</i>
		Yes	AtMYB4	Ethylene, Jasmonic acid, Salt, SA	<i>Arabidopsis thaliana</i>
		Yes	AtMYB6	Ethylene, Jasmonic acid, salt, SA	<i>Arabidopsis thaliana</i>
		Yes	AtMYB7	Ethylene, Jasmonic acid, salt, SA	<i>Arabidopsis thaliana</i>
		Yes	AtMYB44	Ethylene, Jasmonic acid, salt, SA	<i>Arabidopsis thaliana</i>
		Yes	AtMYB73	Ethylene, Jasmonic acid, salt, SA	<i>Arabidopsis thaliana</i>
		No	GmMYB76	Salt	<i>Glycine max</i>
		No	GmMYB92	Salt, cold	<i>Glycine max</i>
No	GmMYB177	Salt, drought	<i>Glycine max</i>		
MYC	bHLH	Yes	AtMYC2	Salt, cold, drought	<i>Arabidopsis thaliana</i>
ZFP	Zinc finger binding domain	–	ZPT2-3	–	<i>Arabidopsis thaliana</i>
		–	Zat12	Oxidative, irradiation	<i>Arabidopsis thaliana</i>
		–	CaZF	Salt	<i>Nicotiana benthamiana</i>
		–	Alfin	Salt	<i>Medicago sativa</i>
		–	SCOF-1	Cold	<i>Nicotiana benthamiana</i>
		–	ZPT-2	Drought	<i>Arabidopsis thaliana</i>
		–	OSISAP1	Cold, salt, drought	<i>Nicotiana benthamiana</i>
		–	OSISAP2	Salt	<i>Nicotiana benthamiana</i>
		–	Zat7	Salt	<i>Arabidopsis thaliana</i>

(continued)

Table 1.1 (continued)

Gene family	Functional domain	ABA induced	Gene	Stress susceptible	Species investigated
NAC	NAC	-	ATAF1	Drought	<i>Arabidopsis thaliana</i>
		-	AtNAC2	Salt	<i>Arabidopsis thaliana</i>
		-	AtNAC19	Salt, drought	<i>Arabidopsis thaliana</i>
		-	AtNAC53	Salt, drought	<i>Arabidopsis thaliana</i>
		-	AtNAC72	Salt, drought	<i>Arabidopsis thaliana</i>
		-	SNAC1	Salt, drought, cold	<i>Oryza sativa</i>

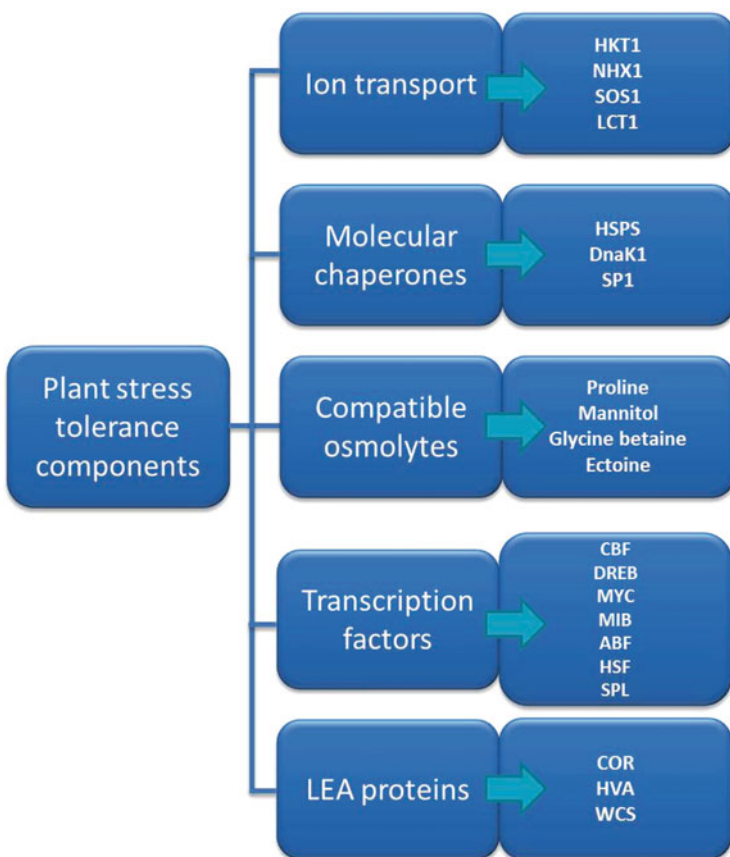


Fig. 1.6 Major cellular components of plant stress tolerance

cis-acting elements DREB and CBF genes, found to be involved in stresses signal transduction pathways (Ishitani et al. 1997; Knight et al. 1999; Lee et al. 2001), belong to a large DNA-binding proteins families containing a conserved EREBP/AP2 domain. Different members of these two families are able to regulate different processes such as stresses response, hormones response and plant development, functioning as trans-acting factors in separate signal transduction pathways (Riechmann and Meyerowitz 1998). A consensus nucleotide sequence, AGCCGCC, known as the GCC-box, has been identified in the promoter region of the pathogenesis-related genes (Ohme-Takagi and Shinshi 1995). DREB and CBF proteins also mediate the transcription of some genes such as *rd17*, *rd29*, *kin1*, *kin2*, *cor6.6*, *cor15*, and *erd10* involved in response to drought, salt and cold stresses (Gilmour et al. 1998; Liu et al. 1998; Thomashow et al. 2001). Overexpression of DREB and CBF genes in engineered plants have led to a significant increasing of stress tolerance (Zhuang et al. 2011). Recently, Morran et al. (2011) isolated in *T. aestivum* grains a new DREB family member classified TaDREB3 transcription factor and developed some transgenic populations of wheat and barley over-expressing both TaDREB2 and TaDREB3 factors. They observed that these transgenic plants showed improved survival under severe drought conditions relative to non-transgenic controls. In addition to drought tolerance, both TaDREB2 and TaDREB3 transgenic plants with constitutive over-expression of the transgene showed a significant improvement in frost tolerance. The increased expression of TaDREB2 and TaDREB3 lead to elevated expression in the transgenic of other CBF/DREB genes and a large number of stress responsive LEA/COR/DHN genes known to be responsible for the protection of cell from damage and desiccation under stresses (Morran et al. 2011). Therefore, this chain reaction in the regulation of expression of several genes involved in drought and salt stresses tolerance shown by DREB and CBF gene families confirms their leading role in determining the resistance of a plant to these stresses.

Several drought-inducible genes are activated by abscisic acid (ABA), confirming that the ABA signaling plays a crucial role in stress response. Two major transcription factors families are involved in ABA signaling: bZIP (a metal ion transport proteins) and MYB (Methionine Tyrosine DNA-Binding domain). Several ABA-inducible genes specifically bind the (C/T)ACGTGGC sequence cis-acting ABA-Responsive Elements (ABRE) in their promoter regions. Key transcriptional regulators of ABA-dependent gene expression are ABFs/AREBs (ABA-responsive Element Binding Factor/Protein), and basic region/leucine zipper (bZIP)-type transcriptional regulators with ABI5 as a typical representative (Finkelstein et al. 2005; Choi et al. 2005; Miura et al. 2009). OST1 and the related SnRK2.2/SnRK2D and SnRK2.3/SnRK2I directly target ABF/AREBs in the nucleus, and ABF2/AREB1 is phosphorylated *in vitro* by this class of ABA-activated protein kinases (Fujii et al. 2007, 2009; Yoshida et al. 2010). SnRK activation is promoted by ABA-mediated inactivation of the PP2Cs, which negatively regulates the protein kinases. Johnson et al. (2002) have deeply studied SnRK and bZIP interaction in wheat (*Triticum aestivum*). In *Arabidopsis* a constitutive expression of ABF3 and ABF4 leads to an increasing of drought tolerance altering the expression of ABA/stress-responsive genes *rd29*,

rab18, *ABI1* and *ABI2* (Kang et al. 2002; Ohta et al. 2003). ABF1 and ABF4/AREB2 are also phosphorylated by Ca²⁺-dependent protein kinases CPK4 and CPK11 (Zhu et al. 2007). Recently, other transcriptional regulators have been identified contributing to ABA-specific transcription. ABI3, among the B3 transcriptional regulators, binding to ABI5, enhances its action. ABI4, an AP2-type transcription factor, and a number of additional transcription factors including other MYC/MYB-type regulators act as positive ABA response regulators (Yamaguchi-Shinozaki and Shinozaki 2006). In addition, the homeo-domain leucine zipper AtHB6 interacting with ABI1 acts as a transcription factor suppressing ABA responses (Himmelbach et al. 2002). More ABA-associated phenotypes were identified in transgenic plants and among these, salt hypersensitivity was identified in ABF3- and ABF4-overexpressing plants during the germination and young seedling stages suggesting a potential involvement of ABF3 and ABF4 in salt tolerance during these developmental stages.

Thence, all these studies confirm the crucial role of transcription factors in stress response and tolerance. In this perspective, the engineering of TFs may contribute to overcome limitations in agricultural practice due to abiotic stresses. However, despite plant engineering with TFs notably increases the expression of downstream stress-associated multiple genes, at the same time can activate non-stress-associated genes that may affect the qualitative and quantitative characteristic that make commercially valuable a crop. Usually, a common negative effect of transgenic plants engineered for TFs consists in a growth retardation (Kang et al. 2002; Abe et al. 2003). Nevertheless, the deriving negative effects can be in part overcome using stress-inducible promoters able to control the expression of the TFs (Kasuga et al. 1999).

1.6 Functional Genomics Approaches to Improve Abiotic Stress Tolerance in Cereals

Currently, even if model species are currently used to transfer in crops the information useful to understand and manipulate stress tolerance due to the great level of abiotic tolerance, cereals are no less so. Information obtainable from *Triticeae* (in particular wheat and barley), are very poor due to the large size and scarce characterization of their genomes, nevertheless their level of abiotic tolerance, respect to the other crops, is considerable. The great diversity present in cereal landraces and wild gene pools represents a key resource to exploit in crop improvement. To date, the advances in genetics and genomics research have been made thanks to model species. The sequencing of the entire genome of *Arabidopsis* and rice have enhanced the capability and usefulness of these models. Mutational analysis and microarrays on *Arabidopsis* have led to the identification of numerous candidate genes involved in a series of stresses comprising drought, salt, freezing, and heat (Liu et al. 2008). The information obtained from these studies have been later successfully applied to improve crop plants. For instance, DREB genes, primarily identified in *Arabidopsis*

(Liu et al. 1998), have been then isolated in wheat, barley, rice and maize and used to improve tolerance to abiotic stress in these crop species (Kizis and Pagès 2002; Morran et al. 2011). Nevertheless, it must be considered that despite the resources offered by model species can result suitable to identify and transfer, engineering crop species, tolerance to biotic and abiotic stresses, the transfer cannot be necessarily successful. In fact, the plant response can be well diverse from the preliminary results when transferred in field, since plants in the field are exposed to multiple concurrent stresses. Another important aspect that must be considered in cereals is the high similarity present among the genomes of the different species, in particular wheat, barley and rye. These relations comprise gene similarity but also a conservation in the order and the positions along the different chromosomes of the different genes as confirmed by clear relationships observed across the grass genomes. This characteristic, called synteny, makes even possible to replace chromosome regions of a particular specie with the same orthologous region in another specie (Moore et al. 1995; Dunford et al. 1995; Foote et al. 1997; Devos 2003). Nevertheless, the presence of syntenous regions depends by the age of evolutionary origin, thence the phylogenetic history of these genomic regions must be considered. In this perspective, a recent origin of a region is often characterized by a not-colinearity among different related species. However, this problem does not seem to concern genes involved in disease tolerance that has demonstrated a good synteny.

1.7 Map-Based Cloning

A wide range of traits and candidate genes for abiotic stresses tolerance have been identified and characterized in grasses. With the advent of molecular markers and through the Marker Assisted Selection (MAS), the attention of the researchers has been focused on mapping studies. To date, several studies have been made in cereals to identify genetic variation and to develop new markers. The starting material comprised wild relatives, landraces and cultivated lines principally of wheat and barley where a great genetic diversity as a source of new variants useful for breeding activity, has been found. The variability found in the genetic traits related with abiotic stress tolerance has permitted to identify and mapping several candidate genes and has confirmed the importance of wild relatives to identify the traits that domestication has canceled in the selected lines. The recent knowledge on candidate genes organization has led to the identification of promising allelic variants that, through the MAS, can be easily transferred into the susceptible commercial lines. Thanks to development of numerous segregating populations such as, Recombinant Inbred Lines (RILs), Near Isogenic Lines (NILs), Double Haploid Lines, F₂ and BC₁ it has been possible to map the positions along the chromosomes among the different genomes of the traits of interest. Several genetic linkage maps have been developed (Chao et al. 1989; Devos et al. 1993; Devos and Gale 1997, Röder et al. 1998; Pestsova et al. 2000; Nachit et al. 2001; Gupta et al. 2002) and in particular for drought tolerance an extensive mapping in different species of cereals has been

made. Thence, the advent and development of molecular markers in quantitative genetics have greatly facilitated the study of complex quantitatively inherited traits by the construction of high density genome linkage maps for crops such as wheat (Xiao et al. 1996). This implies that the use of molecular markers and interval mapping is a powerful approach, that permits the identification and genetic mapping of loci controlling complex traits like grain yield and its contributing traits having great importance in plant breeding. The role of genetic engineering in the development of improved cultivars is still limited considering the unpredictable effects of ectopic gene expression on yield and other important agronomic traits due to the complexity of the morpho-physiological mechanisms occurring during the adaptive process to abiotic stress. Recently, through the advent of new generation genomic approaches, a better understanding of this complexity is still possible and consequently the possibility to directly manipulate the genome resources to building up the desired phenotype is coming close. Considering that the genetic traits involved in tolerance to abiotic stress are mainly quantitative (Blum 1988; Richards 1996), the identification of Quantitative Trait Loci (QTLs) ruling the genetic variability of the traits controlling such tolerance and the consequent manipulation to use in MAS is of crucial importance. The knowledge of the number and effects of QTLs can help breeders to understand the genetic control of these traits and to design more efficient selection strategies for improvement (Broman and Speed 1999).

1.8 Quantitative Trait Loci for Tolerance to Abiotic Stress in Cereals

1.8.1 Quantitative Trait Loci for Drought Tolerance

Components of drought tolerance have been mapped in almost all major cereals and in particular for wheat, several validated QTL have been localized on chromosomes 2, 4, 5 and 7. Among these, several QTL have been identified through the evaluation of yield and yield component measurements under water-limited conditions (Quarrie et al. 2006; Maccaferri et al. 2008; Mathews et al. 2008; von Korff et al. 2008; McIntyre et al. 2009). Yield represents the most important trait to breeders and up to now many studies have focused on it. Nevertheless, it is very hard to discern accurately with respect to water use and consequently identify candidate regions and genes. In fact only few studies have identified QTL associated with specific components of drought response (Table 1.2, Fig. 1.7).

Despite the advances made in building molecular markers specific for genes of interest and the development of several high saturation genetic maps, the genomic portions associated with individual QTL are still too much extended and so incompatible for screening in a breeding program. Although reproductive organs and roots play a key role in drought tolerance, genetic studies concerning their structure and function are very poor or even lacking. Nevertheless, it is known the important effect of drought on reproductive processes (Barnabas et al. 2008). Even if it has

Table 1.2 Mapping position of the more recent QTLs controlling tolerance to different abiotic stresses

Type of stress	Chromosome location
Barley	
Drought	Almost 73 QTLs
Cold	–
Frost at anthesis	2H, 5H
Seedling desiccation at germination	7 QTLs
Manganese efficiency	4HS
Boron tolerance	2H, 3H, 4H, 6H
Wheat	
Drought, low temperatures, salinity	5 and 7 groups
Drought, salinity, nutrient deficiency	18 QTLs
Cold tolerance	5A
Sprouting resistance	5AL, 6A, 3B, 7B
Boron tolerance	7B, 7D
Preharvest sprouting	6B, 7D
Cold and vernalization	5A, 5D
Aluminium tolerance	4DL
Water deficiency	5A, 5B, 5D
Flag leaf senescence	2B, 2D
Drought, Agropyrum	3E, 5E, 7E
Na/K discrimination	4D
Flooding tolerance	15QTL

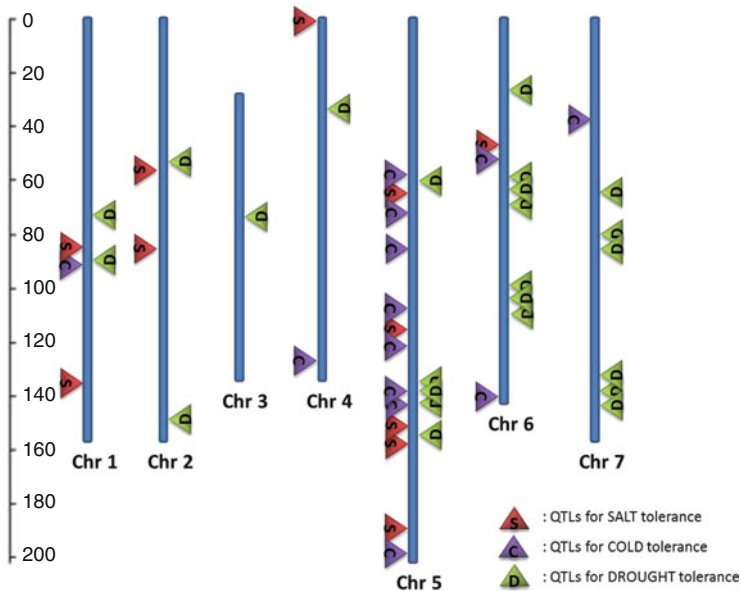


Fig. 1.7 Abiotic tolerance QTLs and major genes mapped on a composite *Triticeae* chromosome map

been confirmed that the floral infertility due to water scarcity, could be a promising target for improvement (Passioura 2007), no QTL studies for this trait have been published in wheat or in other cereals.

The identification of candidate genes and the development of markers associated with root growth and architecture could be very useful to improve root traits through MAS. In this perspective, the engineering of root system capability to extract water from the soil also seems to be an important goal for genetic analysis. In fact Manschadi et al. (2006) have confirmed, through the modification of some root traits that an increase of 10 mm of water up taken during grain filling would increase yield by 500 kg ha⁻¹, representing a 25% increase of the average wheat yield in Australia (2,000 kg·ha⁻¹). Study of QTLs for root architecture and related traits have received great interest (Lafitte et al. 2002). A rather large set of QTL data for root traits has been obtained from the evaluation of different rice mapping populations. Due to the difficulty of properly scoring root traits, MAS could be conveniently deployed for tailoring root architecture (Shen et al. 2001). Champoux et al. (1995) have investigated the connection between QTLs associated with drought tolerance and QTLs associated with root morphology. This study identified 14 chromosomal regions involved in field drought avoidance/tolerance and 12 of these QTL regions influenced also root morphology. Lilley et al. (1996) have investigated for the presence of QTLs associated with osmotic adjustment and dehydration tolerance. One major QTL for osmotic adjustment and two of the five QTLs influencing dehydration tolerance were found to partially overlap with QTLs for root morphology, thus indicating a genetic association among these traits. Both osmotic adjustment and dehydration tolerance were negatively correlated with root morphological characters associated with drought avoidance.

In maize, as well as in other cereals, the most critical stage in terms of deleterious effects of drought on yield is just prior to and during flowering (Westgate and Boyer 1985; Jones and Setter 2000; Saini and Westgate 2000). Drought at flowering delays the extrusion of silks and increases the interval between pollen shed and silking (i.e. anthesis-silking interval: ASI) as indicated by the negative association of ASI and grain yield under drought (Bolaños and Edmeades 1993, 1997; Agrama and Moussa 1996; Chapman and Edmeades 1999). Among the traits that can influence tolerance to drought in maize, extensive work has been carried out to identify QTLs for the concentration of ABA in the leaf (L-ABA) and their associated effects on other drought-related traits and yield (Lebreton et al. 1995; Tuberosa et al. 1998; Sanguineti et al. 1999). In barley, Ellis et al. (2002) observed that carbon discrimination ($\delta^{13}\text{C}$) of the shoot tissue is more heritable than other seedling traits similarly to what was previously reported in wheat (Ehdaie and Waines 1994). A RIL population has been extensively investigated by Teulat et al. (1997, 1998, 2001, 2002, 2003) to identify QTLs for drought-related traits, including $\delta^{13}\text{C}$, grain yield and its components. Even if the authors considered six Mediterranean environments characterized by a large heterogeneity in water availability, 24 QTLs were consistently detected in the different environments, particularly for plant height and kernel weight. Talamè et al. (2004) investigated a population deriving by a cross between *H. vulgare* x *H. spontaneum* under rainfed conditions in three Mediterranean

countries in order to identify agronomically favorable alleles, in QTL region, derived by the wild parental line. From this study, the 81 putative QTLs were found to influence growth habit traits, heading date, plant height, ear length, ear extrusion, grain yield and/or 1,000-grain weight, and it was observed that in 43 cases (53 %) the wild parental line contributed with alleles carrying favorable effects. Nevertheless, despite the majority (65 %) of favourable alleles belonged to *H. vulgaris*, at six QTLs the alleles increasing grain yield were carried by *H. spontaneum*. Quarrie et al. (2005) have extensively investigated for the presence of QTLs influencing water use efficiency and yield in bread wheat. QTLs on chromosomes 2A (near Xpsr375.1), 2B (near Xcbd453) and 4D (near Xpsr575.1) were identified (Quarrie et al. 2005). Consequently, in a subsequent study, a number of significant QTLs for yield traits were found under both drought and irrigated conditions. QTLs for grain yield coincident with water treatments were identified on chromosomes 1A and 7A (Quarrie et al. 2006). Other yield QTLs were unique to the rainfed or irrigated treatment confirming that the QTLs controlling the variability in grain yield under severe drought stress conditions differed from those acting under irrigated conditions.

1.8.2 Genetics of Drought Tolerance in Low-Yielding Environments

The empirical selection for yield under water-limited condition has been widely and successfully used in the traditional improving of wheat drought tolerance. The modern commercial cultivars are the result of this activity, but nowadays it has become insufficient (Tester and Langridge 2010). At the present time this activity has been replaced by the establishment of physiological ideotypes followed by the identification of the resources where find the variation for these traits and the transferring of the selected traits into elite varieties (Reynolds et al. 2009; Richards et al. 2010). Lastly, the final approach consists in MAS activity for desirable alleles identified at QTLs specific for drought tolerance.

1.8.3 Quantitative Trait Loci for Salt Tolerance

The main QTLs for salt tolerance in cereals have been identified in barley and rice. In barley, different genes influencing different degrees of salt tolerance have been identified (Richards et al. 1987; Slavich et al. 1990). In particular, genes controlling Na⁺ influx and concentration in cells can be distinguished (Schachtman and Liu 1999). In parallel, two typologies of QTLs have been identified controlling salt tolerance at germination and seedling stages (Mano and Takeda 1997), while QTLs for salt tolerance at the seedling stage were located on chromosomes 2H, 1H, 5H, and 6H. These sites differed from those of QTLs controlling salt tolerance at germination, suggesting that salt tolerance at germination and at the seedling stage

was controlled by different loci. In rice, salt tolerance seems to be controlled by two different kind of QTLs: QTLs for the total quantity of ions in a shoot and QTLs affecting ion concentration in the shoot overlapping the QTLs for vegetative growth (i.e. vigor) (Koyama et al. 2001). Concerning QTLs involved in ion uptake, it has been found that these QTLs are able to control independently Na^+ uptake, K^+ uptake and Na^+/K^+ selectivity. The major QTLs for Na^+ and K^+ uptake have been mapped on different chromosomes in accord with the different uptake pathways for these ions (Fig. 1.7). On this base, QTLs for Na^+ transport are more likely to act through the control of root development, whereas QTLs affecting K^+ uptake are likely to act through the structure or regulation of membrane-sited transport components (Koyama et al. 2001).

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Chapter 2

Precision Nitrogen Management for Sustainable Corn Production

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Abstract Nitrogen (N) management in corn (*Zea mays* L.) production is a challenge for economic, agronomic and environmental reasons. Recovery of N by crops grown under most cropping systems is low. The inefficient use of N fertilizer represents approximately \$680 million to \$1 billion of direct economic losses annually for Canadian farmers alone. In humid environments such as northeast USA and eastern Canada, corn yield response to N amendments is poorly correlated with soil mineral N content before planting because of great spatial and temporal variability. Therefore it is important to develop new technologies for precision N management. In this chapter we have reviewed the major achievements in N dynamics in a soil-crop-atmosphere continuum, with emphasis on technologies developed for precision N management for sustainable corn production.

Adoption of improved N management practices in corn production can increase both grain yield and N use efficiency (NUE) as well as minimizing N loading of the environment. Corn growth and development is driven by heat, which can be expressed as growing degree days or crop heat units. It takes about 6 weeks for a corn crop to reach quick growth stage. Excess N in the soil after preplant fertilizer application is subjected to leaching, run-off or emission losses, as N uptake during this part of the corn crop life cycle accounts for less than 15 % of plant total N. Up to date research indicates that it is of critical importance to understand the physiological mechanisms of crop NUE. This includes N uptake, translocation, remobilization and its relationship with grain yield which will maximize productivity and profitability as well as minimize N loading of the environment. An optimization between grain yield and NUE through precision N management is physiologically possible and practically achievable for sustainable corn production.

Keywords Corn or maize • Nitrogen fertilizer • N loss • Precision N management • Variable rate application • Nitrogen use efficiency

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Abbreviations

CHU	Crop heat units
NRF	Grain N removal factor
EORN	Economically optimum rate of nitrogen
FIE	Fertilizer-induced emission
GDD	Growing degree days
GTI	Generalized thermal units
LAI	Leaf area index
MRMR	Minnesota relative maturity rating (days)
NDVI	Normalized difference vegetation index
NHI	Nitrogen harvest index
NIE	Nitrogen internal efficiency
NRE	Nitrogen recovery efficiency
NUE	Nitrogen use efficiency
SI	Saturation index
TAN	Total ammoniacal nitrogen
VRA	Variable rate application

2.1 Introduction

Maize or corn (*Zea mays* L.) is a member of the grass (Poaceae) family and is generally considered to have originated from a grassy weed found in Central and South America. As a C4 tropical grass, efficient at converting sunlight into carbohydrates, corn is grown primarily for grain, and secondarily for fodder and raw material for industrial processes. Corn grain is used for both human and animal consumption. In the recent decade, corn grain has been used increasingly as a feedstock for biofuel production and, more recently, for cellulosic conversion of corn straw into ethanol has been considered being one of renewable energy sources.

2.2 Importance of Corn Production in Canada and Worldwide

Corn is the third most important grain cereal crop in the world after wheat and rice. In the early 1990s, more than 50 % of the total world area planted with corn was in Latin America, Africa and Asia, but production in these regions accounts for less than 35 % of the total world grain corn production (Russell 1991). Corn is the most widely grown grain crop in the Americas, with estimated 37 million hectares of

planted area and 382 million metric tonnes of grain yields produced in the United States alone (USDA 2014). Approximately 40 % of the crop (i.e., 130 million metric tonnes) has recently been used for corn ethanol. According to the Food and Agriculture Organization of the United Nations, 885.3 million metric tonnes of corn grain was produced across the globe in 2011 (FAO 2014).

Corn production in Canada ranks the third largest grain cereal after wheat and barley. Annually, Canada produces more than 8 million metric tonnes of grain corn with a farmgate value of over \$2.8 billion from approximately 1.2 million hectares (Statistics Canada 2014). Although field corn is grown in every province, approximately 96 % of the crop is grown in the provinces of Ontario and Quebec. Field corn produced in Canada is harvested as grain (80 %) or silage (20 %). Most corn grown in Canada is used for animal feed, and only about 1.5 % is grown as sweet corn, the kind we find at the market as corn-on-the-cob or canned kernel corn (Ma et al. 2007). The remainder is processed into a wide variety of ingredients such as corn fructose or starch, as well as for fuel and alcohol production. Subsidies to the biofuel industry, along with a public perception that corn ethanol could be a factor in reducing greenhouse gas emissions has provided a dramatic boost to the corn industry in the recent decade. Since its introduction to the fields, transgenic corn or genetically modified corn that is resistant to certain pests, such as Bt corn resistance to European corn borer and corn rootworm, and herbicides, e.g. Roundup Ready® corn resistant to glyphosate, has now accounted for more than 85 % of the corn planted in the United States (James 2009; Zhu and Ma 2011).

2.3 Physiological Adaptation of Corn

Corn is essentially a crop of warm regions with adequate rainfall or grown under irrigation. The crop grows from 50 °N to 40 °S and from sea level to 4,000 m in the Andes and Mexico. The majority of the crop is grown in the warmer parts of the temperate regions and in humid subtropics. The optimum rainfall for corn ranges between 460 and 600 mm in the temperate regions and between 600 and 900 mm in the tropics. Corn is more efficient in water use than other C3 cereals and legume crops such as soybean. However, corn is very sensitive to drought stress from tasseling to approximately 2 weeks after silking (Otegui et al. 1995). Corn could germinate in cool soils (Dwyer et al. 2005), but the optimum soil temperature for seed germination and early seedling growth is between 26 and 30 °C. The most favorable temperature at tasseling is 21–30 °C. High temperature normally increases the rate of grain filling, but greatly reduces the duration of grain-filling, whereas a low temperature causes an inverse response (Jones et al. 1981). It takes several days for the crop to recover and return to its normal photosynthetic capacity (Ying et al. 2000, 2002) after a cool night (<10 °C) during the grain filling period (Dwyer et al. 1995b). Therefore, corn yield is lower at high temperatures compared to low temperatures (Tollenaar and Lee 2002).

2.4 Corn Maturity and Phenology

Estimation of corn development rate is needed to market hybrids of specific maturity, to zone production areas and to schedule stage-sensitive operations such as herbicide spraying (Dwyer et al. 1999a). Best management practices in corn production therefore rely on an accurate rating of hybrid maturity. Rating of hybrid corn maturity and zonation of production areas in North America employ several systems, including Growing Degree Days (GDD; Wang 1960), Crop Heat Units (CHU; Brown and Bootsma 1993), and Minnesota Relative Maturity Rating (MRMR; Peterson and Hicks 1973; Dwyer et al. 1999b). In Canada, Crop Heat Units are commonly used for rating corn and soybean maturity while in the United States, corn producers and agronomists are more familiar with the GDD maturity rating. Regression equations are now available for exchanging these maturity expression systems (Table 2.1).

There are several approaches to describe the phenology of corn growth and development. The Iowa scaling of corn development uses the leaf ligule to rate the vegetative stage. For example, V1 stands for the first fully expanded true leaf with a clear ligule, and V11 is the stage where the 11th leaf is fully expanded with a clear ligule. And the R stages designate the silking and grain filling period, e.g. R1 – silking; R2 – kernel blister stage, R6 – physiological maturity (Ritchie et al. 1993). The BBCH method, using a decimal code for the description of the growth stages of cereals including corn (Lancashire et al. 1991), is based on the well-known Zadoks code for cereals (Zadoks et al. 1974) and is now getting popular.

Table 2.1 Fitted coefficients and their standard errors (in brackets) from simple linear regression ($y = a + bx$) and coefficients of determination (r^2) for each pairing of systems including (A) in which independent variable (MRMR) is set for each hybrid and has zero variance and (B) in which independent variable has associated variance; $n = 28$ for all comparisons

	x	y	a	b	r^2
A.	MRMD	GDD (°C)	-12.34 (75.78)	13.21 (0.810)	0.91
	MRMD	CHU (°C)	776.1 (104.7)	23.00 (1.12)	0.94
	MRMD	GTI (°C)	276.4 (55.49)	10.36 (0.59)	0.92
B.	GDD (°C)	MRMD	0.934 (5.680)	0.076 (0.005)	0.91
	CHU (°C)	MRMD	-33.74 (6.183)	0.043 (0.002)	0.94
	GTI (°C)	MRMD (°C)	-26.67 (6.873)	0.097 (0.006)	0.92
	GDD (°C)	CHU (°C)	768.7 (39.14)	1.765 (0.032)	0.99
	CHU (°C)	GDD (°C)	-435.6 (30.03)	0.567 (0.010)	0.99
	GDD (°C)	GTI (°C)	280.5 (16.24)	0.790 (0.013)	0.99
	GTI (°C)	GDD (°C)	-355.6 (26.53)	1.268 (0.021)	0.99
	CHU (°C)	GTI (°C)	-63.8 (19.02)	0.447 (0.007)	0.99
	GTI (°C)	CHU (°C)	142.7 (40.46)	2.236 (0.033)	0.99

Adapted from Dwyer et al. (1999b)

Abbreviations: MRMR Minnesota relative maturity rating (days), GDD cumulative growing degree days, CHU cumulative crop heat units, GTI generalized thermal units

For the spring-seeded crop in northeastern USA and Canada, corn growth can be divided into three phases. It takes approximately 6 weeks from emergence to the 6th fully expanded leaf stage (V6). During this period, the soil is characterized with rising temperatures and the corn crop takes up only a small portion of its total N, about 10–15 %. From the V6 to the tasselling-silking period, it takes approximately another 6 weeks, but 70 % or more of its total N is taken up during this phase. Crop N uptake continues during the final third of its life cycle, i.e. from silking to physiological maturity, but this accounts only for approximately 15 % of plant total N (Ma and Dwyer 1998).

2.5 Genetic Improvement of Corn

According to Tollenaar and Wu (1999), corn genetic improvement has led to enhanced tolerance to abiotic stresses, such as tolerance to crowding and reduced barrenness. This leads to a 40 % or more increase in plant population density during the past four to six decades, and larger increases in grain yields when the crop encounters weedy, moisture, or nutrient stresses. Genetic improvement has also led to greater tolerance to cool spring soil temperatures, thereby allowing earlier planting by nearly 2 weeks (Table 2.2). Current hybrids have been developed to recover more quickly from a cool spell encountered during the grain filling, resulting in a longer effective grain-filling period than older hybrids (Dwyer et al. 1995b; Tollenaar and Wu 1999).

As corn crop growth rate depends on the amount of intercepted photosynthetically active radiation, the leaf area per unit ground area, i.e. leaf area index (LAI) plays an important role in dry matter production. Optimum population density is thus the basis of sustainable production (Giunta et al. 2009) since dry matter production is a linear function of the cumulative absorption of solar radiation (Monteith 1977; Sinclair and Muchow 1999; Akmal and Janssens 2004). Analysis of historical corn hybrids under both normal and stressful conditions reveals that corn yield improvement is the outcome of integration of genotype improvement by agronomic management (Tollenaar and Lee 2002). The major agronomic innovations for corn production in Canada can be simplified in Table 2.2.

The use of transgenic corn hybrids is a new technology adopted by many countries in recent decades. New genetically modified hybrids, such as Bt corn, are tolerant to

Table 2.2 A simplified summary of some major agronomic innovations for corn production in Ontario, Canada

Year	1930	1990	2010
N fertilizer (kg ha ⁻¹)	30	150	150
Population density (plants m ⁻²)	3.0	6.5–7.0	7.5–8.0
Planting date	25-May	15-May	05-May
Yield (Mg ha ⁻¹)	1.0–3.0	7.5–8.0	9.5–10.0

larva from both the first and second generation of European corn borer and are thereby not susceptible to stalk lodging. Roundup Ready® corn is resistant to glyphosate herbicides so that the application of wide spectrum herbicides is now possible up to the V4–V5 growth stages of corn development. Transgenic corn hybrids are gaining the interest of growers and seed companies because of their favourable yield response to increasing plant population (Seydou et al. 2000; Stanger and Lauer 2006), effective control of European corn borer and corn rootworm damages (Ma and Subedi 2005; Subedi and Ma 2007), reduced dependence on multiple field operations for weed and pest control, and consequent suitability for growing under reduced and no-till conditions (Zhu and Ma 2011). The yield of Bt hybrids is 10 % higher compared to isoline hybrids under the European corn borer infestation (Lauer and Wedberg 1999). Bt hybrids also have higher test weight and less aflatoxin than non-Bt hybrids (Wiatrak et al. 2005). In a recent study, it is estimated over the past 14 years, that Bt corn has a cumulative benefits of \$3.2 billion for corn growers in the United States, and because of area-wide suppression of European corn borer, there is also an additional \$1.9 billion of benefits to non-Bt corn growers. European corn borer population has now declined in every major production states due to the adoption of Bt corn hybrids (Hutchison et al. 2010).

In Canada, under natural infestation conditions, Bt hybrids against European corn borer showed no yield advantages over non-Bt counterparts (Ma and Subedi 2005; Yanni et al. 2011), but corn rootworm Bt hybrids out-yielded its non-Bt near-isoline hybrid or non-Bt hybrid treated with insecticides (Ma et al. 2009).

2.6 Nitrogen Cycle

2.6.1 *Forms of Soil Nitrogen and Nitrogen Cycle*

The majority of Earth's atmosphere (approximately 78 %) is N, making it the largest pool. Despite its abundance in the atmosphere, N is often the most limiting nutrient for plant growth, and is by far the greatest on-farm expense of the three primary nutrients – N, P, K for corn production. Total N of the soil takes into account all the N sources found in the soil: both inorganic and organic. Plants are only able to take up inorganic N that is in the form of ammonium (NH_4^+) and nitrates (NO_3^-) in the soil, via their roots. However, over 90 % of the soil N is in organic forms (Keeney and Nelson 1982), and the majority of the ammonium is trapped in clay (Foth 1977). Only about 2 % of soil N is readily available for plant uptake, such as NO_3^- and exchangeable NH_4^+ . Nitrates in the soil, are added either through fertilization or by mineralization of soil organic N. Nitrate is the main form of soil mineral N, usually eight to ten times more than NH_4^+ (Ma and Dwyer 1999), but can be readily leached out of the root zone through surface runoff or washed away by soil water (Foth 1977). Therefore, maintaining the amount of soil N needed for the plants is done by the natural processes of mineralization and complimented by the addition of chemical fertilizers and farm manures.

The N cycle (Fig. 2.1) represents one of the most important nutrient cycles found in terrestrial ecosystems. This cycle is the process by which N is converted between its various chemical forms. This transformation can be carried out through both biological and physical processes. Important processes in the N cycle include fixation, mineralization, nitrification, plant uptake and grain removal, denitrification, nitrate leaching and gaseous emissions.

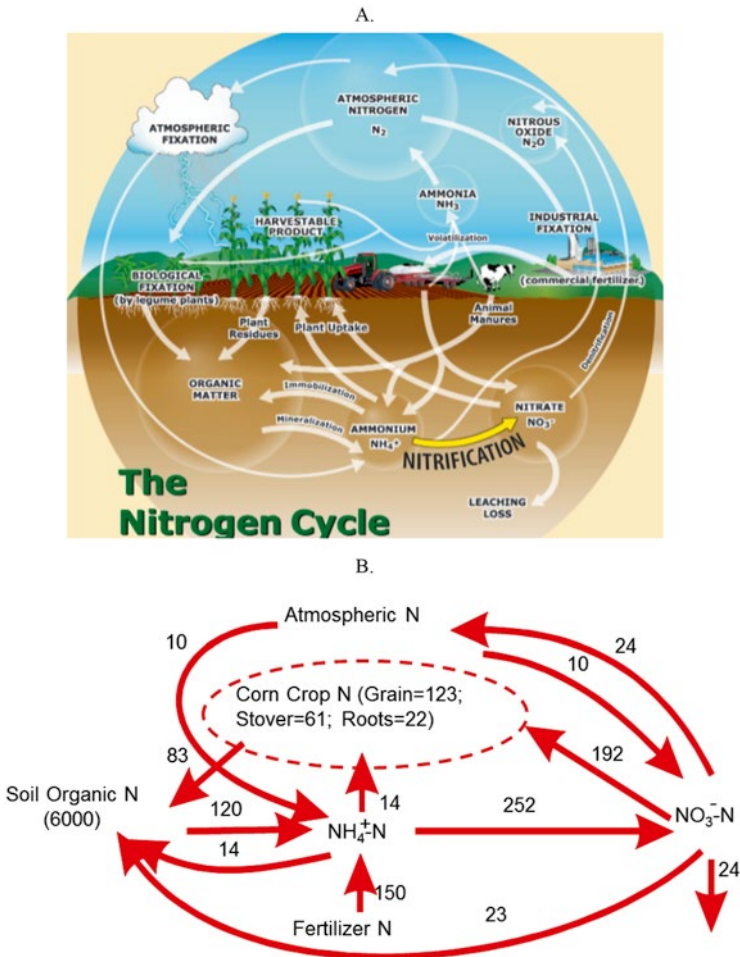


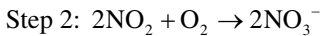
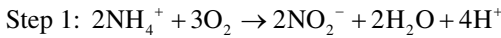
Fig. 2.1 (a) The N cycle, adapted from the International Plant Nutrition Institute (IPNI 2014), and (b) A simulated N cycle in a corn-soil-atmosphere ecosystem in Ontario, Canada. The majority of the soil N is in the organic form. During the corn growing season, about 120 $kg\ N\ ha^{-1}$ was released from soil N mineralization (Ma et al. 1999a, b; Wu et al. 2008) and farmers typically apply 150–250 $kg\ ha^{-1}$ of N fertilizer. This inorganic form of N will be cycled through an open system and suffers from leaching, volatilization, emission and fixation besides removal with the harvest grain out of the system

2.6.2 Soil Nitrogen Mineralization

Mineralization is the process where soil microorganisms transform organic N to inorganic N ($\text{NH}_4^+/\text{NH}_3$) (Foth 1977). NH_4^+ is only a transitory product since it is quickly oxidized into nitrites and then nitrates through nitrification process by other forms of bacteria found in the soil.



Mineralization occurs in two steps, where specific microorganisms found in the soil perform each step (Foth 1977).



Therefore, nitrate is the main inorganic N source for plants whose roots take N up by mass flow (Foth 1977).

2.6.3 Ammonia Volatilization

Ammonia volatilization represents a main form of N loss through an agroecosystem. Ammonia volatilization is a physical process which is mainly influenced by the concentration of soil total ammoniacal N (TAN; $\text{NH}_3\text{-N}$ plus $\text{NH}_4^+\text{-N}$) and by the resistance to NH_3 movement from the soil matrix (Sommer et al. 2004). Several factors regulate the concentration of TAN, such as soil temperature (Fisher and Parks 1958; Fenn and Kissel 1974), soil moisture (Vlek and Carter 1983; Clay et al. 1990), soluble C (Chantigny et al. 2004), soil pH (Fenn and Hossner 1985), and the amount of N fertilization (Ma et al. 2010b). These factors affect the dynamics of soil TAN concentration, and its subsequent depletion through nitrification (Malhi and McGill 1982; Sommer et al. 2004). Soil resistance to NH_3 volatilization is associated with rainfall events, soil disturbance and compaction, and fertilizer application methods, especially placement depth (Sommer et al. 2004). The NH_3 -volatilization rate in the field is the highest at 2–7 days after urea application (Clay et al. 1990; Ma et al. 2010a). Cumulative NH_3 losses could be up to 48 % of surface-applied urea N (Pacholski et al. 2006). In eastern Canada, NH_3 volatilization induced by N fertilizer in corn fields through soil-atmosphere interface ranged from 0.1 to 47 kg N ha⁻¹ after preplant N application and from 0.6 to 20 kg N ha⁻¹ after sidedress at the V6–V8 growth stage, respectively (Ma et al. 2010a). Under the optimum rate of fertilizer N and using the right application method, it is possible to limit the NH_3 volatilization to within a small range of 0–0.5 % of N fertilizer.

2.6.4 Nitrogen Leaching

Excessive rates and inappropriate methods of application or inefficient use of N fertilizer may have adverse effects on ground water through leaching of soil NO_3^- -N. High NO_3^- levels in ground water can cause adverse health concerns, especially for infants. It can also cause excess plant and bacterial growth, which upon death and decay can deplete much of the oxygen in river and lake water. A considerable amount of research has been conducted on NO_3^- -N leaching and redistribution in soil. De Jong et al. (2007) estimated that N loss via leaching and concentration of NO_3^- -N in the leached water in Canada ranged from 5.1 kg N ha⁻¹ in 1991 to 6.4 kg N ha⁻¹ in 2001. The actual concentration of NO_3^- -N in the ground water is affected by the amount of fertilizers applied for the previous crop production, soil texture, seasonal rainfall (percolation), tillage operations and crop rotations. Elevated post-harvest soil NO_3^- -N usually provides evidence that N was applied in excess for corn uptake (Ferguson et al. 1991; Andraski et al. 2000; Ma et al. 2010b). Nitrate remaining in the post-harvest soil profile represents a potential risk for leaching after crop maturity and has been shown to be closely related to N fertilization rate, seasonal precipitation and soil texture (Gehl et al. 2006).

There are different ways which can minimize NO_3^- leaching in the ground water. As N is lost from cropping systems via a number of pathways, a single solution of N management is unlikely (Binder et al. 2000). Precise matching of application rates with crop needs could reduce residual soil NO_3^- -N available for leaching (Bausch and Duke 1996; Vyn et al. 1999; Andraski and Bundy 2002; Ma et al. 2010b; 2014; Rambo et al. 2010). In a multi-site-multi-year study in Ontario and Quebec, it was found that NO_3^- -N leaching occurred once the crop reached physiological maturity or after corn harvest, and downward movement of soil NO_3^- -N was influenced by the amount and timing of fertilizer N application to corn, and by the precipitation during the corn growing season (Ma et al. 2010b).

2.6.5 Nitrous Oxide Emissions

Atmospheric N_2O is one of the major greenhouse gases (GHG), responsible for the rapid change in global climate. Its concentration has increased from about 273 ppb in 1750 to 319 ppb in 2005, a concentration increase of 0.2 ppb year⁻¹, and more recently, at the unprecedented increase of 0.8 ppb year⁻¹ (IPCC 2007). Nitrogen fertilization is one of the main sources of anthropogenic N_2O emission to the atmosphere (Cole et al. 1997).

Assessment of the sustainability of a crop production system should consider both the economic crop yield and the environmental impact. Robertson et al. (2000) suggest that soil mineral N availability, rather than N fertilization *per se*, regulates the N_2O flux from agricultural soils. Therefore, mineralization, fertilization, and

crop uptake of N collectively determine the overall N_2O emission. When fertilizer is applied to the soil, ammonium-N is fairly rapidly converted to nitrate-N through nitrification process under favourable conditions. In contrary, nitrate-N can be reduced through denitrification to various forms of gaseous N and emitted to the atmosphere (Fig. 2.2). Different groups of soil microorganisms contribute to the production and consumption of N_2O , but biological denitrification is the dominant process for N_2O and NO emissions. On average, fertilizer-induced emission (FIE) factor in Canada is estimated to be 1.18 % (Helgason et al. 2005) or 1.19 % (Gregorich et al. 2005), but this can vary widely due to differences in cropping system, soil type, climate, and fertilizer management (Ma et al. 2010b). The large variation in FIE highlights the difficulty in estimating N_2O emissions and the potential opportunity for mitigation through appropriate N management practices (Adviento-Borbe et al. 2007). A new concept of specific emissions or crop yield-based emissions, expressed as kg N_2O -N/kg grain/kg N fertilizer, is therefore

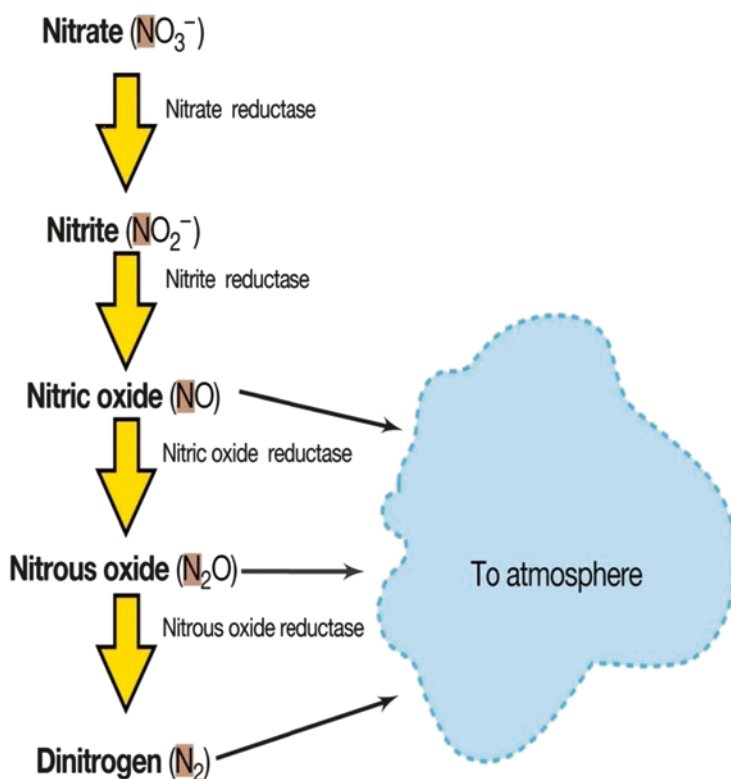


Fig. 2.2 Diagram of soil N_2O emissions through denitrification processes. This biological reduction of NO_3^- and NO_2^- to gaseous N_2 is a four step process: (1) Reduction of nitrate to nitrite, (2) Reduction of nitrite to nitric oxide, (3) Reduction of nitric oxide to nitrous oxide, and (4) Reduction of nitrous oxide to dinitrogen gas

created to link the quantities of N₂O emissions with corn yield (Ma et al. 2010b). By extrapolating this concept and analyzing a long-term experimental data, the total GHG emissions and the C footprints of leguminous-based rotational vs. continuous corn production systems have been modeled. The experimental data has clearly indicated that it is possible to maintain high productivity while reducing GHG emissions and the C footprints with a moderate N fertilizer rate to corn in rotation with forage or grain legumes (Ma et al. 2012).

2.6.6 Nitrogen Removal

Information on N removal by grain corn production is not only required for proper soil N management, but also a prerequisite for environmental risk assessment (Ma et al. 2006). In general, with moderate fertilizer N rate (100 kg N ha⁻¹), grain N removal is a factor of 12.0 kg Mg⁻¹, i.e. 12.0 kg N is removed with each Mg grain yield harvested. The independence of grain N removal factor (NRF) on corn maturity indicates that it is possible to select hybrids with large values of grain NRF for specific growing regions. In a multi-site-year study, large variations in the NRF values of 7.0–16.7 kg Mg⁻¹ were observed among years, N treatments, rotation systems, and hybrids (Fig. 2.3). This indicates that selection of hybrids with greater grain NRF values may also benefit the environment by utilizing applied N fertilizer more efficiently. Caution must be taken when using NRF to assess impact of corn

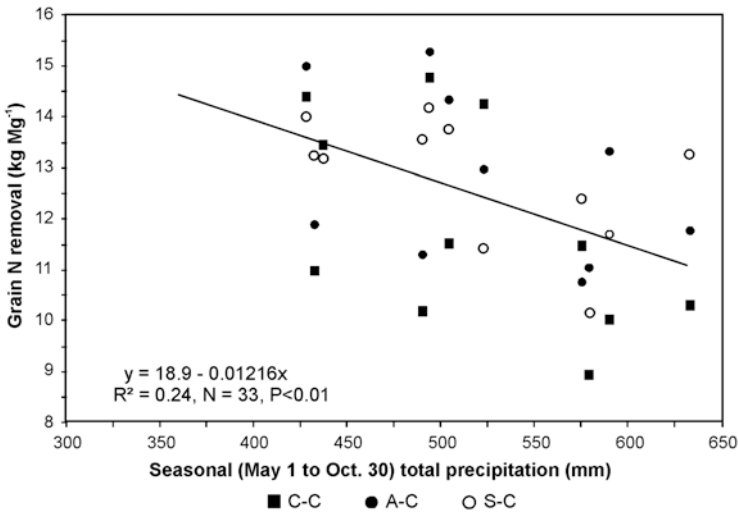


Fig. 2.3 Soil N amendments on the grain N removal factor as affected by growing season total precipitation in three cropping systems: C-C, continuous corn monoculture; S-C, corn and soybean in a biannual rotation; A-C, corn and alfalfa in a biannual rotation (Adapted from Ma et al (2006))

production on the environment, as a generalized NRF index cannot be representative for diverse growing conditions, input level and hybrid types (Ma et al. 2006).

Corn crop production is an open ecosystem and N input/output dynamics vary largely. This represents both a challenge and an opportunity for improving corn crop productivity, NUE and the overall N economy as well as minimizing N load of the environment.

2.6.7 Carbon Footprint of Corn Production

Global warming resulting from greenhouse gas (GHG) emissions of agricultural origin has been considered as one of the important current environmental impact issues (Robertson et al. 2000; IPCC 2007). As a result, GHG emissions and their effect on global warming have inspired the quantification of the C footprint of many human activities (Hillier et al. 2009). It has been documented that more than 75 % of total C footprint from crop production results from N inputs (Hillier et al. 2009). Nitrogen applied in the form of inorganic fertilizers and manure is not always used efficiently by crops (Cassman et al. 2003). In general, corn is more responsive to N fertilizer than other grain cereals, and the practice by farmers of applying more N than the crop requirement often results in relatively high NH_4^+ - and NO_3^- -N concentrations near the soil surface (Ma et al. 2010b). Consequently, a relatively large emission of N_2O can occur in corn production systems (Chantigny et al. 1998; MacKenzie et al. 1998). In eastern Canada, Zebarth et al. (2008) studied the effect of rate and timing of fertilizer N application to corn on N_2O emissions. They found that while the delay of fertilizer application to sidedress and reduction in N fertilizer application reduced soil mineral N levels, there was no effect of these practices on N_2O emissions from soil. In contrast, Ma et al. (2010b) reported that rates of N_2O emissions increased exponentially once the fertilizer application succeeded the recommended optimum rate of N. It is clear that various fertilization regimes exert different influences on the nutrient concentrations at various stages of crop growth, which therefore cause different amounts of N_2O to be produced and released from the soil.

Clearly, it is possible to reduce leaching loss of available N (i.e., NO_3^-), and hence reduce direct and indirect emissions of N_2O generated by soil microbes largely from surplus N (Smith et al. 1998) through improving crop N uptake efficiency. Practices that improve NUE include adjusting application rates based on precise estimation of crop needs (i.e., precision farming), matching N application with corn N uptake, placing the right amount of N more precisely into the soil in the right location of the field, using controlled release fertilizer forms, and avoiding excess N application (Cole et al. 1997). Accordingly, fertilization strategies with both high corn yield response and low N_2O emissions could be developed for the more humid regions such as eastern Canada (Ma et al. 2010b).

2.7 Precision Nitrogen Management

2.7.1 *Corn Nitrogen Requirement*

Corn production requires adequate nutrient supply, especially N. Nitrogen is a component of amino acids, nucleic acids and thereby proteins in all living organisms. Nitrogen fertilizer affects corn dry matter production by influencing leaf area development, leaf area maintenance and photosynthetic efficiency (Muchow 1998), and consequently grain yield. A plant's physical appearance readily demonstrates this nutrient's importance. Plants with insufficient amount of N have dwarfed growth and yellowish leaves (Foth 1977), leading to partial or complete barrenness of ears (Subedi et al. 2006).

Nitrogen is one of the most limiting nutrients, lowering corn yield and quality in various parts of the world (Miao et al. 2007), because most plants can only take up N in two inorganic forms, ammonium ion, NH_4^+ , and nitrate, NO_3^- , from soil solution. In addition, inorganic N sources are subjected to loss through emissions due to microbial activities and leaching due to poor soil conditions. Nitrogen fertilizer requirements depend on many factors, including yield goal, soil residual N, soil organic N mineralization potential, soil type and environmental factors such as rainfall, soil temperature (Schlegel and Havlin 1995; Wu et al. 2008). Precise estimation of soil N supply to corn through soil organic N mineralization plays a key role in implementing N best management practices for economic consideration and environmental sustainability (Wu et al. 2008). The viable options to reduce ground water contamination are by maximizing the quantity of fertilizer N removed by the crop or by minimizing the quantity of residual fertilizer N after harvest (Keeney 1986).

2.7.2 *Nitrogen Use Efficiency*

Nitrogen use efficiency (NUE) by crop plants is generally defined as the ratio of the difference in plant N uptake between fertilized and unfertilized treatments over the amount of N fertilizer, i.e. the difference method. A more precise but expensive approach is of using labeled ^{15}N fertilizer to trace the fate of applied N (Liang and MacKenzie 1994). In general, NUE values vary from 30 to 50 % under various forms of crop and soil management (Raun et al. 2002). The rest of the N is lost through volatilization, denitrification, leaching, surface runoff and stabilization into soil organic matter and clay colloids (Malhi et al. 1996). The inefficient use of N fertilizer represents approximately \$680 million to \$1 billion of direct economic losses annually for Canadian farmers alone. In eastern Canada, the apparent fertilizer N recovery generally ranges between 30 and 70 % depending on many factors such as soil type, soil inorganic N content, N fertilization rates, timing of N application, soil water and environmental factors (Ma and Dwyer 1998; Ma et al. 2004; Ma and

Biswas 2015). Generally, apparent N recovery decreases with increasing N rate. The use of timely-applied adequate N fertilizer is one way to get maximum yield while improving N recovery efficiency (Ma et al. 2005; Ma and Biswas 2015).

Determination of NUE in crop plants is an important approach to evaluate the fate of applied chemical fertilizers and their role in improving crop yields. Definitions of NUE have been grouped or classified as agronomic efficiency, physiological efficiency, agro-physiological efficiency, apparent recovery efficiency, and utilization efficiency (Fageria and Baligar 2005). Agronomic NUE for grain corn has been defined as the grain yield per unit of N available from the soil, including N fertilizer (Moll et al. 1982). It is the product of N uptake efficiency (N-uptake/N supply from the soil) and N utilization efficiency (grain yield/plant N-uptake). Traditionally, NUE is calculated as the difference in plant total N between fertilized and unfertilized control plots as the fraction of the amount of N applied to the soil (Ma and Biswas 2015). This so-called difference method is considered to be biased because soil conditions and its mineral N content have a large impact on NUE. Nitrogen harvest index (NHI) is defined as the partitioning of total plant N into grain. While NHI appears to be under genetic control, other indices of NUE are found to be largely affected by crop management practices and environmental factors.

Improving NUE is more difficult than for any other fertilizer nutrients as N mobility in soil-plant systems is both high and variable, and many sources of addition and loss pathways of N in soil-plant systems complicate N balances and N use by plants (Raun and Johnson 1999; Hirel et al. 2007). Nitrogen use efficiency of corn production is low as are other cereal grain productions in most agroecological regions. Plant N losses have been accounted for 52–73 % of labeled N (^{15}N) in corn (Francis et al. 1993). This is a serious concern regarding N loss in the field and its negative impacts on soil, water and air quality (Ma et al. 2010a, b). Optimization of fertilizer N input and breeding plants with better NUE is one of the main goals of research on plant nutrition (Raun and Johnson 1999; Hirel et al. 2007). Development of fertilizer N application strategies is critical to improve NUE and grain yield of corn while minimizing the loss of N to the environment for sustainable corn production (Raun et al. 2002; Ma et al. 2010b; 2014).

2.8 Nitrogen Management Strategies for Corn Production

Nitrogen is one of the most cost inputs in corn crop production (Ma and Dwyer 1998). As discussed earlier, the accumulation of soil mineral N is highly susceptible to leaching (Vyn et al. 1999) and can potentially threaten groundwater supplies. Corn N management practices should ideally fertilize the crop by replenishing the inorganic N pool to soil fertility levels that can support economic crop growth and yield. This indicates that soil N inputs need to be tailored to meet the specific crop N requirements to maintain soil N fertility levels without exceeding crop N requirements. Therefore, N management strategies should lead to a balance between N input and crop N uptake to mitigate N loss via various mechanisms. Concurrent

improvements in both NUE and corn yield through an integrated management system are identified to be associated with the improved N recovery efficiency (NRE), and the latter is originated from larger root biomass at silking and greater portion of post-silking N uptake (Wang et al. 2014).

Excessive N fertilizer use in corn production has resulted in widespread nitrate pollution of ground water and economic losses for farmers. In addition, reliance on N fertilizers produced with large amounts of fossil fuels may not be economically and environmentally sustainable. Although leaching of nitrate to the ground water is inherent in growing corn, several techniques are available for more environmentally and agronomically sound management.

2.8.1 Remote Sensing

Remote sensing provides an opportunity to assess the in-season N status of corn. Knowing the crop's N status relative to the N status of the crop under optimum N conditions gives one the potential to make in-season N rate applications or adjustments for optimizing N efficiency, corn production, and profitability (Ma et al. 1996, 2005, 2014; Rambo et al. 2010). Remote sensing of canopy reflectance has great potential to help develop a new generation of N recommendations in corn production (Fig. 2.4). Much of the information can be gathered through trials conducted at many different sites and years. However, a more immediate problem with the acceptance of this technique is that there is no established methodology for quantitatively describing

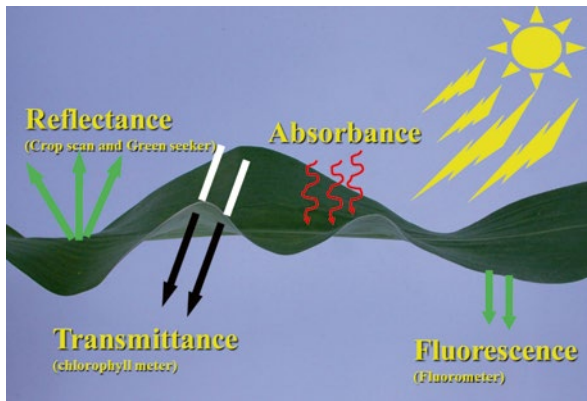


Fig. 2.4 Illustration of the principles of using leaf optical sensing to develop new instruments: When sunlight reaches leaf surface, portion of the radiation at specific wavelengths is absorbed by the leaf, portion of it is reflected back to the atmosphere, another part is passing through the leaf, and a small portion of the absorbed light by chlorophyll is also emitted as fluorescence. The quantities of light reflectance, absorbance and transmittance are closely related to leaf chlorophyll content, and the majority of plant nitrogen is in the chloroplast. By measuring reflectance or transmittance, it is able to determine the plant N status indirectly

the accuracy and precision of remote sensing for assessing crop N levels, transforming these assessments to fertilizer recommendations, and evaluating the benefits of using these recommendations. Although qualitative methods are adequate to show the potential of remote sensing and in-season applications of N, quantitative methods are needed to assess the benefits obtained in production agriculture. Available reports clearly support the conclusion that remote sensing offers more flexibility for improving N management in corn production (Ma et al. 1996, 2014; Daughtry et al. 2000; Sripada et al. 2008; Scharf et al. 2011).

Canopy reflectance measurement with active sensors is a relatively new method of remote sensing. It is similar to that of natural light reflectance with passive sensing technologies (Rambo et al. 2010). Active canopy sensors utilize their own light source and measure light reflectance in real-time at the canopy level. The main advantage of an active sensor is that the reflectance data is independent of the fluctuating sunlight environment (Solari et al. 2008, 2010). Canopy reflectance data with the GreenSeeker (NTech Industries, Ukiah, CA) instrument indicates that active sensors are useful in improving N use efficiency in many crops, including corn, when compared to uniform N rate application (Scharf et al. 2011; Ma et al. 2014). Many indices can be calculated from the visible and near-infrared reflectance data collected with active sensors. Some indices emphasize specific plant canopy characteristics such as leaf area index, leaf chlorophyll, whole plant biomass, plant density, canopy temperature, and canopy moisture. An example is the normalized difference vegetation index (NDVI), with evaluation and N rate prediction models developed in corn (Ma et al. 2014; Sripada et al. 2008). Other indices have been developed that emphasize canopy N status. An example is the chlorophyll index developed by Solari et al. (2008), with recent development of an algorithm for determining corn N sidedress (Solari et al. 2010). LAI is best predicted by NDVI compared to green normalized difference vegetation index (GNDVI). Available results indicate that green simple ratio index and GNDVI are more accurate in predicting corn yield compared to simple ratio and NDVI (Shanahan et al. 2001).

2.8.2 Precision Nitrogen Management

The concept of precision N management relies on targeting N inputs to corn crops according to locally determined requirements. Precision N management is therefore dependent on acquisition and interpretation of data on spatial and temporal variation of crop N status. The main components of precision N management consist of the right N source and the right amount of N, applied at the right time of crop development and in the right locations of the field (i.e., the 4-R approach).

Nitrogen fertilizer source can affect plant N uptake, N-use efficiency (NUE), grain yield and loss of N through various channels (Fox et al. 1986; Ma et al. 2010a, b). While urea is considered the most important N source for well-managed corn production systems, other forms of N fertilizer, such as ammonium sulphate, ammonium nitrate, calcium ammonium nitrate, have gained popularity in corn production under no-

tillage (Bandel et al. 1980). It has also been shown that NUE is greater with urea-ammonium nitrate or ammonium nitrate than with urea on no-till corn (Touchton and Hargrove 1982; Fox et al. 1986). Available results suggest that ammonium sulphate results in higher NUE and grain yield of corn compared to urea and calcium ammonium nitrate. However, there has been limited research to evaluate the effect of fertilizer N source on plant NUE and grain yield of field corn production.

Applying the proper rate of N is the single most important N management practice a farmer can do to improve economic return in corn production while minimizing N loss to the environment. Finding the right amount of fertilizer N to add to a crop means first estimating how much N is available from the soil and then adding fertilizer N to meet the crop's total N need. Because uncontrollable factors like precipitation and temperature affect the amount of N released from soil organic N mineralization as well as the amount of N required by a crop, the optimum amount of fertilizer N can change within a field, from location to location, and from year to year.

Time of N application is often the second most important N management decision related to profitability in corn production and loss of nitrates to ground and surface waters. Based on continued research since the late 1980s, application of N at spring preplanting plus sidedressing at the V6 growth stages is preferred for less risk, greater profitability, and reduced loss of nitrate to the environment (Ma et al. 2004, 2005, 2014; Ma and Biswas 2015). Because of the narrow window between snow thaw and the drying of the soil in late March and April and the beginning of planting in early May, many questions are being asked about split applications and whether diagnostic criteria are available to assist in making N rate decisions for in-season application of N. Research plot studies indicate that split application of N to corn often increases NUE and grain yield in eastern Canada (Ma et al. 2004, 2005, 2014; Ma and Biswas 2015; Subedi and Ma 2009).

The third component of precision N management in corn, right location in the field, reflects local/site-specific edaphic and climatic conditions. Soil N availability depends on soil inorganic N, soil N mineralization, soil type and environmental factors (Schlegel and Havlin 1995; Ma and Dwyer 1999; Wu et al. 2008). Precise estimation of soil N supply to corn through N mineralization plays a key role in implementing best N management practices for economic consideration and environmental sustainability (Wu et al. 2008). On the other hand, site-specific microclimate conditions determine crop N requirement, plant N uptake, NUE and yield. Therefore, precision N management strategy must be in synchronization with site-specific soil available N pool and prevailing weather conditions of the target agricultural zone to maximize NUE in corn.

2.8.3 Crop-Based Nitrogen Indicator

Great spatial variation in soil N fertility has long been recognized (Cohn et al. 1994; Solie et al. 1999) and several strategies have been developed to address this variation in different scales when fertilizer N is applied. Inherent factors such as

microtopography (Kitchen and Kanwar 1995; Dharmakeerthi et al. 2005) and soil texture (Shahandeh et al. 2005) determine soil total N and its mineralization (Campbell et al. 1984; Dharmakeerthi et al. 2005; Wu et al. 2008) and hence lead to spatial variation in N supply to corn (Eghball and Varvel 1997; Ma and Dwyer 1999; Eghball et al. 2003; Ferguson et al. 2002; Beauchamp et al. 2004).

Spatial variability in soil's N supplying power is probably the primary factor controlling variable N fertilizer needs. Therefore, applying the right amount of N in the right location of a field at the right physiological stage of corn development is a significant challenge (Tilling et al. 2007). Up-to-date research attempts have been made to develop new methods based on leaf and canopy optical properties (Fig. 2.5) such as transmittance and reflectance (Blackmer et al. 1994; Ma et al. 1996; McMurtrey et al. 1996; Rodriguez et al. 2006; Rambo et al. 2010). For example, the chlorophyll meter (SPAD-502 Chlorophyll Meter, Minolta Camera Co. Ltd., Japan) was designed on the basis of the quantitative relationships between leaf transmittance/absorbance and leaf chlorophyll content (Dwyer et al. 1991; Markwell et al. 1995). The use of plant characteristics to predict N deficiency in corn has received much attention, especially for determining the correct time and the amount for side-dressing N (Piekielek and Fox 1992; Raun et al. 2002; Scharf and Lory 2009; Rambo et al. 2010; Ma et al. 2014).



Fig. 2.5 Illustration of using field measurements of canopy reflectance signatures, expressed as the normalized difference vegetation index (NDVI) and the information is then used for on-the-go variable rate of N application to corn at the quick growing stage (the sixth to ninth fully expanded leaf stage)

In general, it is known that reflectance in the near IR (NIR) range ($\lambda=700\text{--}1,300\text{ nm}$) is determined by the optical properties of the leaf tissues: their cellular structure, and the interfaces existing between air-cell wall and protoplasm-chloroplasts (Kumar and Silva 1973; Grant 1987). These anatomical characteristics are affected in turn by external factors such as water and/or nutrient status (Thomas et al. 1971; Blackmer et al. 1994), and infection with pathogens. Reflectance in the visible red range ($\lambda=550\text{--}700\text{ nm}$) is negatively correlated with leaf greenness (Ma et al. 1996), and thereby it can be used to estimate leaf chlorophyll (Benedict and Swidler 1961; Thomas and Oerther 1972; Thomas and Gausman 1977) and carotenoid (Filella et al. 1995; Thomas and Oerther 1972) levels, and by extension the photosynthetic competence of the crop (Ma et al. 1995). Field studies with corn indicate that the majority of leaf N is in the chloroplasts (Peterson et al. 1993), and thereby leaf chlorophyll content is a good indicator of leaf N status (Dwyer et al. 1995a, b). Similarly, canopy reflectance, expressed as the normalized difference vegetation index (NDVI), measured at sidedress time (i.e. V6–V8 growth stage), integrates leaf greenness and plant biomass (Ma et al. 1996), and therefore can be used as a rapid, non-destructive and reliable alternative approach for estimating corn N requirements compared to the tedious soil test methods (Ma et al. 1996, 2005, 2007; Rambo et al. 2010).

The economically optimum rate of N (EORN) required for corn can vary spatially due to variations in soil characteristics (Fig. 2.6) which affects the available soil

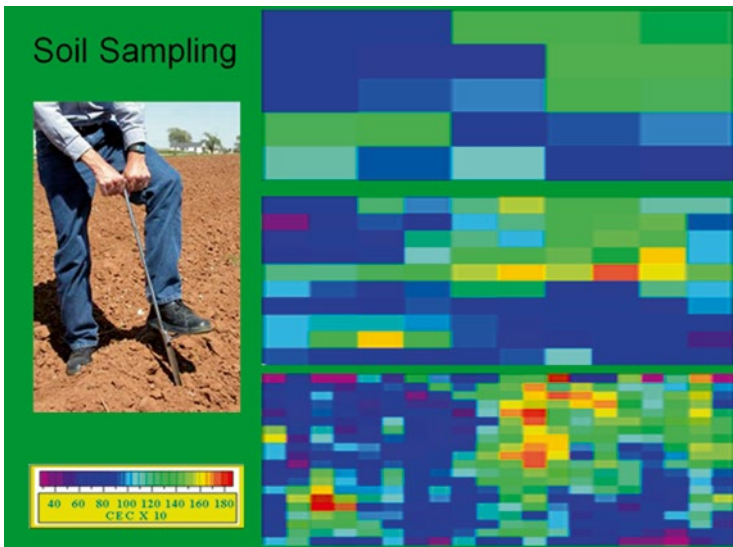


Fig. 2.6 Illustration of grid soil sampling in a 21-ha field: with 1 ha per sample and 21 samples from the field, the soil cation exchange capacity (CEC) shows some spatial pattern. Soil CEC shows a higher spatial variation if 83 samples are taken, and a fine resolution of soil CEC is revealed with 333 samples taken from the same field. The large spatial variability reduces the effectiveness of using soil test as a tool for fertilizer recommendation for improved corn crop productivity and nitrogen use efficiency (NUE)

moisture storage capacity, and temporally due to the interactions of environmental factors such as temperature, precipitation and its seasonal distribution (Mamo et al. 2003). Knowledge of both soil factors and crop N requirements is a prerequisite for the development of best management practices to maximize the yield response to fertilizer N (Muchow 1998). Recent development in canopy optical sensing and automation technologies provides the opportunity to apply N variably according to plant spatial variation at the field scale. Using optical sensors and then calibrated economically optimum rate of N has shown to be beneficial in making effective N management decisions (Shanahan et al. 2008). Compared with uniform fertilization, this new technology is expected to have the potential of crop yield increase (Ma et al. 2014) or reduction in N loading of the environment (Dinnes et al. 2002; Raun et al. 2002).

2.8.4 Variable Rate Application

Historically, most farmers have treated a field as a unit, and have applied management practices uniformly in large geographic regions without consideration to in-field variability (Power et al. 2001; Schmidt et al. 2002). Current N recommendations are generally based on previous yields, expected yields and pre-planting soil tests. This leads to over-fertilization in some areas while under-fertilized in others (Mamo et al. 2003). Yield variation in a corn field may be caused by many factors, including spatial variability (Fig. 2.6) in landscape position, soil structure and texture, crop production and field operation history, soil physical and chemical properties and nutrient availability. The first step in site-specific N management is to examine the field history, in particular its effect on N mineralization, and to take account of anticipated effects of soil texture, drainage and precipitation. It has been argued (Roth et al. 1992) that N rates should be tuned to yield expectations (i.e., application of 20 kg N per expected tonne of grain). There are good reasons to decrease rates of fertilizer N in locations of a field where yield expectations are low. However, simple recommendations based on yield expectations alone have been questioned. For example, a field with low yield expectations is associated with relatively low N recovery and a limited mineralization capacity (Davis et al. 1996). Besides, variable N management based on historical precedents do not necessarily account for weather conditions in the current season and therefore a strategy of “on-the-go” adjusted N application is needed.

Variable rate application of N based on an in-season assessment of crop N status may offer producers increased grain yield, profitability, and NUE. The strategies used in the VRA are assessing site characteristics that affect soil nutrient dynamics. Variable N management requires indicators of the N status of the soil-crop system (Ma et al. 2005). Indicators provide information on either the necessary adjustment of N rates in subsequent seasons or support decisions on N use in the current season. Various crop and soil-based indicators have been developed and tested during the last decade. Crop-based indicators (e.g. chlorophyll meter, canopy reflectance map),

and real-time site specific yield monitors are gaining popularity in recent years. Variable responses to the amount of N fertilizer application in the same region or field have been more common than other nutrients in corn (Magdoff et al. 1984; Scharf et al. 2002; Andraski and Bundy 2002). Crop N status at the time of N sidedress is more relevant to corn producers than a soil test. However, an ideal indicator has to have a reproducible relationship with N status of the soil-crop system and must be able to detect both deficiency and excess of N. The assessment must be cost effective and rapid enough to inform growers of a timely supplement of N. Therefore, there is a need for new diagnostic tools that provide a better prediction of economically optimum rate of N in management zones of a field. Adoption of this technology has been hampered by the difficulty of classifying fields into management units, the high cost of sampling soils on a grid basis, and the variability of soil and plant properties in the landscape. Further research on tools that detect nutrient variability will make this technology more economic and practical for growers.

There are also some disadvantages of on-the-go N management strategy. Although young corn plants need an ample supply of N, on-the-go strategy requires a timely detection of crop N requirements to avoid yield reductions. In addition, extra operation costs, equipment, and suitable soil and weather conditions are needed to apply adequate N to the corn crop. In a standing crop such application methods may damage the root systems although crop damage can be minimized by sidedressing in between corn rows. Recently, an on-farm trial including 55 site-years of data showed sensor-based N applications outperformed producer-chosen rates of N for corn in the USA (Scharf et al. 2011). Similarly an on-farm study conducted in Ontario (Ma et al. 2014) showed that the vehicle based optical sensors, including GreenSeeker® can estimate N needs of corn in a whole field (Fig. 2.7). This was done by reading the canopy reflectance at around the V6–V8 growth stage and calculating corn crop N needs using an algorithm. The algorithm first calculated a saturation index (SI), i.e., the canopy reflectance (NDVI) in the target location as a fraction of maximum NDVI measured from the whole field. A previously developed curvilinear relationship between SI and N rates was then used to determine the N needed for the specific locations (Ma et al. 2014). This algorithm resulted in N recommendation rates that were similar to the economically optimum rates of N, calculated with a yield-N rate response curve or the provincial N calculator (Ontario Ministry of Agriculture, Food and Rural Affairs). Therefore, the optical sensor system, which eliminated soil sampling and analysis, could be as satisfactory as the provincial N recommendation system for a whole field, but had the advantage of time- and cost-saving. The NDVI readings are the reflections of corn growth conditions, which in turn are from the results of soil N supplies along the monitoring directions (vehicle way). The optical sensor system could detect the spatial variations in soil N supply and allow the on-the-go application of the recommended N variably using the algorithm. Therefore, the optical sensor system can be used as an alternative system of N recommendation for corn growers in the more humid regions such as northeastern USA and Canada.

An economic analysis based on the on-farm study conducted in Ontario has revealed that sensor-adjusted in-season N application resulted in savings up to \$70



Fig. 2.7 A photo taken for sensor-based variable N rate application in a commercial corn field (Paul Raymer, The Farm Office in Ontario): Reflectance sensors are mounted in the front of tractor and instant collection of canopy N status data is used with a computer to determine the amount of N fertilizer needed for the specific locations. With variable rate controlled pump, precision N fertilizer is delivered to the specific locations in the field. How does this work? At planting, an N-rich strip ($\sim 200 \text{ kg ha}^{-1}$) is created for comparison, and rest of the field receives the regular start-up fertilizer. At sidedress stage, N-rich strip is mapped first and the resulting NDVI is used to adjust the algorithm for each field. Algorithm, programmed into the on-board computer, is used to control the variable rate of fertilizer application according to the plants requirement

per ha for rainfed corn (Ma et al. 2014). Further investigation into this technique revealed that the variable rate strategy produced similar grain yields to those of the uniform rate strategy when the same amounts of N fertilizer were used in both cases. Improvements in algorithm and equipment are therefore, warranted to enhance the ability of this technique to address the variations of soil mineral N in smaller scales.

2.8.5 Matching Improvement in Nitrogen Use Efficiency with Increased Yield

The efficiency of N uptake and N-use relative to the production of grain requires processes associated with absorption, translocation, assimilation, and redistribution of N operate effectively (Moll et al. 1982). Despite N requirement for many physiological processes of crop and grain yield formation, N application often results in decreases in NUE in many crops including corn. For grain crops such as corn, NUE has been defined as the grain yield per unit of N available from the soil, including N

fertilizer. Generally NUE is higher in plants grown with low N compared to those grown with high N (Ma and Dwyer 1998). The use of N by plants involves several steps, including uptake, assimilation, translocation and, when the plant is aging, recycling and remobilization. Accordingly, NUE is the product of N uptake efficiency and N utilization efficiency, which is the optimal combination between N assimilation efficiency and N remobilization efficiency. Among these processes, uptake of N in higher amounts by plants and its translocation to grain is crucial for increasing yields. Agronomic, physiological, apparent N recovery and N utilization efficiency have significant positive associations with grain yield. However, plant N accumulation and grain yield generally show a positive quadratic relationship (Cassman et al. 2002; Fageria and Baligar 2005), which means that diminishing returns appear in the conversion of plant N to grain as yields approach yield potential ceilings. This suggests that there is a need to optimize NUE and grain yield for sustainable corn production and integrated agronomic management practices might be the choice for concurrent improvements in corn grain yield and NUE (Wang et al. 2014, 2015).

2.9 Conclusions

Nitrogen management for grain corn production continues to be a challenge for economic, agronomic and environmental reasons. Nitrogen use efficiency in grain corn production under most cropping systems is less than 50 %. This low recovery is associated with N losses from $\text{NO}_3\text{-N}$ leaching, NH_3 volatilization, surface runoff, and denitrification. Adopting improved N management practices can increase grain yield and NUE in corn. These improved practices include creating favorable environmental conditions for the crop, which lead to higher N uptake and utilization, and consequently higher yields. Management practices such as adequate rates, appropriate sources, efficient methods of application, and application timing which synchronizing crop N requirements are important N management strategies. Nitrogen recommendations based on yield expectations should be associated with soil N pool and mineralization capacity, plant N recovery, N uptake and NUE. Variable rate N management based on an in-season assessment of crop N status using optical sensing technologies may offer producers with an increased grain yield, profitability, and NUE. In addition, variable N rate management based on historical precedents should account for weather conditions in the current season. Therefore, there is a need for a strategy of “on-the-go” adjusted N application.

Remote sensing of canopy reflectance has great potential to help develop a new generation of N recommendations in corn production. However, the problem with the acceptance of this technique is that there is no established methodology for quantitatively describing the accuracy and precision of remote sensing for assessing crop N levels, transforming these assessments to fertilizer recommendations, and evaluating the benefits of using these recommendations. Quantitative methods are needed to assess the benefits of this technology in precision N management for sustainable corn production. In order to maximize agronomic NUE for corn

production, a deeper understanding of physiological NUE involving a number of physiological processes such as N uptake, translocation, remobilization and its relationship with grain yield is important. An optimization between grain yield and NUE must be achieved for sustainable corn production. In addition, a better understanding of N interactions with other nutrients and environmental conditions may be useful in developing sustainable N management strategies for corn production.

An ideal indicator must have a reproducible relationship with the N level of the soil-plant system and be capable of detecting or predicting either a deficiency or an excess of N. Simultaneously, it should also work quickly enough to allow management to correct deficiencies during the current season. The differential responses in corn grain yield to variable rate strategy guided by canopy NDVI mapping, using different algorithms across the states, highlights the need for improvement in both the NDVI – N rate algorithm and the equipment. Measurements of canopy reflectance are able to assist in NDVI mapping on many plants in different locations of a field to have adequate evaluation of the spatial variability. It is possible to use relative NDVI adjusted for reference crop for guiding variable rate application of N that accounts for the spatial variability. The integration of these characteristics can be applied to develop new instruments and/or derive suitable algorithms for more effective monitoring of the plant and canopy N levels as indicators for side-dressing N fertilization in corn. The precision farming approach is not as simple as plug-and-play. However, with technology and equipment improvement, variable rate strategy will become reality in the near future to improve both NUE and grain yield, as well as sustaining our environment.

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Chapter 3

Soil and Crop Management for Sustainable Agriculture

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Abstract Sustainable management systems have continuously been promoted as an alternative to conventional farming practices for improving soil health and crop yield. However, benefits of these systems largely depend on the type of soil and climatic conditions prevalent locally. Loss of fertile topsoil due to water, wind, or tillage erosion, or due to management practices such as over-tillage of fragile soils, and removal of too much residue, can lead to reduced soil organic carbon (SOC) content, degraded soil health, loss of soil resiliency, and lower crop productivity. Conservation practices have been promoted to improve SOC content. Some of these practices include: conservation tillage, cover crops, organic farming, grazing management, and manure management. These conservation practices are designed to maintain C in sufficient areas and improve C in deficient areas. Conservation tillage is one of several management practices for increasing SOC and improving soil health of agriculture lands. The no-till (NT) system, in general, improves SOC content which is a strong indicator of soil health and crop productivity and strongly influences various ecosystem services. In contrast, intensive tillage generally stimulates soil C losses through enhanced decomposition and erosion. However, contrasting results have been obtained by different researchers under a variety of environmental conditions. Many of these findings are incomplete because they are based on information collected from a single site and soil type and measured over a relatively short duration. Process-based models can be used to integrate and extend the findings from multiple empirically-based research studies to aid in the prediction of the effects of conservation practices on SOC and greenhouse gas emissions over long periods of time, under a variety of environmental conditions, and for exploring novel management strategies to improve SOC and mitigate greenhouse gas emissions. Literature collected from research conducted globally by different

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researchers have shown that conservation practices often are helpful in improving SOC content, reducing greenhouse gas emissions, and improving crop productivity. However, monitoring of these parameters should be considered over a longer duration under a variety of environmental conditions and integrated with process based models.

Keywords Soil organic carbon • Cover crops • Manure • Grazing • Drainage • Organic farming • Greenhouse gas emissions

3.1 Introduction

Land management practices and land use have changed tremendously to feed a growing population. Subsequently, this conversion has decreased the soil organic carbon (SOC) of soils which in turn has reduced soil quality and crop productivity. Some of these land management practices include intensive tillage, overgrazing, deforestation, and improper fertilizer application that disturb soil quality and health and deteriorate the environment. These practices often significantly result in soil degradation, an increase in soil greenhouse gas (GHG) emissions, and impaired water quality. Further, these practices decrease SOC, a strong indicator of soil health and crop productivity since it strongly influences various ecosystem services, crop productivity, and soil properties. Loss of fertile topsoil due to land use change, environment (water or wind erosion) or management problems (intensive tillage of fragile soils, removal of too much residue) can lead to degraded soil health, loss of soil resiliency, and lower crop productivity. Thus, improved soil and crop management systems are strongly needed to maintain and build SOC levels for sustainable production. There are several alternative (conservation) practices that can improve soil, air and water quality (Fig. 3.1), however, their benefits depend on local environmental conditions. This chapter will focus on land management practices that influence sustainable production. Accordingly the present chapter has been divided into the following sub-headings: Land management systems and their impacts on soil and crop productivity; Impacts of climate change on sustainability of agriculture; and Conclusions and future directions.

3.2 Land Management Systems and Their Impacts on Soil and Crop Productivity

3.2.1 *Types of Conservation Tillage Practices*

Conservation tillage is defined as any cropland system that leaves at least one-third of the soil covered with crop residue after planting. Examples of conservation tillage include: no-tillage (NT), strip-till (ST), ridge-till (RT) and mulch-till (MT).

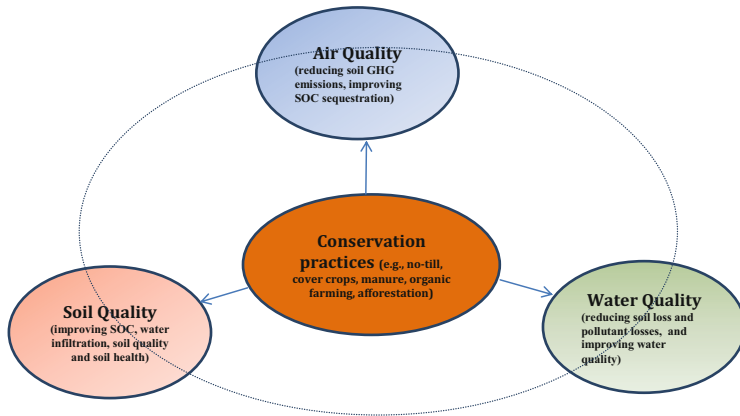


Fig. 3.1 Schematic diagram shows that conservation practices improve soil, air and water quality

Mulch tillage is a “type of conservation tillage where full-width tillage involving one or more tillage trips which disturbs the entire soil surface and is done prior to and/or during planting” (Conservation Technology Information Center, CTIC). This type of tillage practice uses tools such as chisels, field cultivators, disks, sweeps or blades (CTIC). *Ridge-tillage* is also a type of conservation tillage where soil is left undisturbed from crop harvest to planting except for strips up to 1/3 of the row width. This type of tillage practice uses sweeps, disk openers, coulters, or row cleaners. *Strip tillage* uses the potential of creating such conditions by combining the benefits of conventional tillage and no-tillage by disturbing the row and leaving the interrow with complete residue cover (Vyn and Raimbult 1993). No-till (NT) or zero-till (ZT) is the most commonly used type of conservation tillage practice where tillage operations are eliminated for crop planting. NT was practiced on 35.5 % of the US cropland area in 2007 and used with eight major crops (Horowitz et al. 2010). Acreage under NT farming systems has continued to increase.

3.2.1.1 Benefits of Conservation Tillage

The elimination of tillage practices has been reported to lower decomposition of residue and increase SOC (Spargo et al. 2008). In contrast, tillage removed crop residue from the soil surface, resulting in a decrease of the soil’s albedo, higher soil temperature, subsequently increasing decomposition of the remaining crop residue (Spargo et al. 2008). Tillage practices also affect soil biochemical and physical properties, consequently influencing SOC and N dynamics such as nitrate leaching, volatilization and denitrification (N_2O emissions). Intensive tillage disrupts aggregates, exposing internally protected soil organic matter (SOM) to microbial activity thus enhancing the decomposition of SOM.

Conservation tillage systems promote the protection of SOC since these systems alleviate disruption of soil macro-aggregates and lower exposures of micro-aggregates

and free organic matter (OM) to microbial decomposition (Bronick and Lal 1995; Jacobs et al. 2009). NT practices are one of several management practices that are helpful in enhancing SOC and improving the soil health of agriculture lands. No-till (NT) systems restore soil C levels, through retention and incorporation of crop residues and reduction in high-disturbance cultivation. Long-term use of intensive tillage (plow-till) practices decrease SOC and water retention compared to that under NT soils under corn- (*Zea mays* L.) corn and corn-soybean (*Glycine max* L.) cropping systems (Kumar et al. 2012b). NT farming systems improve soil aggregation which plays a significant role in maintaining soil structure and sustaining soil fertility (Bronick and Lal 1995). Further, when these conservation tillage systems were used for longer duration (47–49 years), soil hydraulic properties such as water retention and infiltration improved as compared to that under plow-till system (Kumar et al. 2012c). A study conducted at Hoytville and Ohio by Kumar and colleagues (2012c) reported that soil water infiltration was higher under NT compared to moldboard plow system. A study conducted by Franzluebbers (2005) using 96 observations in the southeastern United States concluded that a change from conventional to NT system resulted in sequestration of $0.42 \pm 0.46 \text{ Mg C ha}^{-1} \text{ year}^{-1}$. Similarly, in another study West and Post (2002) estimated ($n=93$ observations collected around the world) from $0.48 \pm 0.13 \text{ Mg C ha}^{-1} \text{ year}^{-1}$. Continuous NT farming helps in improving the soil quality parameters including improved soil structure, SOC stock, increased infiltration and reduced runoff and erosion (Spargo et al. 2008). In contrast, continuous plow-till (PT) or intensive tillage farming increases soil bulk density because of the usage of heavy machinery load. This substantial increase in soil bulk density increases penetration resistance and reduces aeration and root growth (Pabin et al. 1998). Crop residues left on the soil surface of NT systems help in water capture and storage, moderation of soil temperature, and maintaining soil water infiltration in the water-limited regions (Graham et al. 2007).

Continuous NT is a sustainable management system, however, it is dependent on local conditions (environmental and soil conditions). West and Post (2002) documented that about 85 % of soil C sequestered with NT systems occurs in the surface soils of 0–7 cm depth. However, these tillage systems need to be compared over longer duration to determine their transitional and long-term impacts on crop growth and soil properties (Vyn and Raimbult 1993). Influences of different tillage systems on SOC vary depending upon climatic conditions, soil type and depth. Sampling depth should be considered when comparing different cropping systems as soil management impacts SOC dynamics below the plow layer (Mikhailova et al. 2000). The SOC stock measurements performed just in the topsoil can underestimate the total SOC stock within the whole soil profile. Soil later below plow layer (subsoil) can function as a C sink, depending on the cropping system (Diekow et al. 2005). There is a possibility that deeper soil layers could sequester more C compared to that under the soil surface layer (Swift 2001).

The NT system, in general, improves soil properties. In a study conducted in Ohio by Kumar et al. (2012b, c) a long term NT system (49 years) improved soil properties (Table 3.1; Fig. 3.2a). However, NT can behave differently under different environmental conditions. For example, in a study conducted in North India under

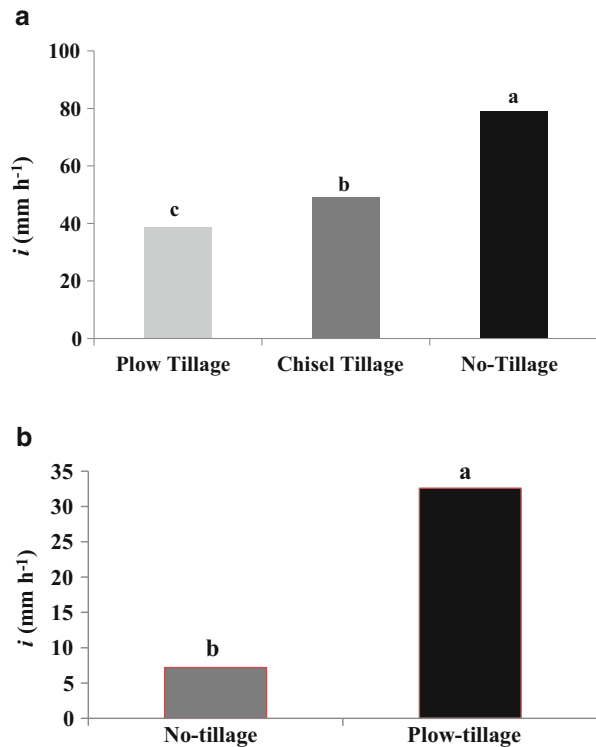
Table 3.1 Soil properties (measured for the 0–10 cm depth) influenced by 47 years of long-term no-tillage (NT) and plow-till (PT) under corn-soybean cropping systems located in Ohio

Parameters	Units	No-tillage	Plow-tillage
Bulk density	Mg m ⁻³	1.31 ^b	1.42 ^a
Soil organic carbon	g kg ⁻¹	19 ^a	14 ^b
SOC stock	Mg ha ⁻¹	21 ^a	17 ^b
Water infiltration	mm h ⁻¹	35 ^a	8 ^b
Macropores	m ³ m ⁻³	0.017 ^a	0.007 ^b
Corn yield in 2011	Mg ha ⁻¹	13 ^a	12 ^b

Source: Adapted from Kumar et al. (2012b, c)

^{a,b}Means with different letters within a row between no-till and plow-till systems are significantly different at P<0.05

Fig. 3.2 (a) Water infiltration as influenced by long-term tillage systems (Source: Adapted from Kumar et al. (2012c)). (b) Water infiltration as influenced by short-term (*bottom*) tillage systems (Source: Data adapted from Kumar et al. (2012d))



rice-wheat cropping system Kumar et al. (2012d) reported that the NT system decreased water infiltration rates in the short term (1 year duration) compared to the conventional (plow) tillage system (Fig. 3.2b). Similarly, crop yield (e.g., corn) may improve, decrease or remain unaffected when using NT systems compared to mold-board plow systems (Table 3.2).

Table 3.2 Corn yield as influenced by no-till (NT) and plow-till (PT) under different cropping systems and environmental conditions

Reference	Soil type	Location	Duration (in years)	Cropping system	NT
Brown et al. (1989)	Poorly drained	Iowa	8	Corn-soybean	-4 %
Colvin et al. (2001)	Poorly drained	Iowa	10	Corn-corn	-20 %
Cox et al. (1990)	Tile drained and undrained	New York	3	Corn	-10 %
Dickey et al. (1983)	No-irrigation	Nebraska	4	Corn	-15 %
Griffith et al. (1973)	Well drained to poorly drained	Indiana	3	Corn	Equal or higher
Ismail et al. (1994)	Poorly drained	Kentucky	20	Corn-corn	5 %
Kapusta et al. (1996)	Silt loam (fine-silty)	Illinois	20	Corn-corn	-3 %
Kitur et al. (1994)	Poorly drained	Illinois	10	Corn-soybean	-7 %
Kumar et al. (2012b)	Poorly and well-drained	Ohio		Corn-soybean	Higher/lower
Wendt and Burwell (1985)	Clay pan soil	Missouri	5	Corn	Equal
Boomsma et al. (Boomsma et al. 2010)	Silty clay loam	Indiana	14	Corn-soybean	-13 %
Chen et al. (2011)	Black soil	Northeast China	7	Corn	-28.4 %

Results adapted from previously published articles

3.2.2 Cover Crops

Cover crops introduce into traditional cropping systems an ability to provide environmental and economic benefits by enhancing SOC sequestration, soil fertility, suppressing weed growth, and reducing soil GHG emissions, ammonia volatilization and nitrate leaching losses (Dinnes et al. 2002). These crops provide various services to agroecosystems (Steenwerth and Belina 2008) including, decreased erosion and runoff, improved infiltration rate and soil nutrient retention, and building SOM (Battany and Grismer 2000). Cover crops are planted after harvesting the main crop and reduce N losses by absorbing residual fertilizer N (Mitchell et al. 2000). Crop rotation with legume cover crops can extract plant-available N that was unused by the previous crop (Smith et al. 2008), thus saving additional synthetic nitrogen fertilizer use, and reducing N₂O emissions (Maraseni and Cockfield 2011). Further, cover crops, when grown in rotation with regular crops are very effective in disease control. Therefore, the amount of pesticide application can be reduced, hence reducing GHG emissions from soils.

Cover crops are very effective in reducing nitrate leaching. In a study conducted by Meisinger and colleagues (1991) nitrate concentrations decreased by 20–80 % in leachate samples in the presence of cover crops. Logsdon et al. (2002) observed that Oat (*Avena sativa* L.) and rye (*Secale cereale* L.) as cover crops can reduce nitrate leaching by 70 % in a corn -soybean rotation. There are different types of cover crops that can be used worldwide depending upon environmental conditions and soil type. Grass cover crops have been efficiently used for reducing nitrate leaching losses as compared to legume cover crops. Non-legume cover crops (grasses) are effective in capturing soil residual N left after crop harvest in the autumn and reducing N losses through leaching compared with legumes or no cover crop. In contrast, legumes can increase N supply and reduce the amount of N fertilizer required for succeeding crops (Meisinger et al. 1991). However, the success of cover crop depends on the coincidence of its N release and uptake of the subsequent crop. Steenwerth and Belina (2008) reported that cover-cropped treatments had higher microbial biomass, higher N transformation rates, and lower soil nitrate values as compared to that without cover crops. Cover crops when used with NT systems improve productivity of degraded soils in the Southeastern USA (Bruce et al. 1995; Sainju et al. 2002). These crops influence the biologically active SOC fractions (microbial biomass carbon) and potential C mineralization that change rapidly over time. These fractions of SOC reflect changes in soil quality and nutrient dynamics (Bremer and Kessel 1992). Schutter and Dick (2002) reported differences in microbial biomass C and potential C mineralization between cover crops and fallow treatments because of the differences in nutrient levels, substrate availability, soil temperature, and water content. Blair and Crocker (2000) reported in Australia that wheat-legume rotations in red clay soils increased labile C fractions but had no effect on total C compared with continuous wheat. Cover crops help in nutrient cycling by providing food for microbes which cycle nutrients more efficiently and by improving soil organic matter which contributes to cation exchange capacity. Newman et al. (2007) reported that higher CEC enhances retention of positively charged micronutrients through soil chemistry complexes.

3.2.3 Grazing Systems

3.2.3.1 Grazing Systems in Croplands

Grazing intensity strongly influences the C and N dynamics in agroecosystems. Dung and urine patches during grazing contribute to accumulation of nitrite (NO_2^-) and emissions of nitrous oxide (N_2O) (Rafique et al. 2012; Velthof and Oenema 1995). Livestock urine has a higher concentration of N than their feces which can be quickly lost through leaching, denitrification or volatilization (Bussink 1992). The increase in grazing frequency significantly increases volatile losses of ammonia

(NH₃) from pastures (Luo et al. 1999). Therefore, managing the grazing intensity (season) can influence the C and N dynamics of soils (e.g., nitrate leaching; Erickson et al. 2010). Cardenas et al. (2010) observed a decrease in nitrate leaching with reduced grazing intensity. The incorporation of cover crops (N-fixing legume species) can reduce N₂O emissions from grassland either partly or completely due to replacement of inorganic fertilizer N by biological N fixation (Williams et al. 2011). The N fixed by legume crops becomes available slowly over time to the companion grass after it is released into soil *via* exudates from legume roots, by mineralization of senesced legume tissues and in excreta after consumption by grazing livestock (Ledgard et al. 2009). The amount of biological N₂ fixation by clover in temperate pastures depends on a number of factors including soil, climate, legume species, and nutrient supply and *grazing* management (Ledgard et al. 2009).

Integration of livestock in arable production can reduce the global warming potential (GWP) of food production. Herbivores in grasslands can modify the soil N cycling (Augustine and McNaughton 1998) and influence plant growth. Grazing can influence plant community, soil properties and cycling of nutrients within the plant-soil system (Ganjegunte et al. 2005). Soil OM is the main reservoir of SOC and N in rangelands and determines soil fertility, soil structure and water retention (Tiessen et al. 1994). The SOC content increased, decreased or remained unchanged under contrasting grazing conditions across different environmental conditions (Pineiro et al. 2010). Some of the factors influencing SOC in these systems were: (i) root contents were higher in grazed than in ungrazed, and (ii) bulk density values were unaffected or increased. A higher root mass has been reported to increase SOC content because of greater C inputs to the soil (Derner et al. 2006).

3.2.3.2 Rotational vs. Continuous Grazed Systems

Grazing impacts soil properties, however, the amount of impact whether it is positive or negative depends on grazing intensity, climate and soil type. In a study conducted in Central Missouri on Menfro silt loam soils continuous grazing increased soil bulk density compared to that under rotationally managed pasture areas (Table 3.3;

Table 3.3 Soil properties influenced by rotationally grazed (RG) and intensively grazed (IG) pasture areas located in Central Missouri

Parameter	Units	Rotationally grazed	Continuously grazed
Bulk density	mg m ⁻³	1.41	1.45
Saturated hydraulic conductivity	mm h ⁻¹	3.98	3.11
Macropores	m ³ m ⁻³	0.042	0.026
Total porosity	m ³ m ⁻³	0.505	0.477
Root length	mm ⁻²	2,185	356
Water infiltration rate	mm h ⁻¹	6.2	3.7

Source: Adapted from Kumar et al. (2008, 2010, 2012a)

Fig. 3.3) (Kumar et al. 2008). This can be attributed to the fact that trampling by herbivores compacted the pasture area. The increased bulk density enhances runoff and reduces water available for plant growth (Savadogo et al. 2007). In contrast, managing grazing in the pasture areas (e.g., rotational grazing system) can improve plant growth and soil properties compared to areas that are intensively grazed. Rotational grazing is a controlled grazing system where pasture area is subdivided into smaller areas (called paddocks) and animals are allowed to graze in these paddocks in a sequence for certain time and moved to another paddock. This type of grazing encourages uniform forage consumption and manure distribution and decreases compacted and eroded areas (Warren et al. 1987). Further, rotational grazing management improves cattle productivity compared to conventional grazing (Warren et al. 1987). Table 3.3 shows the data collected from soils under rotationally grazed and continuously grazed areas in Central Missouri. These data have been extracted from previous published manuscripts by Kumar et al. (2008, 2012a). Data show that soil properties are better (reduced bulk density, higher infiltration, macropores, hydraulic conductivity) under rotationally grazed areas compared to that under continuously grazed areas.

3.2.4 Organic Farming Systems

Organic farming is one of several alternative practices that are often believed to have generally beneficial impacts on the environment compared to conventional farming. Adoption of these low-input organic cropping practices often include reduced energy use and synthetic input costs (Légère et al. 2013). Organic farming (as described by *International Federation of Organic Agriculture Movements*, IFOAM, 2008; www.ifoam.org) has four principles: (i) *health*: organic agriculture sustain and enhance health of ecosystems and organisms, and production of high quality food without using fertilizers, pesticides, animal drugs and food additives, (ii) *ecology*: organic agriculture must be adapted to local conditions, ecology, culture and scale and inputs used in this farming system should be reduced by reuse, recycling and efficient management of materials and energy to improve environmental quality and conserve resources, (iii) *fairness*: organic agriculture should provide good quality of life, contribute to food sovereignty and reduction in poverty and aims to produce a sufficient supply of good quality food and other products, and (iv) *care*: organic agriculture should prevent significant risks by adopting appropriate technologies and rejecting unpredictable ones for example genetic engineering.

Soil fertility in organic farming is improved through applications of compost and manure (C content varies 20–40 % on dry-weight basis) and by the use of cover crops (Mitchell et al. 2000). Mixed organic farming is highly efficient in recycling of manures from livestock and of crop residues by composting. Leguminous crops in organic farming deliver additional N in sufficient quantities. Further, organic farming enhances high reliance on internal N cycling (Søren et al. 2006), and reduces N volatilization, GHG (e.g., N₂O) emissions (Bruce and Uwe 2001), and nitrate



Fig. 3.3 Intensive grazed areas (a) and rotationally grazed areas (b) in the small watersheds located at Horticulture and Agroforestry Center, New Franklin, Missouri (Photo by Sandeep Kumar)

leaching. Higher levels of organic inputs result in higher rates of organic matter and nutrient turnover (Thomas and Olof 1999). Since organic crop systems are limited by the availability of N, these systems aim to balance their N inputs and outputs and their N efficiency. Therefore, soil GHG fluxes are less in organic farming compared to conventional farming systems (ITC 2007). The global warming potential (GWP) (GHG emissions in CO₂ equivalents/land area/product) of organic farming is considerable smaller than that of conventional farming systems (ITC 2007). A study was conducted by Robertson et al. (2000) in Midwestern USA comparing conventional and organic farming systems. This study observed that GWP of organic production

with legume cover crop was 64 % lower than conventional farming system. In a 9 years study conducted at Beltsville, Maryland (USA), Teasdale et al. (2007) reported that organic farming improved soil fertility and was superior to no-till farming, despite the use of plough. Further, organic farming systems that involve recycling and nutrient use in a mixed-farm approach offers many ecological benefits (ITC 2007).

Achieving efficient weed management in low-input organic farming is a great challenge. As outlined by Tuomisto et al. (2012), the major challenge for organic farming to improve overall sustainability is to increase yields without causing harm to the environment. This is partially attributed to soil nutrient deficiencies and problems with pests, diseases and weeds under organic farming systems (Drews et al. 2009). Therefore, Tuomisto (2012) concluded that there is a strong need to breed new crops and animals breeds that can cope with organic farming systems.

3.2.5 *Afforestation*

Afforestation (the conversion of cultivated land into forest) is one of the viable options for sequestering C in terrestrial ecosystems (Lamb et al. 2005). The Intergovernmental Panel on climate change (IPCC 1999) reported that the potential of C sequestration through afforestation in the USA is about 117 Tg C year⁻¹ (ITg = 10), and the rate of C sequestration in US forests is 0.3–0.7 Pg C year⁻¹ (Sarmiento et al. 2010). Forest can change ecosystem dynamics and play an important role in the hydrology and global C cycle (Schipper et al. 2010). The conversion of forest to pasture or cropland, or conversion of pasture to cropland has been found to decrease soil organic carbon (SOC) stocks, whereas, the opposite conversions usually leads to increased SOC stocks (Falloon et al. 2006). Forests store larger amounts of carbon in biomass than cultivated lands (Freibauer et al. 2004). Soils under native forests generally have high infiltration capacity and hydraulic conductivity, and subsequently enhancing the base flow (Buytaert et al. 2007). Land use conversion from grassland to forest reduces soil erosion (Maclaren 1996), increases plant available nutrients such as phosphorus and sulfur compared to grasslands (Alfredsson et al. 1998). Growing trees in the footslope of pasture or croplands can reduce sedimentation of streams and improve soil properties. In a study conducted by Kumar et al. (2011) agroforestry trees when grown at the edge of small watershed can significantly reduce runoff compared to the watershed with no trees.

3.2.6 *Manure Management*

Animal manures as N fertilizers account for about 38 % of the entire N applied on a global basis (Bouwman et al. 2002). Animal manure contains significant amount of nutrients, yet, application of this manure is often performed for disposal rather

than for crop fertilization (Jarecki et al. 2009). It has been well reported that SOC improved by manure application. Manure is frequently applied in the fall when there is no crop in the field with subsequent leaching and gaseous losses (Jarecki et al. 2009). Application of fertilizer N and manures can increase agricultural emissions (Williams et al. 2011). Soil N₂O emissions are especially likely to be very high when manure is treated as waste and not recycled as fertilizer for crops. Surface-applied ammonium fertilizers and livestock manures to agricultural lands are the main sources for volatile losses of N (Sharpley et al. 1998). Efficient methods of incorporation and appropriate rate of these manures can minimize volatile losses of N. Rochette et al. (2001) observed an 80 % reduction in NH₃ volatilization due to immediate incorporation of organic manure into soil. The mixing of manures rapidly with soil using tillage implements is an effective method for reducing ammonia losses (Klarenbeek et al. 1991).

3.2.7 Drainage Management

Drainage is important in soils to improve gases flux and diffusion in soils. A total of about 33 % of the cultivated area is sub-surface tile drained in the Midwestern USA (Power et al. 2000). Subsurface tile drainage improves aeration, increases the availability of nutrients, enhances crop productivity (Cannell et al. 1985; Lal and Taylor 1970), and allows timely farm operations (Nangia et al. 2010) especially in poorly-drained soils. Soil gas fluxes in poorly drained soils depend upon soil type, crop management, and other environmental conditions. Managing these soils with subsurface drainage can enhance crop yields and reduce the yearly yield variability (Zucker and Brown 1998). Therefore, effective drainage has now become a routine practice in the Midwest USA (Nangia et al. 2010). Drainage influences the GHG emissions through influence on mineralization, denitrification and nitrification. However subsurface drainage when combined with high rates of N fertilization can result in significant loading of N in drainage discharge (Kaspar et al. 2007; Randall et al. 1997; Jha et al. 2010). Managing subsurface drainage in poorly drained soils can improve the crop productivity and reduce GHG emissions when used as part of an integrated production system that manages for N efficiency.

3.2.8 Example of Northern Great Plains, South Dakota, USA

In the South Dakota, a state in the Northern Great Plains, a majority of arable land has been managed with intensive tillage since the beginning of the twentieth century or earlier (Carpenter-Boggs et al. 2003). More recently (within the last 20 years) there has been a reduction in the intensity of tillage operations. Past intensive tillage practices continue to affect the soil properties and crop productivity in the state.

Land use change has had a significant impact on SOC. It has been reported that crop residue harvesting in South Dakota has increased from 10 % in 2007 (Janssen et al. 2008) to about 60 % in 2010 (Mamani-Pati et al. 2010). In the northern Great Plains of USA, a water limited region, standing crop residue on soil surface reduces wind speed and traps snow and provide moisture to soil (Nielsen et al. 2005). In an analysis conducted by Clay et al. (2012) it was reported that C is currently being sequestered in surface soils of many regions of Northern Great Plains. This was attributed to a combination of past SOC mining that occurred following homesteading, producers recently adopting NT and reduced tillage, and a gradual crop yield increase due to high-yielding crop varieties.

3.3 Impacts of Climate Change on Sustainability of Agriculture

3.3.1 Greenhouse Gas Emissions

Global surface temperature has increased by 0.88 °C since the late nineteenth century according to IPCC (2007). The observed climate changes are caused by the anthropogenic greenhouse gas (GHGs) emissions (Cheng-Fang et al. 2012). Agriculture is responsible for a significant amount of these emissions that primarily include carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O). Nitrous oxide (N₂O) is the most important stratospheric ozone-depleting GHG and is expected to remain as the largest throughout the twenty-first century (Ravishankara et al. 2009) as the current soil and crop management practices are continued to be utilized. N₂O has approximately 300 times the global warming potential of CO₂ on a mass basis (IPCC 2006) and these emissions are estimated to increase at the rate of 0.25 % per year (IPCC 2001). In the Midwestern USA, agricultural activities contribute about 25–33 % of total soil N₂O emissions of the entire USA (Li et al. 1996; Mummey et al. 1998). Nitrogen (N) inputs to agricultural land in the form of fertilizers, manure, leguminous crops, and crop residues contribute about 90 % of N₂O emissions (Søren et al. 2006). Nitrogen from mineral fertilizer is the major source of N for crop growth. However, when available N exceeds plant requirements, excess reactive N moves freely into water resources or into the atmosphere and negatively impacts the environment. Thus, alternative practices are needed that can help minimize N losses from agroecosystems. These practices (e.g., conservation tillage, organic farming, and manure management) are helpful in reducing N losses. GHG emissions are changing with change in land use across the US region. As more land is placed into cultivation SOC is decreasing resulting in an increase in emissions. Soil GHG fluxes can be reduced by controlling the flow of C and N in the agroecosystems (Smith et al. 2008). Inorganic fertilizers applied to crops influence soil C sequestration. Fertilization increases crop biomass and microbial decomposition of crop residues thus affecting N availability (Green et al. 1995). Application of N

fertilizer removes carbon from the atmosphere through increased plant biomass production while at same time enhancing soil biological activity which increases CO₂ emissions. The net effect of N fertilization on carbon in field organic carbon may be positive or negative depending on collateral land management practices.

3.3.2 Land Use Management Impacts on Soil GHG Fluxes

Tillage practices affect soil biochemical and physical properties, consequently influencing the release of CO₂ (Oorts et al. 2007). However, these effects vary and depend on the duration of tillage practice, soil type, climate and management practices and litter accumulation (Six et al. 2004). Innovative practices such as NT farming, mixed cover crops, improved crop varieties, improved nutrient management plan are beneficial in reducing these fluxes from soils. These innovative practices generate higher inputs of residue C and enhance soil C storage (Follett 2001). However increased SOC and higher microbial activity on the soil surface of NT compared to CT can increase CO₂ emissions. CT is generally thought to increase CO₂ emissions by exposing organic matter to more oxidizing conditions of the topsoil thus accelerating the decomposition of aggregate-associated soil organic matter (Reicosky et al. 1997).

Conservation tillage (reduced or no-tillage) is one of several management practices for increasing the SOC stock and reducing the GHG emissions from agroecosystems. Conservation tillage under the right conditions can restore soil carbon levels through retention and incorporation of crop residues and reduction in high-disturbance cultivation. Conservation tillage also reduces overall GHG emissions through a reduction in farm machinery operations.

Sometimes contrasting results can be observed due to differences in practice duration and soil type. For example, MacKenzie et al. (1998) reported increased N₂O emissions following reduced or no-till management on fine-textured soils including clay and silty clay loam, and imperfectly drained clay loam (Ball and Ritchie 1999), and silt loam (Baggs et al. 2003). Increased N₂O emissions rates were attributed to the availability of more plant materials, which are sources of carbon and energy for heterotrophic denitrifying organisms (Dalal et al. 2003). Grandy et al. (2006) showed no differences in flux due to tillage on fine/coarse-loamy soils. Therefore, studies should be conducted under different environmental conditions to gain an improved understanding of the underlying processes. Some researchers reported that NT is effective in reducing SOC losses but not in increasing the total carbon stock, primarily because of warmer winters that are likely to increase the rate of SOC mineralization (Chan et al. 2009). In some situations, no-till practice and stubble retention enhances weed population and growth, and large amount of herbicides, which have high global warming potential compared to other agrochemicals are needed (Lal 2004). Therefore, crop rotations and cover crops are encouraged to overcome these issues. When evaluating carbon gains from management systems it is also important to document the initial state of the system. Even though conservation

systems may gain carbon relative to intensively tilled systems under some conditions they may still lose carbon relative to a well-defined initial state (Pikul et al. 2008; Olson 2013).

N supplied by commercial fertilizers affect soil CO₂ flux by increasing C input from enhanced plant productivity and crop residues returned to soil (Paustian et al. 1997). Application of N fertilizer can increase crop biomass and influence the microbial decomposition of crop residues by affecting N availability, however, the effects of N fertilizer application on soil C vary with soil type. Excess amounts of N that are unused by crops emit significant emissions. Nutrient efficiency can be improved by (i) timing and amount of N applications, avoiding excess N applications, placement of N fertilizers at a certain distance where roots can extract most of the fertilizer. The practices that deliver N to the crops more efficiently often suppress N₂O emissions from those soils (Bouwman et al. 2002).

Crop rotations with cover crops promote SOC sequestration by increasing the input of plant residues and providing a vegetation cover during critical periods (Bowman et al. 1998), subsequently GHG emissions from soils managed under cropping systems can be reduced. Cover crops can improve SOC and extract plant-available N which was unused by the previous crop, subsequently reducing GHG (especially N₂O) fluxes. Cover crops, when grown in rotation with regular crops can be very effective in disease control if consideration is given to matching cover crop to disease etiology. Often the amount of pesticide application can be reduced, hence reducing the GHG emissions. Crop rotations with legume crops (West and Post 2002), for example, reduce the N inputs (Smith et al. 2008), however, legume-derived N can be a source of N₂O (Rochette and Janzen 2005). Legumes can fix atmospheric nitrogen and also make available to the companion/next crop, thus saving additional synthetic nitrogen fertilizer use, and hence reducing GHG emissions (Maraseni and Cockfield 2011). Crews and Peoples (Crews and Peoples 2004) argued that biologically-fixed nitrogen is ultimately derived from solar energy and should have a lower impact compared to nitrogen fertilizer which requires significant amounts of fossil fuels. However Maraseni and Cockfield (2011), concluded that this is still a debatable issue amongst the scientific community. Cover crops provide beneficial and economical means for supplying organic matter, enhancing soil fertility, suppressing weed growth, attracting beneficial insects, spiders and predatory mites, and reducing nitrate leaching losses to the groundwater during periods between crops (Mitchell et al. 2000).

3.3.3 *Process-Based Models*

Soil GHG fluxes are spatially and temporally variable because of heterogeneous climate, land management, and soil types across space (Smith et al. 2008). Calculations based on the amount of GHG emissions emitted from agricultural soils are mostly based on the default methodology developed by Intergovernmental Panel on Climate Change (IPCC). However, these calculations do not account for the

range of management practices that could potentially reduce GHG emissions (Rafique et al. 2011). Further, it is almost impossible to continuously monitor soil GHG fluxes across all possible combinations of crop rotations, management practices, soil types, and microclimates of the region (Gryze et al. 2010). Therefore, biogeochemical process models such as DeNitrification and DeComposition (DNDC; Li et al. 1996), Daily Century (DayCent; Metherell et al. 1995; Parton et al. 1987, 1994), and many others are useful tools to simulate gas exchange for different permutations (Del Grosso et al. 2006). These models are helpful in predicting GHG fluxes from different land uses and exploring strategies for mitigating these fluxes. The development of process based biogeochemical models has been used to simulate soil N₂O (and other GHG) fluxes at field, national and global scales (Donna et al. 2010). These models use mechanistic equations based on substantial long-term research to represent growth, nutrient, water, soil, and GHG dynamics. Such models have been, and are still being revised by prominent researchers so that these can be used effectively and adapted to different climate, soils and management scenarios applicable to specific regions. Performance of any such model depends on whether the models accurately reflect the relationships between ongoing processes in soils and climates to which the model is being applied, and to how well these models were calibrated for the studied site (Pete et al. 1997).

3.4 Conclusions

There is a great need to explore sustainable management systems that can deal with climate change and meet increased population demand for food. Conventional management practices have proved to impair environmental quality. Some of these practices include intensive tillage, improper nutrient management, and increased fertilizer input, uneven grazing that are affecting the soil quality and crop productivity. Over the years use of long-term no-till systems, cover crops, rotational or managed grazing are some of the alternative systems that are beneficial in improving soil properties and health. However, these systems depend on the local conditions. It has been demonstrated that incorporation of innovative management practices (such as introduction of mixed cover crops in corn-soybean rotation, grazing management, and organic farming, and managing manure rates) to existing agronomic regimen of Midwestern agriculture have potential to increase SOC and to reduce GHG fluxes from soils while improving the crop productivity. In order to achieve this potential more information is needed about fundamental mechanisms and how these mechanisms interact to create system efficiencies. Since, soil properties, GHG emissions, and SOC vary due to weather, soil type and landscape position, long-term monitoring of soil is needed for stronger conclusions and improved models. Process-based models are helpful in providing and extending estimates of SOC sequestration and developing strategies for mitigation GHG emissions in different environmental conditions. However the utility of process based models is highly dependent on a thorough understanding of casual mechanisms and their interactions. In conclusion, future

research is needed to focus on long-term monitoring of SOC and GHG analyses under a wide range of environmental conditions and this information will need to be further evaluated using well designed process-based models that use robust GIS data to explore environmentally viable mitigation strategies.

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Chapter 4

Rice Pest Management and Biological Control

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Abstract Insect pests and disease infestations are the primary constraints in rice (*Oryza sativa*) production systems. The rice stem borer and brown plant hopper have the largest share. Concerns about the sustainability of conventional agriculture have prompted widespread introduction of integrated pest management, an ecologically-based approach to control of harmful insects and pests. Integrated pest management is intended to reduce ecological and health damage from chemical pesticides by using natural parasites and predators to control pest populations. The main points of this review are: (1) integrated pest management (IPM) practices in rice have been developed in different ways in many countries of the world, but these practices vary with the socioeconomic and environmental characteristics of each country. (2) Integrated pest management has been a valuable model for organizing research and extension efforts worldwide. (3) Biological control relies on the interaction of organisms with the target pests and the environment. It is therefore more complex than certain traditional pest control practices such as the use of chemical pesticides. (4) Destruction of natural enemies by certain insecticides supports the contention that insecticide use, especially early in the crop season, upsets natural enemy

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control of insects such as Brown planthopper and also creates heavy selection pressure for strains of pests that can overcome previously resistant rice cultivars. (5) Overall, results show the superiority of integrated pest management over chemical method or farmers' own practices as indicated by the yield data and economic analysis. It is recommended that minimizing the pesticides applications and adopting different IPM practices against insects and pests, larger consideration should be given to farmer and their educational formats. The present article will shed light on different techniques of integrated pest management especially on biological control methods in rice crop.

Keywords Integrated pest management • Pesticides • Biological control • Rice

4.1 Introduction

Rice, as the single most essential human energy source, gives food to about half of the world's population (IRRI 1989), so it was crucial that predicted 1980's Asian famines (Ehrlich 1968) were averted by the development of Green Revolution high yielding rice and technology between 1965 and 1975. Great advances were made in irrigated rice which provided over 72 % of total yield and will remain outstandingly important (IRRI 1989); with ecologically comparable rainfed lowland rice, it produces more than 92 % of the world's crop, of which over 91 % is grown in Asia. Rice production plays an important role in the economic development of many countries, and any crises that decrease the production of this commodity can adversely affect these countries. In almost all rice -producing countries of the world, insect pests and crop diseases are considered the major factors that contribute to a decrease in rice production.

Unfortunately, pest problems enhanced with the intensification of irrigated rice production, which included increased investments such as insecticides. More than 200 million tonnes of rice are lost every year due to biotic and abiotic factors (Khan et al. 1991). Many devastating diseases of rice, such as tungro and yellow dwarf disease, are transmitted by insect-borne viruses (Heinrichs et al. 1985). The most destructive insects for rice are the lepidopteran stem borers (*Tryporyza incertulas* and *T. innotata*) and the rice leaf folder (*Cnaphalocrocis medinalis*) which cause annual losses in the order of ten million tonnes. Complete crop failure is rare, but, occasional outbreaks can destroy between 60 and 95 % of the crop (Yambao et al. 1993; Pathak and Kehan 1994).

In particular, insecticide use preceded outbreaks of secondary pests, notably the brown planthopper, *Nilaparvata lugens* (Stal) (Hemiptera: Delphacidae), that was previously of minor significance (Kenmore 1991). The intensification of insecticide use against increasingly large secondary pest outbreaks posed other problems, notably induced pest resistance, such that some large rice production schemes, as in the Solomon Islands, were abandoned (Rombach and Gallagher 1994). Moreover,

insecticide poisoning of farm workers became a serious issue (Teng and Heong 1988) and chemicals used against rice pests induced insecticide resistance in human disease vectors that breed in flooded fields (Way 1987). In pest management the challenge is to make natural non-chemical controls collectively more effective, so largely dispensing with the need for costly insecticides that, besides exacerbating some pest problems, can also be environmentally damaging and may involve virtually unresolvable dilemmas for farmers need based use of insecticides (Goodell 1984). In response to rising concern about the sustainability of conventional agriculture, we have to promote Integrated Pest Management (IPM) throughout the world. IPM has no standard definition, but comprises approaches that range from carefully-targeted use of chemical pesticides to biological techniques that use natural parasites and predators to control pests (Sorby et al. 2003).

Predators consume a huge amount of preys, and do not harm plants. They can attain equilibrium in pest control. Predators play a significant role in the control of agriculture pests. They have not usually been treated as an important biological control agent, because there is so little information on the ecological role of predators in pest control. Kim (1992) reported that predators in rice fields start to build up their populations in the mid of July, 40–45 days after transplanting. The same pattern is seen in Japan, which has a similar agricultural environment (Kobayashi and Shibata 1973). Spider is an important predator which helps to regulate the population densities of insect pests (Kajak et al 1968; Fox and Dondale 1972). It has been confirmed from the research work of different researchers that spiders in rice fields can play an important role as a predator in reducing the populations of insect pests of rice crop (Hamamura 1969; Gavarra and Raros 1973; Choi and Namkung 1976; Kobayashi 1977; Chiu 1979; Holt, et al. 1987).

Indiscriminate use of pesticides can easily disrupt the natural balance between insect pests and their natural enemies. Thus, although insecticides may be needed in some cases, they must be used judiciously in order to maximize the effect of the natural control agents (Mueller 1980; Reissig et al. 1986). It is fundamental to recognize that a small number of insect pests occurring at levels which cause no economic damage are helpful to provide food to maintain population of beneficial species at levels which can prevent damaging pest outbreaks. It is extremely costly to mass rear predators for release in rice fields. Conservation of bio-control agents is prime important and can be achieved by limiting broad spectrum insecticides use, or by applying insecticides which are selectively toxic to pests but not to predators (Thomson 1994; Shepard et al. 1995).

Insect pest control through conventional methods has often been found to be difficult under intensive cultivation of crops. Emphasis has been given in the recent past on the importance of pest management involving a sound knowledge of ecology of the key pests. One of the important components in pest management is biological suppression of insect pests employing pathogens like viruses, fungi, bacteria, protozoa and nematodes as bio-control agents. The pathogens cultured *in vitro* are employed either for short-term control equivalent to chemical control or long-term biological control (Jayaraj 1986).

The aim of this review is to highlight the importance of Integrated pest management practices in agricultural field especially recent developments biological control methods within an ecological framework in rice crop and also to point out a possible scope for providing an ecologically based Integrated pest management programs. In practice, the objective of the review is to point the opportunities for the farmer to optimize the use of the diverse natural controls as an alternative to dependence on pesticides.

4.2 Integrated Pest Management

Twenty-five years after its first enunciation, Integrated pest management is recognized as one of the most robust constructs to arise in the agricultural sciences during the second half of the twentieth century. Integrated pest management is an extensive approach for pest management which use all the existing skills, techniques and practices such as cultural, genetic, mechanical and biological methods including chemical pesticides use as a last option in harmonious and compatible manner in order to restrain pest inhabitants below economic injury level, based on regular crop pest inspections and monitoring. Live and lets live is the philosophy behind Integrated pest management. Integrated pest management approach has been acknowledged worldwide for achieving sustainability in agricultural.

All over the world markets globalization and increased traveling of people are allowing for increasing numbers of invasive species to be brought into countries (Perrings et al. 2000; Clercq et al. 2011). Suitable reactions to these pests are needed and development and implementation planning should be arranged. It is necessary that the option which poses the least threats while maximizing benefits is needed and that the strategy may include all components related to integrated pest management strategies (Wright et al. 2005). The integrated pest management (IPM) concept has gradually gained acceptance and has been adopted over the past three decades as an eco-friendly pest management approach suitable for sustained production of a commodity (Wu and Guo 2005). Integrated pest management practices in agriculture can be defined as an optimum combination of pest management methods implemented in farmers' fields that minimizes economic yield loss of a crop caused by insect pests without resulting in toxic effects to other organisms (Guo 1998).

From this review, it observed that Integrated pest management practices in rice have been developed in various ways in many countries of the world, but these practices vary with the socioeconomic and environmental characteristics of each country. Research on insect management of rice has resulted in the development of viable Integrated pest management technologies for the production of rice, especially in the United States, Europe and Asia.

4.3 History

Soon after World War II, when synthetic organic insecticides became widely available, a few, far-sighted scientists documented that haphazard use of the new synthetic organic insecticides would verify to be problematic. According to Smith and Smith (1949) reported that Californian entomologists developed the concept of “supervised insect control”, for example, responded with the concept of ‘supervised control,’ in which insect control was to be supervised by qualified entomologists, and insecticide applications were based on conclusions reached from periodic monitoring of pest and natural-enemy populations. This was viewed as an alternative to calendar-based insecticide programs. The first program was initiated 60 years ago in supervised control and targeted alfalfa caterpillar, *Colias eurytheme* Boisduval; it was overseen by the late KS Hagen who went on to a distinguished career in biological control.

After a period of decade, the troubles with haphazard use of insecticides were becoming clear, including pest resistance, target pest resurgence, secondary pest outbreaks and environmental contamination. It was in this setting that four entomologists at University of California presented the concept of ‘integrated control,’ which was stated as ‘to identify the best mix of chemical and biological controls for a given insect pest’ (Stern et al. 1959). This was one of the first understandable definitions of ‘integrated’ in pest management. The concepts of economic threshold and economic injury level were also introduced by these authors. For managing spotted alfalfa aphid, *Therioaphis maculata* (Buckton), on alfalfa grown for hay purposes, first integrated control program was devised. However, integrated control as originally formulated had a relatively slight center of attention. However, the challenging thought of ‘pest management’ gained support in some quarters of 1960s; it was wider and included large numbers of suppressive strategies such as host plant resistance, cultural control and semio-chemicals.

Though, integrated control and pest management steadily became synonymous, even if each remained largely insect oriented. Early 1970s until the incorporation of all classes of pests, modern concept of IPM was born (Kogan 1998; Prokopy and Kogan 2003). Overall Integrated pest management has been a valuable model for organizing research and extension efforts worldwide over the past 30 years.

4.3.1 Principles of Integrated Pest Management

Currently, programmes running in country like Africa and Latin America apply the term integrated production and pest management, and the integrated production and pest management principles are:

- To grow a healthy soil and crop;
- Protect natural enemies;
- Monitor the field regularly such as soil, water, plant, pests and natural enemies
- That farmers should make every effort to become experts.

Within these principles, economic decision-making remains at the hub of rice integrated pest management but the approach also incorporates good farming practices and active control of pest within a production context.

4.4 Biological Methods

Since the late 1940s, much of the insect, pest damages were managed by the use of synthetic chemical insecticides. Insecticides are the comparatively easy to apply and have generally provided successful control of pests (Susan et al. 2001). It is likely they will always be a part of pest management programs. Unluckily insecticides have several undesirable characteristics. Some degrees of risks are commonly presented by them to the replicator and other people who may get in touch with them; they can leave toxic residues that some find undesirable; water and soil contamination caused by them and affect flora and fauna, marine life and other non target organisms. They can also meddle with beneficial organisms for example insects beneficial for pollination and also against natural enemies of insects; and in this way insects can build up resistance to insecticides, effectively eradicating those resources as pest management options. For this reason, there is mounting interest among farmers to investigate different methods that diminish pesticides use (Susan et al. 2001).

Biological control represents one option to the use of pesticides. It is the conscious use of valuable live organisms; know as natural opponents for the control of different insects and pests. Almost all pests have natural enemies and many insects and pests can be successfully restricted by managing these natural enemies (Susan et al. 2001). Though biological control will not control all pests, it can be the base of an approach called integrated pest management, which connects a variety of pest control methods in ecologically protected system. In general biological control can be useful, inexpensive and secure, and it should be utilized more broadly than it is today. This study displays that biological control relies on the interaction of organisms with the target pests and the environment. It is therefore more complex than certain traditional pest control practices such as the use of chemical pesticides.

4.5 Biological Control Methods in Rice Crop

Rice belongs to the grass family, along with wheat and maize, is one of the three crops on which the human species largely depend. Around the global level rice now become one of the most important crops, as in most countries of the world it is used as a staple food and will continue to be for the foreseeable future. According to the FAO (2004), the crop is grown in at least 114 mostly developing countries and in Asia and Africa is the main source of income and providing jobs for more than 100 million households. Countries like China and India contribute together for more than half of world's rice area, while, along with Indonesia consume more than three-fourth of the worldwide rice production (Hossain 1997; MacLean et al. 2002).

In recent years, concern over food shortage is increasingly sensed, more in developing countries where rice production did not equivalent to the increasing population. Rice export was shortened or suspended by some of the third world's leading producers (Cotula et al. 2009). While on other hand decreased land availability and for high production with increased demand, interest is converted towards use of higher fertilizer inputs and cropping. As a result such efforts in turn increased pest populations (Heong 1996) and losses caused by pests are a major threat in order to achieving high rice yields (Waddington et al. 2010). In tropical Asian countries Insect pests are responsible for great yield loss of various crops (Savary et al. 1997). Uptill now in rice field about 128 species of insects have been reported. According to Kalode (2005), out of 128 species only 15–20 insect are considered as economically obnoxious species.

Brown planthopper (*Nilaparvata lugens*) is the potentially very damaging secondary pest for rice crop. In the past, disastrous losses have occurred by large-scale sudden occurrence of this small but mighty insect (IRRI 1979), although these sudden occurrence were mainly pesticide induced – triggered by pesticide subsidies and policy mismanagement (Kenmore 1996). However, the brown planthopper still remain a restricted problem, especially in those areas where pesticide overuse and mistreatment are common, and can therefore be well thought-out as an ecological hub around which both ecological understanding and management are vital to get gainful and constant rice cultivation. For all Integrated pest management educational programs, brown planthopper has also become the major entrant because it is always needed to take protective actions against an outbreak during crop management (Figs. 4.1 and 4.2).



Fig. 4.1 Brown plant hopper present on paddy rice leaf



Fig. 4.2 Brown plant hopper (BPH) causes draining out of plant fluid and nutrients by continuous sucking during high infestation and rice plants become yellow and finally die

Brown planthopper populations are generally under control by using great extent of natural enemies. During the 1970s with regular use of insecticides outbreaks in the tropics were reported. More application of the insecticide, faster will be resurgence of brown planthopper populations – which results rice plants dehydration to the large-scale, a symptom recognized as “hopperburn”. With the application of insecticides get rid of both brown planthoppers and their predators. However, their eggs are relatively unharmed which present inside the stem and, when these hatch, their nymphs grow in an environment which is free of predators. It is observed that in those field which are unsprayed; the brown planthoppers population does not rise to any significant level, so it is suggesting the significance of biological control.

Stem borers are also significant insect pests of rice crop. Among all the borers, yellow stem borer, *Scirpophaga incertulas* Walker (Lepidoptera: Pyralidae), dispersed all over India, are well thought-out as the most damaging species (Catling et al. 1987; Ooi and Shepard 1994). Of the total borer population yellow stem borer contributes about 89.50 % in the northern parts of West Bengal (Biswas 2006). Paddy is grown by selecting high-yielding varieties in temporal and spatial continuum, along with using great inputs of fertilizers and insecticides but apart from careful management due to shortage of appropriate scientific understanding in the northern



Fig. 4.3 Stem borers on paddy rice leaf



Figs. 4.4 and 4.5 Neck rot symptoms on the rice panicle. Symptoms (rotten neck) appear at the base of the panicle (Figs. 4.4 and 4.5). Such attack often results with the killing of the entire panicle. Infected panicles appear white and are partly or completely unfilled

districts of West Bengal. For this cause evaluation of validity of different management components of the nationally given broadly defined pest management protocol at ‘local level’ is thus found crucial (Satpathi et al. 2005) (Figs. 4.3, 4.4 and 4.5).

From this review it is observed that destruction of natural enemies by certain insecticides supports the contention that insecticide use, especially early in the crop season, upsets natural enemy control of insects such as Brown planthopper *Nilaparvata lugens* (Stal) (Hemiptera: Delphacidae) and also creates heavy selection pressure for strains of pests that can overcome previously resistant rice cultivars. Such situations create outbreaks of secondary pests and impair biological control of some key primary pests such as stem borers.

An experiment was conducted by Kaushik using widely cultivated paddy variety *Swarna mashuri* (MTU 7029) during five consecutive kharif cropping seasons (winter crop) of 2003–2007 at Raiganj Uttar Dinajpur, West Bengal, in order to validate rice-IPM module to suppress yellow stem borer population at regional level. Following were the results obtained by Kaushik during his experiments.

Table 4.1 demonstrated that non-significant difference was observed between the Integrated pest management and non- Integrated pest management plots during 2003 and 2004 for Dead heart (%) and White head (%). However significant differences were noted for the subsequent years. In Integrated pest management plots the damage extent was 7.13–11.72 % for Dead heart and 4.12–7.78 % for White head. While for non- Integrated pest management plots the resultant values were 10.59–12.13 % for Dead heart and 7.63–8.05 % for White head. Pooled data of 5 consecutive years showed that the Integrated pest management plot significantly reduced (8.55 Dead heart and 5.41 % White head) incidence of yellow stem borer over the non- Integrated pest management plots (11.25 % Dead heart and 7.69 % White head) (Kaushik 2012).

According to Kaushik (2012) revealed that in Integrated pest management plots occurrences of spider and coccinellid beetle populations were 8.92–14.22 and 7.83–10.78 individuals/10 hills respectively in Table 4.2. While the corresponding values were 8.57–.77 for spider and 7.76–8.08 for beetle populations were observed in non- Integrated pest management plots. Integrated pest management module influenced the abundance of both spider and beetle number significantly in all the experiment years except 2003. Whereas results from pooled data elaborated that in Integrated pest management plots significantly more spiders (11.39 individuals/10 hills) and beetle (9.44 individuals/hills) numbers were recorded as compared to non- Integrated pest management plots (Table 4.2).

The data presented in Table 4.3 revealed that a significant impact was observed in consideration of both grain and fodder yield in Integrated pest management module over non- Integrated pest management module. While results from Pooled data showed that 33.41 and 29.48 q/ha grain yield were obtained in Integrated pest management and non- Integrated pest management plots while the fodder yield in Integrated pest management and non- Integrated pest management plots was 35.84 and 33.18 q/ha respectively. The range values for grain production in Integrated pest management was 30.47–35.57 q/ha and 28.92–330.21 q/ha for non-Integrated pest management field. During 2003 and 2004 non-significant impact of Integrated pest management module on fodder yield was observed, while the effect was statistically significant for the remaining years. Integrated pest management plots showed an overall increase of 13.33 % for grain and 8.01 % for fodder generation were observed in Integrated pest management plots as compared to non- Integrated pest management plots (Kaushik 2012).

Data showed in Table 4.4 that Integrated pest management plot is economically better as compared to non-IPM plots. Under Integrated pest management plots net return (Rs 12,470/ ha) was relatively higher in comparison to non-IPM plots (Rs 9,567/ha). Further the input expenditure was comparatively lower in IPM plots (Rs 6,297/ha) than non- Integrated pest management plots (Rs 77,778/ha). Ultimately

Table 4.1 Occurrence of dead heart and white head in both integrated pest management and non-integrated pest management modules

Treatments	Dead heart (%)					White head (%)						
	2003	2004	2005	2006	2007	Pooled	2003	2004	2005	2006	2007	Pooled
IPM	11.72 (3.50)	10.81 (3.36)	6.73 (2.69)	6.49 (2.64)	7.13 (2.76)	8.55 (3.01)	7.81 (2.88)	7.78 (2.88)	3.71 (2.05)	3.64 (2.03)	4.12 (2.15)	5.41 (2.43)
FP	12.13 (3.55)	11.08 (3.40)	11.41 (3.45)	10.82 (3.36)	10.59 (3.33)	11.25 (3.43)	8.05 (2.92)	8.01 (2.92)	7.56 (2.84)	7.21 (2.78)	7.63 (2.85)	7.69 (2.86)
S.Em (\pm)	0.87	0.91	0.23	0.19	0.24	0.15	0.91	0.83	0.25	0.31	0.19	0.15
C.D. at 5 %	2.51	2.63	0.61	0.50	0.62	0.41	2.61	2.47	0.73	0.90	0.55	0.43

Adapted from Kaushik (2012)

Figures in the parenthesis are square root transformed values

IPM integrated pest management, FP farmer practices (non Integrated pest management)

Table 4.2 Occurrence of predatory spider and coccinellid beetle (individuals/ten hills) in both in both integrated pest management and non-integrated pest management modules

Treatments	Spider(s)					Coccinellid beetle(s)					Pooled	
	2003	2004	2005	2006	2007	2003	2004	2005	2006	2007		
IPM	8.92 (3.07)	9.89 (3.22)	11.27 (3.43)	12.64 (3.62)	14.22 (3.84)	11.39 (3.45)	7.83 (2.89)	8.92 (3.07)	9.36 (3.14)	10.31 (3.29)	10.78 (3.36)	9.44 (3.15)
FP	8.57 (3.01)	8.53 (3.00)	9.01 (3.08)	9.02 (3.09)	8.07 (2.93)	8.64 (3.02)	7.76 (2.87)	7.52 (2.83)	7.81 (2.88)	8.02 (2.92)	8.08 (2.93)	7.84 (2.89)
S.Em (±)	0.12	0.19	0.13	0.15	0.11	0.08	0.19	0.15	0.12	0.15	0.12	0.14
C.D. at 5 %	0.33	0.55	0.35	0.41	0.31	0.20	0.52	0.41	0.32	0.43	0.32	0.40

Table 4.3 Yield of paddy under Integrated pest management and non-integrated pest management modules during 2003–2007

Treatments	Grain (q/ha)					Fodder (q/ha)						
	2003	2004	2005	2006	2007	Pooled	2003	2004	2005	2006	2007	Pooled
IPM	30.47	32.29	33.83	34.89	35.57	33.41	33.56	34.82	35.69	36.92	38.21	35.84
FP	28.92	29.11	29.89	29.27	30.21	29.48	32.98	32.04	33.08	33.54	34.27	33.18
S.Em (\pm)	1.42	1.06	0.89	0.91	0.56	0.76	0.91	0.89	0.72	0.98	1.23	1.09
C.D. at 5 %	4.06	3.02	2.40	2.33	1.18	2.08	2.33	2.37	2.06	2.34	3.19	3.07

Adapted from Kaushik (2012)

IPM: integrated pest management, FP: farmer practices (non Integrated pest management), q/ha: quintals per hectare

Table 4.4 Economics of Integrated pest management and non-integrated pest management modules during the experimental work

Treatments	Field management										Net (a-b)	CBR value
	Expenditure (a)					Income (Rs/ha)						
	A	B	C	D	E	Total	Grain	Fodder	Total			
	i											
IPM	2,121	1,575	224	356	1,239	782	6,297	36,751.0	16,128	52,879	12,470	1:1.98
FP	996	4,290	1,012	787	-	774	7,778	32,428.0	14,931	47,359	9,567	1:1.23

Adapted from Kaushik (2012)

A fertilizer (i-organic, ii- inorganic), B pesticide, C Irrigation, D pheromone trap installation and *Trichogramma* sp. parasitoid release, E manual field maintenance including harvesting, Rs Indian rupees, CBR value cost benefit ratio value

the Cost Benefit Ratio was superior in IPM plot (1:1.98) than the non- Integrated pest management plots (1:1.23) Kaushik, (2012).

Satpathi et al. (2005) have also reported that substantial increase of grain production was observed from the southern part of Bengal by adopting of rice- Integrated pest management protocol. Now it is clear that insecticide application destroy large proportion of natural enemies along with their food supply, keep the field open for pest build up by secondary and resurgent pests like the brown planthopper, *Nilaparvata lugens* Stal (Kenmore et al. 1984) and green leaf hopper *Nephotettix* spp. (Kiritani 1988). Apart from causing pest out breaks, insecticide application is supposed to have accelerated the brown planthopper adaptation to resistant varieties by preference the survival and reproduction of virulent individuals (Gallagher et al. 1994). Apart from these, insecticides applications have significantly changed the faunal composition in rice and contaminated the environment eventually resulting in pesticide residues in food and mother's milk (Kiritani 2000).

Doing these type of experiments farmers will gain knowledge about predators and when they learn the role of these natural enemies, so they will less liable to use insecticides. Though, worldwide rice Integrated pest management was evolved through continued hard work of numerous entomologists, due to different reasons, its adoption has not achieved the desired level. The greater influence of agrochemical manufacturers and distributors in awkward the holistic adoption of Integrated pest management for its slow implementation is one of the major reasons (Murray 1994; Brader 1982).

According to Morales (2004) that Integrated pest management has always encompassed a wide range of practices and is ecologically based. The previously studies were restricted or were based on single or a complex of the pests and their natural enemies in rice. A holistic approach of rice ecosystem was either never considered vital or unnoticed. As a result, there were periodical insect outbreaks, the justifications for which are to be investigated in depth (Litsinger 2009).

Table 4.5 provides some indication of the myriad parasitoids and predators that exist in the tropical rice agro-ecosystem. In this table, the predators and parasitoids that attack yellow stemborers, brown planthoppers and green leafhoppers are listed. The predator complex that feeds on eggs and larvae of leafhoppers is nearly the same as for stemborers. A major exception is the cricket, *M. vittaticollis*, which is a voracious feeder on the eggs of leafhoppers. Leafhopper egg predation averaged about 50 % in both direct-seeded and transplanted rice in field experiments. Kamal (1981) reported 70-percent leafhopper mortality by predators. The parasitoid complex for leafhopper larva and pupa is richer than that for eggs. Rao et al. (1969) listed 32 species of larval and eight species of pupal parasitoids for rice fields in India. In Table 4.5 generalist nature of some predators is clear.

The wolf spider, *L. pseudoannulata*, consumes the larvae and adults of stemborer, leafhopper larvae and the nymphs and adults of Brown plant hopper and green leafhoppers. While, mirid, *C. lividipennis*, eats the eggs and nymphs of the Brown plant hopper, also feeds on the green leafhopper, and the eggs of leafhoppers. The grasshopper, *C. longipennis*, feeds on the eggs of stemborers and leafhoppers. Parasitoids have a tendency to be more specific in their tastes. Settle et al. (1996)

Table 4.5 Predators and parasitoids of common insect pests

Stage attacked			
	Egg	Larva	Pupa
Yellow stem borer			
Parasitoids	<i>Tetrastichus schoenobii</i>	<i>Temelucha philippinensis</i>	<i>Tetrastichus ayyari</i>
	Ferriere	Ashmead	
	<i>Trichogramma japonicum</i>	<i>Stenobracon nicevillei</i>	
	Ashmead	(Bingham)	
	<i>Telenomus rowani</i>	<i>Bracon chinensis</i>	
	(Gahan)	Szepligeti	
Predators	<i>Conocephalus longipennis</i>	<i>Lycosa pseudoannulata</i>	<i>Also attacks adults</i>
		(Boesenberg and Strand)	
		<i>Micraspis</i> spp.	
		<i>Ophionea</i> spp.	
		Ants	
		<i>Microveli douglasi atronilinea</i>	
		<i>Mesovelia vittigera</i>	
	<i>Limnogonus fassarum</i> (F.)		
Leaffolders			
Parasitoids	<i>Copidosomopsis nacoleiae</i>	<i>Temelucha philippinensis</i>	<i>Xanthopimpla</i>
	(Eady)	<i>Cotesia augustibasis</i>	Flavolineata Cameron
	<i>Trichogramma japonicum</i>	(Gahan)	<i>Tetrastichus ayyari</i>
	(Ashmead)	<i>Trichomma enaphalocrosis</i>	Rohwer
		Uchida	
Predators	<i>Conocephalus longipennis</i>	<i>Lycosa pseudoannulata</i>	
		<i>M. vittaticollis</i>	<i>Micraspis</i> spp.
		<i>Cyrtorhinus lividipennis</i>	<i>Ophionea</i> spp.
		Reuter	Ants
		<i>Micraspis crocea</i>	Water bugs
		Mulsant	
Brown planthopper			
Parasitoids	<i>Oligosita yasumatsui</i>		
	(Viggiani and Subba Rao)		
	<i>Anagrus</i> spp.		
		<i>Pseudogonatopus</i> spp.	<i>Pseudogonatopus</i> spp.

(continued)

Table 4.5 (continued)

Stage attacked			
	Egg	Larva	Pupa
Predators	<i>Cyrtorhinus lividipennis</i>	<i>Cyrtorhinus lividipennis</i>	
		<i>Lycosa. pseudoannulata</i>	
		<i>Migrovelia douglasi</i>	
		<i>atrolineata Bergroth</i>	
		<i>Synharmoni octomaculata (F.)</i>	<i>Synharmoni octomaculata (F.)</i>
	<i>Paederus fuscipes Curtis</i>	<i>Paederus fuscipes Curtis</i>	

reported 765 species of arthropods in 4 years of its sampling in the irrigated rice fields on the island of Java. Out of 765 species, only 40 % or 306 species were predators and 24.4 %, 187 species, were parasitoids. However, for only 16.6 % or 127 species herbivore species were reported.

In summary despite the use of chemical pesticides among farmers who take advantage of biological control has not been completely eliminated, but with a significant decrease in, farmers are not benefiting from this technology. Overall, results showed the superiority of IPM over chemical method or farmers' own practices as indicated by the yield data and economic analysis.

4.6 Integrated Pest Management Against Pests

Certain pests like rats, snails and birds etc. also caused great damage to different crops so to control the damages caused by these insects it requires greater community level planning and action. Among these pest rats cause severe damage to rice plants in field. It moves around locally to rice areas. It preferred the rice plant after the panicle has come out. In order to suppress the population of rats in crops, farmers often used different strategies such as different pesticides etc. Rat management strategies have comprised determining the major species of rat present in order to make sure that baits are proper and then rising community-level mapping methods to plan and carry out continuous trapping along feeding routes, fumigation or holes digging of rat, modification of appropriate habitat and set up early season bait stations using second-generation anticoagulant baits (Gallagher et al. 2009). However some farmers used highly poison chemicals like zinc phosphide and unlabelled aldicarb (Temik), so in most countries the use of these chemicals are strongly dejected because of subsequent deaths of children and small livestock (Gallagher et al. 2009).

According to FAO (1998) reported that educational activities on rat biology and behavior to improve strategy development and participation in programs can include in community programs. Before booting stage, year-round community-level management is well thought-out to be the major step to rat management (Buckle and

Smith 1994; Leung et al. 1999). In Malaysia an innovative owl habitat program has been successful in increasing number of owl as a result of which decreasing rat populations in rice and plantation crops. In rice fields a trap-and-barrier system with plastic has also achieved excellent results (Murakami et al. 1991).

Apart from rats, another pest that can be very damaging to rice is birds. They are danger at that time when come in large groups. In sub-Saharan Africa, red-billed quelea (*Quelea quelea*) and different species in Asia in rice ecosystems are known as constant problems. Netting is used in most Asian countries in rice and also in different crops to catch huge numbers of birds for sale as food. While for some species large number of nest destruction is also achievable. These methods have successfully decreased pest bird populations to very low numbers in Asia. The catching method may fetch profit to local people in terms of income or some time providing additional dietary protein in country like Africa, but the impact on pest bird populations has been small (Gallagher et al. 2009). In northeast Asia during the ripening period, some fields are sheltered by being covered with bird nets, which are widely available.

In fields the nets are also often used to save seed production from harm. Some time various forms of bird-scaring are used in order to keep fields safe from birds in both Asia and Africa. In Asia used video, cassette tape or reflective ribbons are widely used to scare birds while in Africa people shouting or hurling dried mud at the birds is common (Gallagher et al. 2009). Poisoned baits use and nesting destruction habitat are discouraged because of the possible negative effects on non-target species in adjacent aquatic environments (Gallagher et al. 2009). Overall, certain pests like rats and birds require greater community level planning and action for their control.

4.7 Future Needs

In agriculture sector Integrated pest management can be expected to continue to be a dominant theme in the future. More work is needed to improve Integrated pest management models in rice. In rice crop the potential utility of resistant cultivars to insects, either alone or in combination with other Integrated pest management tactics, by and large, remains unexploited. In spite of the accessibility of thousands of resistance sources against insects, host plant resistance studies have not made any major impact on rice crop production.

According to Teetes (1985) the reasons attributed are failure of entomologists and plant breeders to complete their assignment after recognizing the insect resistant germplasm, lack of farmers interest to accept and use resistant cultivars to insect; tendency to separate crop production and crop protection, and failure to generate adequate information about the pests and resistant cultivars. Besides from resistance cultivar, for all types of pests, especially weeds and fungal pathogens, environmentally friendly methods of control are essential to lessen their ever-increasing environmental impact as intensification of production continues.

For major changes already some countries are working. For example, pesticide use in Seoul's watersheds has been banned in Republic of Korea and massive organic agricultural investments is promoting by the Korea government in order to make certain clean water and high levels of production. While, other communities are in state of movement from maximization of grain to diversification such as rice–fish–vegetable culture in order to fulfill the food and nutritional security and this development is likely to raise as demand for more profitable non-grain products grows and levels of nitrogen are necessarily reduced to lower the environmental impact and incidence of expensive-to-control fungal pathogens (Gallagher et al. 2009).

In more countries Integrated pest management program development is required. These programs should guarantee that our educational systems (both formal and non-formal) respond adequately to the future needs of lessening the agriculture impact on the environment while enhancing yields – Integrated pest management/ Integrated production and pest management is, of course, a main feature of such education. Research work is required in association with field programs to engage farmers in finding answer of problems that faced in Integrated pest management/ Integrated production and pest management systems as economic changes alter the way rice is cultivated. Major improvements are required in the area of Integrated pest management policy relating to removing toxic compounds and subsidies for toxic pesticides.

However a lot of work is needed for subsidizing the commercialization of locally produced products such as pheromones, attractants, natural enemies, pest-exclusion netting (for birds), high-quality seed, resistive cultivar to insects and pests, improved disease resistance and balanced soil fertility products so that farmer will get maximum benefits from these steps.

4.8 Conclusion

In recent years great progress has been made in Integrated pest management program in rice crop, although additional data on the potential of natural enemies, microbial insecticides, and cultural practices are still needed. However, insect pests will continue to challenge rice production throughout the world. The reduction in chemical pesticide use associated with biological control method is increasing the abundance of some beneficial insects and improving the natural control of specific pests. Our evidence suggests that IPM adoption increases profits for rice farmers, since pesticide costs are reduced with no countervailing reduction in output. The reported incidence of sickness is lower for IPM farmers. It is concluded that for control of insect pests, spraying should be avoided in order to protect the natural parasite and predator populations. It is necessary to take on IPM strategies and tactics that are compatible with conservation. Susceptible varieties should be eliminated after the outbreak of a disease or insect while, multiple host plant resistant varieties having resistance to nematodes, diseases and insects need to grow.

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Chapter 5

Sustainable Rice Production

Gurbir S. Bhullar

Abstract Rice is an important staple food for more than half of the world's population and its demand is expected to increase with increasing population, more so in developing countries mainly Asia. Green revolution technologies transformed agricultural practices resulting into a considerable increase in the productivity of rice in the later part of twentieth century. However, rice agriculture is currently faced with multiple challenges such as declining or stagnant yields, lack of water availability, contamination of natural resources due to excessive use of agrochemicals, biodiversity losses, greenhouse gas emissions and losses due to extreme climatic events. There is a strong need to adapt agricultural practices in order to increase the production of rice in a manner that is economically viable and environmentally sustainable.

Here I review the technologies that are already available and could contribute significantly towards achievement of this objective. Using modern high throughput technologies, breeders are developing rice varieties, which possess traits for climate resilience along with higher yields. Effective management of limited water resources is of high value e.g. rainwater harvesting, groundwater recharge and timing of paddy transplantation with the onset of rain can greatly contribute towards sustainability of rice production and a well maintained canal system could reduce seepage losses by 30–40 %. Agronomic practices like laser levelling of fields, dry seeding and alternate wetting drying are known to considerably reduce irrigation water requirement. System of rice intensification is advocated to increase rice yields by 50 % while halving the water requirement and input costs, particularly in resource-poor conditions.

Integrated nutrient management, adjustment of fertilizer dosage, placement and application timings increases nutrient use efficiency while minimising degradation of natural resources through runoff and leaching. Integrated pest management and observation of threshold values could reduce environmental and health costs such as undesired effects on non-target organisms, development of pesticide resistance and residues in the food products along with minimising the input costs. To obtain full

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benefit from the available technologies, a strong commitment from extension and policy institutions is highly important. Use of modern information technologies for timely communication of information such as weather forecast could assist farmers in decision making for various farming activities.

Keywords Rice • Paddy farming • Sustainability • Water management • SRI • Integrated nutrient management • Greenhouse gas emissions

5.1 Introduction

Rice is one of the most important staple foods, cultivated (largely flooded) on more than 140 million hectares and with a current annual consumption of over 4.5 billion tonnes. A major increase in rice production in the coming decades will be indispensable in order to feed the burgeoning global population (Brar and Khush 2013). Agricultural productivity is faced with multitude of challenges such as changing environmental conditions, diminishing natural resources and biodiversity losses. Modern agricultural practices, though credited for ensuring food security during second half of the last century, are also criticised for associated environmental and health costs. Rice as a staple food gains more importance with the high rates of population growth, particularly in less developed countries of Asia – which is the major rice producing as well as consuming region. Although challenge is significant and multifaceted, there are a number of technologies, which could offer opportunities to attain sustainability in our production systems. This chapter will discuss the challenges facing paddy farming and will explore opportunities for sustainably increasing the productivity of rice.

5.2 Food Security

Ensuring sustained supplies of enough quantities of nourishing food for the global population is probably the biggest challenge facing modern mankind. Historically rice has always played an important role in food security. Since the collection of rice grains from wild till the domestication and later improvement of cultivars, rice has accompanied the great human civilisation in Asia – mainly China and India – through thousands of years (Chen 1997; Fuller 2011). It is no surprise that most of the rice consuming populations live in this part of the world. However, with increasing globalisation, migration and diversity in food choices, rice consumption is also growing in other parts of the world. Currently, rice is main staple food for over three billion people, which accounts for nearly half of the world's population (FAOSTAT). Per capita consumption of rice varies considerably among different regions; though in many rice producing/consuming countries, as many as 70 % of the calories in the

daily diet are supplied by rice alone. Besides, in Asian cultures rice forms an integral part of life with a significant role in many cultural, social and religious activities.

Continuing with the current trends, global population is expected to cross the nine billion mark by the middle of this century (FAO 2002). Given the fact that population is increasing at a higher rate particularly in rice consuming parts of the world, the demand for rice is going to increase. This inevitably calls for increasing production of rice to feed the additional billions.

5.3 Climate Change

The other big challenge of our times is posed by the ongoing environmental changes. Agricultural production heavily depends upon weather and the on-going climatic changes are bound to influence it. At the same time, agricultural activities have a significant contribution towards climate change.

5.3.1 Extreme Events /Floods/Droughts

Increased intensity and frequency of extreme weather events (such as droughts and floods) is predicted to threaten the agricultural production, more so in underprivileged parts of the world (IPCC 2007; Nguyen 2006). Humanitarian crisis of eastern Africa caused by severe drought in the past years and food crisis in Sahel region of Africa caused due to drought followed by severe flooding are some of the recent evidences to the potential of the problem.

5.3.2 Greenhouse Gas Emissions

Agriculture being the largest anthropogenic activity covers major share of the terrestrial use by man and hence exerts profound influence on earth's environment (FAO 2002; Smith et al. 2007). Besides air and water pollution – with runoff from agricultural areas containing nutrients and pesticide residues etc. – agriculture is also a major source of greenhouse gas emissions. For example, in 2005, agriculture was estimated to account for 60 % of nitrous oxide (N_2O) and 50 % of the methane (CH_4) emissions from anthropogenic sources (Smith et al. 2007). During the twentieth century alone, an expansion of agricultural land and increasing use of N fertilizers, increased the global N_2O emissions from 11 Tg N year⁻¹ in 1850 to 18 Tg N year⁻¹ in 1994 (Kroeze et al. 1999); and the continuing trend towards high input production systems is likely to increase emissions even further (Neill et al. 2005). Along with N_2O , rice is a major source of methane as it contributes about two-thirds of 'natural' emissions and one quarter of total emissions of methane globally (Laanbroek 2010).



Fig. 5.1 Measurement of greenhouse gas emissions by closed chamber method in a paddy field in India

Methane is microbially produced under anaerobic conditions in inundated paddy soils; and rice plants influence its emissions by affecting various processes responsible for production, transport and oxidation of methane. With major rice production concentrated in the region, it is no surprise that more than 80 % of the methane produced during rice cultivation is contributed by South and East Asia (Smith et al. 2007) (Fig. 5.1).

5.4 Biodiversity

Loss of species at alarming rates is the third equally big challenge, critical to the survival of our own species on this planet (Diaz et al. 2006). Biodiversity losses have gained more and more attention in second half of the past century. As estimated by the Millennium Ecosystem Assessment report, earth's ecosystem has seen rapid losses due to human activities resulting in degradation of 60 % of the ecosystem services in the past half century (Millennium Ecosystem Assessment 2005). About half of wetlands and more than one third of forests and mangroves have been lost due to anthropogenic activities mainly agriculture (Hussain et al. 2012). Conversion of natural habitat to agriculture is only one way of loosing biodiversity. There are a number of other ways by which biodiversity is lost as a side effect of

agricultural activities. For example, nutrient rich runoff from agricultural fields causes eutrophication of streams, reservoirs and wetlands, which results in dominance of highly productive plant and algal species and eventual extinction of oligotrophs and other aquatic organisms from the ecosystem. Intensification and modernisation of agricultural practices have led to severe decline in farmland biodiversity both at spatial and temporal scales (Benton et al. 2003). Large-scale monocultures characterised by cultivation of single cultivars of particular crops possessing one or more agronomically or economically important traits married to specific management practices has resulted in so called 'genetic erosion' (Cebolla-Cornejo et al. 2007; van de Wouw et al. 2010). Loss of pollinators and natural enemies of insect pests due to excessive application of agrochemicals has been well established (Kremen et al. 2002). On the other hand casually managed traditional agricultural systems, such as 'Dehesas' in Spain and the paddy farms of Thailand, some of which are thousands of years old, are good evidence of highly sustainable systems (Edwards et al. 1999). Such biologically diverse systems, despite producing lower yields of the main crop, have high overall productivity and are resilient to climatic extremes (Berg 2002). There is a growing understanding about the importance of biodiversity in sustainable agroecosystems (Hajjar et al. 2008). Losey and Vaughan (2006) estimated that the economic value of four ecological services (dung burial, pest control, pollination, and wildlife nutrition) provided by insects is at least worth \$57 billion annual, alone in the United States. Such estimates highlight the need to invest in conservation of ecosystems for the continued provision of these services.

5.4.1 Rice and Associated Flora and Fauna

Rice fields harbour a number of organisms throughout various growth stages, which range from minute organisms such as zooplankton to larger invertebrates, vertebrates (birds and mammals) and various plant (weed) species. The diversity of species present in a paddy field can vary depending upon various factors such as management intensity and production regime (Bambaradeniya et al. 2003). A preliminary study reported 77 species of invertebrates, 45 species of vertebrates and 34 weed species present in a paddy field in Sri Lanka during one cropping season. The greatest number of invertebrate species (68) was supported by vegetative and reproductive stages of crop growth (Aquatic phase) and application of insecticides considerably decreased their number (Bambaradeniya et al. 1998). It has been shown that repeated application of herbicides with a particular mode of action can result in shift of weed species composition (Bhullar 2004). In addition, it is worth mentioning that adoption of rice cultivation in new areas such as Indo-gangetic plains of north India (Punjab & Haryana) has resulted in severe changes in biodiversity and ecology of these areas.

5.5 Diminishing Natural Resources

Modern agricultural practices involve intensive use of high-energy inputs such as nutrients, irrigation water and fuel to operate farm machinery etc. As increasing population poses greater demand on agriculture for food, fibre and industrial products, assured supply of agricultural inputs is imperative. There are concerns about the feasibility of continuing current agricultural practices as we have already surpassed peaks of production for both petroleum and phosphorus. Therefore, alternative production practices need to be explored.

5.5.1 *Water and Land*

Water and land are the most important resources for production of any crop. Assured irrigation is even more critical for rice, as production of every kilo of unmilled rice requires about 2,500 l of water to be supplied to the field, less than half of which is used in evapotranspiration by the rice plant and rest is lost to seepage and percolation etc. (Bouman 2009; Zwart and Bastiaanssen 2004). Due to heavy water consumption, rice crop is well suited to humid tropical regions, where rainfall fulfils large proportion of the water requirement. Rice was introduced as a cash crop in north Indian states of Punjab and Haryana having well-developed canal irrigation system. As the canal irrigation system gradually declined and other resources such as power supply became available, there was a rapid shift towards groundwater extraction with tube wells to fulfil the irrigation demands (Raina and Sangar 2004). Currently, groundwater irrigation accounts for more than half of the irrigated area of Haryana and more than 73 % of the irrigated area of Punjab. During past 40 years (1970–2010), area under rice increased from 0.39 to 2.80 Mha in Punjab and from 0.3 to 1.2 Mha in Haryana and at present these two states contribute more than half of the total rice procurement by government of India (Statistical Abstract of Punjab and Haryana). Higher cropping intensity, cultivation of rice and extraction of water at a faster rate than it could be replenished by natural means (such as percolation of rain water) resulted in rapid decline in water table. In recent years, some places have seen alarmingly high rates of decline e.g. in central Punjab water table declined about a meter annually between 2000 and 2006 (Sudhir-Yadav et al. 2013). Not only the availability of water has been hampered but also the quality of water has been significantly deteriorated. Contamination of potable groundwater and freshwater resources due to agricultural runoff and leaching of agrochemicals is a widespread problem across agricultural zones (Agrawal et al. 1999; Scanlon et al. 2007).

As growing population poses greater demand on food resources, mainly in the rice consuming regions of Asia, the production of rice has to be increased, which would be limited by the increasing scarcity of water. Novel methodologies need to be developed to cultivate rice with minimal use of water and alternative cropping systems and cultivation technologies need to be designed to fulfil the human needs in a sustainable manner.

5.6 Stagnant Yields

Adoption of nutrient responsive, high yielding semi-dwarf varieties gave a significant push to the food grain production in the second half of past century, ushering many nations into green revolution. This big leap was made possible with coordinated efforts of researchers, farmers and policy institutions. The package of new crop varieties coupled with availability of fertilizers, irrigation and credit for purchase farming equipment and inputs resulted in miraculous increase in food production termed as 'green revolution'. But in the recent years the yields have shown either a stagnant or even decreasing trend (Kataki et al. 2001). The yield increase achieved during the second half of past century was largely input based and the production technologies ignored sustainability factors such as soil health and ecosystem balance. Sustained growth in yields could only be achieved by revising the production systems in order to include practices that promote sustainability.

Higher food demand posed by ever increasing population and the ongoing environmental changes makes this task even more challenging. A number of biotic (insects and disease etc.) and abiotic (droughts, floods and salinity etc.) stresses pose a major constraint to rice productivity. Ongoing global climatic changes are predicted to increase the intensity and frequency of extreme weather events, which will not only have direct effect on productivity but may also increase the incidence of disease and insect outbreaks. Therefore, adaptive technologies need to be developed and deployed on field.

5.7 Improved Varieties

The yield increase attained in the past decades is largely credited to the high yielding rice varieties produced through conventional and molecular breeding (Khush 1999). Rice mega variety IR8 was released in 1966, having a yield potential of 10 t ha⁻¹, it became the single variety of any food grain cultivated on the largest area. Since then, significant progress was made to develop rice varieties with improved grain quality and/or resistance to multiple disease and insects, however, the yield potential could not be improved much further (Brar and Khush 2013). Further enhancing the yield potential of rice varieties is a big challenge currently facing rice researchers. A number of different approaches have been proposed for the improvement of productivity related traits such as increasing photosynthetic efficiency and increasing sink size. Modern biotechnological tools offer better understanding of genes controlling yield related traits and are being employed, for example in molecular marker assisted breeding and identification of genes and quantitative trait loci controlling yield promoting factors.

In view of the changing environmental conditions and diminishing water resources, development of climate resilient rice varieties is being realised as critically important research priority. To cope with increased incidence of extreme weather

events and resource limitation, we need rice plants, which can tolerate drought and/or flood (submergence) conditions and stresses of extreme temperatures, salt and nutrient deficiencies. As rice varieties are known to differ for their potential to influence greenhouse gas emissions, combining the traits for lower emissions into the varieties with higher productivity would be equally important to improve the sustainability of our rice cultivation systems (Aulakh et al. 2000). A number of different breeding and biotechnological strategies are being used to develop improved rice varieties which are summarised by Brar and Khush (2013). In addition, participatory plant breeding approaches have also been shown to offer opportunities for involving farmers' skills in the selection of cultivars with desired traits (Forster et al. 2013).

5.8 Efficient Water Management

Effective management of limited water resources is the key to solve problem of water scarcity. This would require coordinated efforts to reduce the water losses during distribution e.g. in canal irrigation system; and improved rainwater harvesting and groundwater recharge. For example, a well maintained canal system can potentially reduce seepage losses by 30–40 % (Wachyan and Rushton 1987). Various strategies for the recharge of groundwater could be implemented at community or landscape level (Hira 2009). Assured availability of canal irrigation during peak time such as transplanting of paddy may significantly reduce dependence on groundwater. As construction of larger dams has ecological and economic consequences, small to medium sized reservoirs made at various locations within the canal system may ensure quick availability of water when needed and also will stabilise groundwater by promoting recharge (Grewel and Dar 2004). Sudhir-Yadav et al. (2013) suggest conjunctive use of surface and ground water for sustained supply of irrigation water and to stabilise the water table.

5.9 Agronomic Practices

A number of simple agronomic practices when effectively adopted at larger scales could potentially offer useful solutions to some of the major challenges. For example, in case of traditional practice of flood irrigation of puddle transplanted rice, well levelled fields may significantly increase irrigation efficiency. Laser levelling of fields prior to transplanting is advocated as a useful technique to achieve adequate flow rates and even distribution of water by flood irrigation, which could potentially reduce irrigation input to rice by more than 300 mm and could also result in higher yields (Humphreys et al. 2010; Sidhu and Vatta 2010). Adjusting the peak demand period i.e. from transplanting to establishment with the onset of monsoon rains

could save significant amounts of irrigation water. As a measure to halt decline in groundwater table, since 2009 it has been forbidden by law to plant paddy before 10th of June in Punjab and before 15th of June in Haryana, because mid April to mid June are the months with highest evaporative demand (Hira 2009; Humphreys et al. 2010).

Several alternative planting and water management methodologies have been tried and standardised in order to save water and energy without compromising yield. Dry/direct seeded rice offers a suitable alternative to puddle transplanted rice in most soil conditions and saves water and energy required for multiple tillage and puddling operations (Sudhir-Yadav et al. 2013). Similarly, alternate wetting and drying can save 10–40 % of irrigation water compared to flood irrigation, depending upon the soil type. In alternate wetting and drying, fields are irrigated to a shallow depth (50 mm) and subsequent irrigation is only applied after previous water has disappeared (Humphreys et al. 2010). The key is to maintain the soil moisture status at a threshold level of 15 kPa in the root zone, which could be easily determined by simple tools such as ‘PAU tensiometer’ and ‘field water tube’ (Sudhir-Yadav et al. 2013). However, for the success of this method, assured source of irrigation is very important. A number of other techniques such as sprinkler and drip irrigation have also been tried for cultivation of rice, but with limited success.

5.9.1 System of Rice Intensification (SRI)

SRI is a package of various practices proven to be effective at various stages of plant growth. All these practices combined together over the whole crop growth period enhance resource use efficiency by maximising output with minimum inputs. Initially developed in Madagascar, SRI practices involve planting of single, healthy and younger (8–12 days old) seedlings in carefully levelled fields, application of organic manures, intermittent irrigation to develop extensive root system and non-chemical (mechanical) weed management. Proponents of SRI have reported 50 % higher yields with significant savings on water (50 %) and other inputs such as seed and pest control (Sinha and Talati 2007; Uphoff 2003). Despite the advocated capability to dramatically enhance production and resource use efficiency particularly in resource-poor conditions, the advantage of SRI over so-called established best management practices (BMP) is contradicted (Deb et al. 2012; Dobermann 2004; McDonald et al. 2006; Stoop et al. 2002).

A similar methodology traditionally used by ‘Basmati’ rice farmers in the Uttarakhand state of India is called “Thakuli” (plate) method. The name “Thakuli” refers to the planting distance, which is equivalent to the size of a plate, i.e. about 30 cm. In this method, 3–4 seedlings are transplanted per hill using 12–15 days old nursery. Significant yield increase has been reported compared to conventional systems, perhaps due to the higher number of tillers per hill (Pande and Akermann 2008) (Fig. 5.2).



Fig. 5.2 Hand weeding of paddy fields at a research farm in India

5.9.2 Nutrient Management

The modern high yielding varieties are highly nutrient responsive and to exploit their full yield potential, soil nutrients need to be supplemented with external input in the form of fertilizers and manures. However, the application of fertilizers has ecological costs, such as groundwater pollution due to leaching, eutrophication of freshwater sources with runoff and greenhouse gas emissions. To minimise these drawbacks while maximising the benefits, a number of methodologies are suggested; e.g. altering the timing and dose of N-fertiliser, deep placement of urea briquettes or super-granules, slow release fertilisers etc. (Singh et al. 1995). Besides, soil incorporation of green manures prior to transplanting, use of compost or organic manures and Azolla-rice system may be more suited for organic production of rice. However, application of both inorganic fertilizers and organic manures may have consequences for greenhouse gas emissions, which also need to be considered while designing sustainable production strategies (discussed below). Integrated nutrient management could offer an optimal solution to maximise nutrient use efficiency while minimising negative ecological impacts.

5.9.3 *Pest Control*

Insect pests, diseases and weeds pose major constraints to maximise productivity. Agrochemicals offer economical and relatively easy control of these problems and are therefore widely used across farming systems. However, their non-judicious use has resulted in development of pesticide resistance, undesired effects on non-target organisms, residues in the food products, contamination of environment and agrobiodiversity losses. Judicious use of agrochemicals can help in effective control of pests while reducing the environmental and health costs. Instead of preventive application of pesticides, which is many times unnecessary, observation of pest populations and application only at threshold levels not only minimises environmental risks but also maximises net returns and promotes biodiversity. This could be achieved by providing information and appropriate training to the farmers via authentic sources. In many cases, farmers are dependent on advice from pesticide dealers or advertisement campaigns by the agrochemical companies, so there always remains a scope of misguiding farmers to sell more products.

Insect and disease resistant varieties could significantly cut-down yield losses while reducing agrochemical inputs and genetic engineering as well as molecular breeding techniques have potential to speed up the process of development of such varieties. However, perceived environmental concerns related to genetically modified crops pose a question.

5.10 Mitigation of Greenhouse Gas Emissions

Agricultural emissions of two important greenhouse gases namely N_2O and CH_4 exhibited a 17 % increase between 1990 and 2005 (Smith et al. 2007). As anaerobic soils in lowland rice form a major source of global CH_4 emissions, several strategies have been tried to mitigate CH_4 emissions from paddy fields. Mid-season drainage is often suggested as a useful strategy to reduce methane emissions from paddy fields (Yan et al. 2009). However, it has two major drawbacks: first, while drainage reduces methane emissions, it substantially increases emission of N_2O (Cai et al. 1997), which has 15 times higher global warming potential than CH_4 ; second, once the paddy field has been drained, often it becomes nearly impossible for the farmer to re-flood it due to lack of enough irrigation water, and this may result in yield loss.

Application of nitrogenous fertilizers, particularly ammonium sulphate, is suggested to mitigate CH_4 emissions (Bodelier et al. 2000), but it has an important trade-off with N_2O emissions. Cai et al. (1997) found a substantial reduction in methane emissions from paddy fields where ammonium sulphate (42–60 %) and

urea (7–14 %) were applied at the rates of 100 and 300 Kg N ha⁻¹; however, the emissions of N₂O significantly increased with the dose of N-fertilizer and were particularly high in case of ammonium sulphate. Moreover, in terms of per unit N content, ammonium sulphate is generally more expensive than urea, but does not offer any added net profit to the farmer. This delicate balance between emissions of CH₄ and N₂O continues to pose a major challenge till a suitable strategy is identified. Various rice varieties have been compared and were found to vary in their effect on emissions of CH₄ and N₂O (Aulakh et al. 2000), but so far no commercial variety has been released that combines both high yield and low emissions.

5.11 Policy and Economics Tools

Economic and political institutions can play a vital role towards the adoption of sustainable technologies as they previously did for green revolution. The enforcement of law forbidding paddy plantation before 10th of June in Punjab and before 15th of June in Haryana was a significant step towards halting the declining groundwater table (Hira 2009). Strong political-will is critical for the deployment of new technologies on field. For example, provision of subsidies such as free power to farmers is criticised for being partially supportive for the overexploitation of ground water resource in some parts of India (Johl 2013; Sudhir-Yadav et al. 2013). Similarly, assured purchase price offered by government of India for major food grains (rice and wheat) and not for other crops is believed to act against the efforts to diversify cropping systems (Johl 2013).

5.12 Diffusion of Information

Timely and appropriate dissemination of information among the stakeholders – in this case farmers and agriculture extension workers – is very important to harvest the full benefit of technologies developed by researchers. Suitable infrastructure for the diffusion of information needs to be in place. A combination of modern and traditional means could be successfully used for this purpose. For instance, print and electronic media could be used to educate farmers regarding long-term benefits of sustainable technologies. Articles in newspapers and discussions on television or radio can also help in developing acceptance towards government policies such as delayed planting date or not to burn crop residues. Village meetings and farmer fairs offer an opportunity to the farmers for a direct interaction with experts. Mobile phone technology being easily accessible even in remote locations is increasingly being used to deliver information to farmers. Timely and precise information on weather forecast could help farmers in decision making for various farming activities e.g. timely forecast of rain could save farmers from unnecessary irrigation and subsequent problem of water logging.

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Chapter 6

Rice, Wheat and Maize Biofortification

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Abstract Cereals are the major energy source for humans throughout the world and hold a prominent position in a balanced diet to meet up carbohydrate demand of the body. Most of the cereals are deficient in micronutrient and vitamins and continuous dependency on cereal based diets resulted in human malnutrition. Biofortification is a novel concept defined as the enrichment of micronutrients through conventional plant breeding and modern biotechnology. In this post genomic era, an enormous amount of genetic information is available for staple food crops. This genetic information could be used to improve nutritional quality of the staple food crops to provide nutritional requirements. During last few decades, cereal biofortification research has been significantly contributed to reduce malnutrition around the world. Knowledge of precise phenotyping and genetics of the traits are prerequisite before starting of a genetic biofortification program. The inheritance of major micronutrients and vitamins in rice, wheat and maize were reported to be polygenic and in most of the cases the quantitative trait loci were mapped in the genome. A few commercial cereal cultivars are developed so far using genetic biofortification technique. The ongoing biofortification programs

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are more competitive as newer perspective of food matrix came into picture. In this article we reviewed an overview of current global cereal biofortification efforts, global malnutrition issues, and the promise of biotechnology techniques to improve cereals as a whole food solution to combat global malnutrition issues.

Keywords Biofortification • Bioavailability • Biomolecules • Cereals • Food matrix • Food legumes • Genomics • Malnutrition • Molecular mapping • Nutritional deficiency • Sustainable food systems

Abbreviations

cDNA	Complementary Deoxyribonucleic Acid
CGIAR	Consultative Group on International Agricultural Research
ILs	Introgression Lines
QPM	Quality Protein Maize
QTL	Quantitative Trait Loci
RILs	Recombinant Inbred Lines
SCN	Standing Committee on Nutrition
UN	United Nations
WHO	World Health Organization

6.1 Introduction

Millions of deaths occur every year due to micronutrient malnutrition (Anonymous 2008). Ironically, obesity and diet-related non-communicable diseases mostly are concomitant with micronutrient deficiencies (Bouis and Welch 2010). Chronic, non-communicable diseases including diabetes, heart disease, and cancer result in over 36 million deaths around the world annually (WHO 2005). These chronic diseases, whose development is largely caused by high-energy, micronutrient-poor diet, are not isolated to developed countries, having caused for more deaths than infectious diseases throughout the world (except parts of Africa) in recent years (UN 2011). Both micronutrient deficiencies and associated diseases are by poor nutritional content of staple cereals and food legumes. Therefore, enrichment of micronutrients in staple food crops is of much importance for nutrition security of human beings, especially, in the developing countries.

Anemia is one of the few common nutritional disorders affecting living and survival of humans. According to the World Health Organisation (WHO), around two billion of the world populations are anemic. Anemia is the body condition where

hemoglobin concentrations in the blood are below thresholds. The threshold varies between gender and ethnicity. There are generally two groups of causes which may induce anemia: dietary iron deficiency, vitamin B₁₂, vitamin A, and/or folate deficiency and infectious diseases such as malaria, hookworm infections, schistosomiasis, and hereditary disorders such as thalassemia (WHO 2007). Iron deficiency may or may not be accompanied by anemia but always has an important negative impact on human health. The effect of Fe deficiency is more pronounced in case of pregnant women and children (WHO 2007). Sometimes this has severe consequences leading to the mortality of the newly borne children or even the fetus during prenatal stage. Children with acute iron deficiency show mental retardedness, laziness and, in case of working persons, reduced capacity to work (WHO 2007).

Zinc deficiency has a prominent role in affecting human health, particularly in children and mothers. For detailed review, see Gibson (2012). Zinc deficiency is reported to cause child mortality by way of increasing the vulnerability to other diseases like diarrhea and pneumonia (SCN 2004; Jones et al. 2003). Women in the developing countries are also increasingly affected with Zn deficiency and associated illnesses (Caulfield et al. 1998). A clinical trial indicated that Zn supplementation in pregnant women's diet has positive impacts on immune status and morbidity of newborns (SCN 2004; Osendarp et al. 2003).

Maternal vitamin A deficiency has profound effect on maternal mortality risk, infant morbidity, and depressed immunological responses (SCN 2004). The initial findings showing correlation between vitamin supplementation and reduced mortality (SCN 2004; Christian et al. 2000; West et al. 1999) are inspiring and need to be studied in detail.

Populations in developing countries including Southeast Asia and Africa consume mostly cereal-based diets. The 'Green revolution,' which averted the occurrence of famine in many areas, was a major factor leading the current cereal based diet (Bouis and Welch 2010). Cereal diet in most cases provides adequate calories but insufficient quantities of micronutrients to the human body. As a consequence of adopting this cereal based cropping systems, many countries observed a drastic increase in malnutrition cases (Welch and Graham 1999) (Table 6.1).

Table 6.1 Status of micronutrient deficiency in the world

Deficiency	Prevalence in the world	Consequences
Iron	111,000 maternal deaths	Reduced cognitive ability. Anaemia, maternal mortality (SCN 2004)
Vitamin A	140 million preschoolers and 7 million pregnant women suffering from deficiency	Night blindness, Xerophthalmia, Keratomalacia & immune system failure (SCN 2004)
Zinc	2 billion people suffering from deficiency	Infectious diseases, poor child growth, maternal mortality, reduced birth weight (WHO 2005)

6.2 Biofortification – Approach to Reduce Nutrient Deficiency

Traditional plant breeding and crop production technologies have immensely contributed over the past to increase the production of food crops, thereby ensuring the food security around the world. One such example is the Green Revolution. Green Revolution dramatically increased the cereal crop production as in wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), and rice (*Oryza sativa* L.), and this contributed to fulfill the protein and energy requirements of the developing nations (WHO 2013). With time the production of cereal crops increased, whereas the production and the consumption of traditional micronutrient-dense crops like pulses decreased leading to major micronutrient deficiencies such as Fe, vitamin A, and iodine deficiencies among the developing nations (WHO 2013). This example shows that the food security solely may not be sufficient to combat leading nutritional problems. As Chandra (1990) describes, “Taken together, micronutrient deficiencies affect a far greater number of people in the world than protein-energy malnutrition”.

Biofortification is the process of enrichment for micronutrients and vitamins in edible parts of staple food crops using traditional plant breeding and modern biotechnology techniques (Welch and Graham 2004). Research shows that consuming biofortified staple food crops significantly increases the target micronutrient concentration in populations (Welch and Graham 1999). Biofortification is identified as a sustainable, less costly, and feasible strategy which aims to reduce micronutrient deficiencies in low-income populations (Welch and Graham 2004; Nestel et al. 2006; Meenakshi et al. 2007). Although agronomic biofortification, is the enrichment of micronutrients through soil amendment, foliar spray or irrigation. Agronomic biofortification though is a common practice it appears to be less effective in the case of some mineral elements such as iron (Fe) and have little promise in reducing deficiencies of such elements in human populations (Bouis and Welch 2010; Tagliavini et al. 2000; Tagliavini and Rombola 2001). On the other hand, genetic biofortification could be a cost-effective way to provide access to nutritional foods to people who are living in the remote underprivileged parts of the world as it requires one time initial investment, and seed multiplication through plant breeding interventions would make it sustainable.

Therefore, food security along with nutrient security should be a key consideration in designing modern-day food systems. Traditional plant breeding and modern biotechnology techniques should be exploited, not only to produce high-yielding starchy crops, but also, to produce micronutrient-dense traditional legumes. One such example is the iron biofortification of food crops (Fig. 6.1).

6.3 Traits of Interest

Initial biofortification research efforts have focused on few micronutrients, Fe and Zn. Over time, other micronutrients like Se and different vitamins like Vitamin A, Folic acid are gaining the attention. Generally, it was observed that genetic and

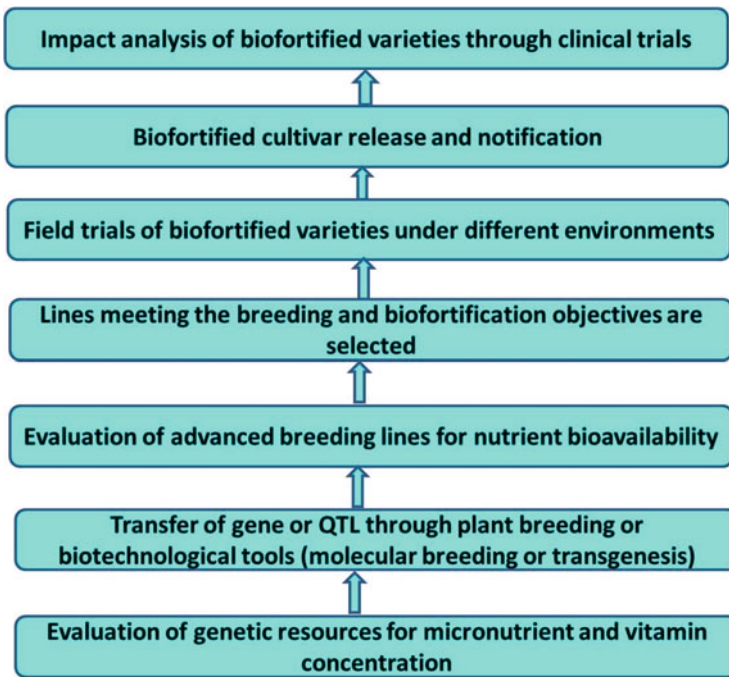


Fig. 6.1 Schematic diagram of multidisciplinary biofortification research. In this flow diagram the entire process of development of biofortified cereal varieties are shown. The process is initiated with selection of lines with high micronutrient or vitamin concentration with optimum food matrix profile. The donor gene or quantitative trait loci is then transferred to the desired line through crossing or marker assisted selection (MAS) or transgenesis. After going through multiple cycle of selections through different filial generations the stabilized advanced breeding line fulfilling the breeding objectives is released and notified for mass cultivation. The impact analysis of released biofortified varieties can be done by clinical trials

environmental interactions are more pronounced in case of secondary metabolites than micronutrients *per se*. These are the traits for which precise phenotyping facility is the prerequisite to improve research conclusions. It has also been observed that these traits mostly have polygenic inheritance and low heritability. This is the reason why molecular breeding tools like marker assisted breeding could be a viable option to breed for these traits. In major cereals like rice, wheat and maize inheritance of micronutrient are today known (Table 6.2). But in case of food legumes more efforts are required to gain genetic knowledge about these traits, particularly number of gene(s)/Quantitative Trait Loci (QTL) controlling these traits and their effects (Thavarajah et al. 2014). The biofortification techniques are separate for traits controlled by single or few genes and by polygenes. Polygenic traits are improved by selecting for region of major importance within plant genome controlling the trait which is known as a major QTL.

Table 6.2 Mapping and tagging of gene(s)/QTL controlling micronutrients in rice, wheat and maize recombinant inbred lines (RILs)/introgression lines (ILs) mapping populations

Crop	Micronutrient	Gene(s)/QTL(s)	Population	Reference
Rice	Fe	7 QTLs for iron concentration	RIL population between two <i>indica</i> varieties, Zhengshan 97 × Minghui 63	Lu et al. (2008)
	Fe	1 QTL for Fe concentration	RIL population between, Bala (<i>indica</i>) × Azucena (<i>japonica</i>) cultivars	Norton et al. (2012)
	Fe	2 QTL for Fe concentration	ILs derived from a cross between, Teqing (<i>indica</i>) and the wild rice (<i>Oryza rufipogon</i>)	Garcia-Oliveira et al. (2009)
	Zn	6 QTLs for zinc concentration	RIL population between two <i>indica</i> varieties, Zhengshan 97 × Minghui 63	Lu et al. (2008)
	Zn	6 QTLs for zinc concentration	RIL population between, Bala (<i>indica</i>) × Azucena (<i>japonica</i>) cultivars	Norton et al. (2012)
Wheat	Fe	2 QTLs for iron concentration	RIL population derived from a cross between wheat cultivars, Xiaoyan 54 and Jing 411.	Xu et al. (2012)
		2 QTLs for iron concentration	RIL population from a cross between <i>T. boeoticum</i> accession pau5088 × <i>T. monococcum</i> accession pau14087	Tiwari et al. (2009)
	Zn	2 QTLs for zinc concentration	RIL population derived from a cross between wheat cultivars, Xiaoyan 54 and Jing 411.	Xu et al. (2012)
		1 QTL for zinc concentration	RIL population from a cross between <i>T. boeoticum</i> accession pau5088 × <i>T. monococcum</i> accession pau14087.	Tiwari et al. (2009)
Maize	Fe	3 QTLs for iron concentration and 10 QTLs for iron bioavailability	RIL population between B73 × M17.	Lung'aho et al. (2011)
	Fe	3 QTLs for iron concentration.	RIL population derived from a cross B84 × Os6-2en.	Šimić et al. (2012)
	Zn	Single QTL for zinc concentration	do	Šimić et al. (2012)

In short, knowledge of precise phenotyping and genetics of the traits are prerequisite before starting of a genetic biofortification program.

6.4 Available Genetic Variability for Nutritional Traits

Genetic variation exists for micronutrient concentration (Welch and Graham 2004; Bouis 2003; Graham et al. 2001) and plant breeding tools can improve for β -carotene, iron, zinc, and other micronutrients in food crops by making selection for appropriate genetic material (Nestel et al. 2006). Mostly micronutrient density and yield is positively correlated unlike protein content and yield. Also, it is possible to combine multiple nutrition traits in single cultivar along with high yield (Nestel et al. 2006).

Knowledge of genetic diversity for a particular trait of interest can be a predictive tool for estimating genetic variation in segregating generation or hybrid progeny. For development of molecular markers linked with the high concentration of micronutrient loci initial large scale evaluation of available germplasm sets of different food legumes is essential. After identification of potential genotypes, suitable mapping population can be developed for particular traits (Talukder et al. 2010; Beebe et al. 2000). For the development and advancement of mapping for micronutrient traits state of the art phenotyping facility for micronutrient analysis is required. In case of few studies so far conducted to map and tag the gene(s)/QTL controlling micronutrient status in legumes and model plants mostly found to be having quantitative mode of inheritance and resulting in identification of gene(s)/QTL capable of explaining moderate amount of phenotypic variation for micronutrient concentration. Inheritance of Fe concentration had been identified in major cereals and food legumes, also associated QTL are mapped in the respective genomes; rice (Anuradha et al. 2012), wheat (Xu et al. 2012; Tiwari et al. 2009), maize (Qin et al. 2012; Lung'aho et al. 2011) (for Fe and Zn), Sompong et al. 2012 (for phytic acid in mungbean), Blair et al. 2005; Gelin et al. 2007; Cichy et al. 2009; Blair et al. 2010a, b; (for Fe and Zn in common bean), Sankaran et al. 2009 (for several mineral elements in *Medicago truncatula*), Waters and Grusak 2008 (for several seed mineral contents in *Arabidopsis thaliana*), Walker et al. 2006 (for phytic acid in soybean). Major biofortification projects are undergoing in various laboratories for introgression of such QTL from donor sources to the high yielding backgrounds.

6.4.1 Cereals: Iron, Zinc and Vitamin A

It was quoted many times that biofortification research had been initiated in cereals with the first report of Opaque mutant of maize which later became the source to develop Quality Protein Maize (Taylor et al. 2012); in this review we will be concentrating on micronutrients and vitamins. To have a look into the protein biofortification efforts and effects in maize reader may go through these articles, Hugo et al. 2010; Akalu et al. 2010; Gunaratna et al. 2010.

6.4.1.1 Iron (Fe)

The Fe content ranges from 8.8 to 16.7 mg/kg in milled rice (*Oryza sativa*) (samples included both *indica* and *japonica* types) and from 11.8 to 22 mg/kg in brown rice (Gregorio et al. 2000). Among different accessions of wheat it ranges from 21.26 to 30.59 mg/kg in bread wheat (*Triticum aestivum*), in macaroni or durum wheat (*Triticum durum*) it ranges from 21.91 to 25.60 mg/kg (Tiwari et al. 2009). *Aegilops ventricosa* accessions were reported up to the concentration of 93.52 mg/kg of Fe (Tiwari et al. 2009). Among the other species and relatives of wheat like *T. boeoticum*, *T. dicocoides*, *T. arraraticum*, *Aegilops longissima*, *Aegilops Kotschy*, *Aegilops peregrine*, *Aegilops cylindrica*, *Aegilops ventricosa*, *Aegilops ovate* Fe concentration ranged from 22.29 to 93.27 mg/kg (Tiwari et al. 2009). The target for plant breeders is to increase the iron concentration in wheat to have a significant effect on nutrition is approximately 30 mg/kg for Fe. This estimate is based on the assumption of daily consumption of 300 g per capita and 90 % of nutrient retention during milling (Cakmak et al. 2010). Fe concentration in maize (*Zea mays* L.) kernel reported to be between 16.8 and 24.4 mg/kg among the tested late maturing cultivars (Oikeh et al. 2003).

Further, target concentration of iron is highly influenced by factors which affect bioavailability of Fe either positively or antagonistically. Anemia or iron deficiency could be tackled by two ways, either increasing the Fe concentration in grain and or by increasing the Fe absorption in the human gut. For increasing higher gut absorption bioavailability of Fe food matrix becomes the important consideration (Hunt 2003; Plaami 1997; Bravo 1998). It was reported that an increase of 67 % Fe bioavailability in hybrid corn (*Zea mays* L.) over the control (44.2 vs. 26.5 µg ferritin per mg of total protein) was associated with a steep 12 % increase in total Fe concentration, which exhibits the influence of other food matrix factors (Hoekenga et al. 2011). The increase of Fe density in food grain alone is insufficient to address the menace of Fe deficiencies around the world (Lucca et al. 2001). Promoters and inhibitors of Fe absorption within the food matrix should be considered with regard to the bioavailability of Fe in food crops (Cook et al. 1972). Phytic acid or inositol hexakismonophosphate, the primary storage form of phosphorus in plants, is the potential inhibitor of Fe absorption in the human gut (Turnbull et al. 1962). Chelation of Fe^{2+/3+} by phytic acid is the reason of reduced absorption of Fe, on the other hand ascorbic acid antagonist this activity of phytic acid depending on the molar concentrations (Siegenberg et al. 1991). Other important inhibitors are fiber, heavy metals, and certain groups of polyphenols (Glahn et al. 2002). Another important group of promoter is prebiotics (Johnson et al. 2013) which modulates microbiome composition and activity in positive way and thereby increasing the gut absorption of micro-nutrients. It was reported using *in vitro* study simulating human colon conditions that cereal and legume flours carrying pre-biotics had considerable positive impact on the colonic microbiota (Maccaferri et al. 2012).

Phytoferritin gene family which regulates the Fe uptake and transport within the plant are investigated by many workers (Strozycki et al. 2010; Curie and Briat 2003) and it was reported to be a 24 subunit protein capable of carrying upto 4,500 Fe atoms (Harrison and Arosio, 1996). The expression of Ferritin genes is affected by

various stresses and factors; excess iron concentration is one of them. It is also important to mention that expression of plant ferritin gene is controlled at the transcriptional level rather than at translational level as observed in case of animals (Lescure et al. 1991). It was also found that ferritin Fe is less affected (Lönnerdal 2009) with other food matrix factors like phytic acid, ascorbic acid, calcium as studied Caco-2 cells. The above mentioned feature makes phytoferritins as potential candidate for inclusion into the biofortification research. The most studied phytoferritin is soyabean ferritin. It was reported that soyabean ferritin iron is better absorbed in human gut (Lönnerdal 2009). To expression of soyabean ferritin gene in cereal like rice had been made, for example, cDNA clone for soybean ferritin gene as was expressed in rice (*Oryza Sativa*) endosperm tissues caused an increase of two to three fold iron concentration in grain (Goto et al. 1999; Lucca et al. 2001; Qu et al. 2005; Vasconcelos et al. 2003). Similarly in wheat (*Triticum aestivum*) two Ferritin genes (*TaFer1* and *TaFer2*) has been isolated and characterized and it was found that biofortification of wheat is possible through the overexpression of one the ferritin genes in wheat endosperm tissue (Borg et al. 2009).

6.4.1.2 Zinc (Zn)

To improve the Zn content of cereal grains, it is important to know the baseline concentration and variability of its deposition in the grains. The Zn concentration ranges from 13.5 to 58.4 mg/kg in milled rice (*Oryza sativa*) (samples included both *indica* and *japonica* types) and from 20.4 to 34.7 mg/kg in brown rice (Gregorio et al. 2000). Over half of the total Zn in rice is present in the endosperm, and polishing will have less severe effect on Zn concentration than other mineral elements (Lu et al. 2013). Zinc ranges from 14.88 to 29.33 mg/kg in bread wheat (*Triticum aestivum*), from 13.68 to 19.60 mg/kg in durum wheat (*Triticum durum*) (Tiwari et al. 2009). *Triticum dicoccoides* accessions were reported up to the concentration of 66.15 mg/kg of Zn (Tiwari et al. 2009). Among other relatives of wheat (*Aegilops longissima*, *Aegilops Kotschyi*, *Aegilops peregrine*, *Aegilops cylindrica*, *Aegilops ventricosa*, *Aegilops ovate*) Fe concentration ranged from 22.29 to 58.61 mg/kg (Tiwari et al. 2009). Screening of Zn concentration in wheat cultivars (current and out-of-production) and land races was conducted; wild relatives contained 3–4 times more Zn in some cases (Gregorio et al. 2000; Chhuneja et al. 2006; Tiwari et al. 2009). In maize (*Zea mays*), it was reported to range from 16.5 to 24.6 mg/kg as forty-nine late maturing cultivars were studied (Oikeh et al. 2003). Bioavailability of zinc is less affected by food matrix factors as compared with iron (HarvestPlus Brief 2006).

While genetic enhancement of seed Zn concentration is a viable approach, it is important to note that the concentration of Zn in grains can be greatly regulated using agronomic practices. Foliar application of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5 %) to commonly grown wheat cultivars nearly doubled the concentration of Zn in the flour. While agronomic biofortification of cereals is successful, genetic biofortification is nevertheless a useful complement in improving Zn concentration in cereal grains.

6.4.1.3 Vitamin A

To combat vitamin A deficiencies, biofortified staple cereals rice and maize have been developed. Vitamin A is synthesized from several provitamin A carotenoids alpha-carotene, beta-carotene, and beta-cryptoxanthin, beta-carotene being the most effectual provitamin, and therefore the most targeted. After its development, 'golden rice' was introduced as the first transgenic rice to provide beta-carotene (Beyer et al. 2002). Using an *Agrobacterium*-mediated transformation, four genes originating from other plant sources were introduced to code for the necessary enzymes in the beta-carotene pathway. Later, it was discovered that one of the genes used coded for the flux-limiting enzyme, and was replaced with the homolog gene *psy* from maize (Paine et al. 2005). This transformation increased the beta-carotene concentration in rice up to 35 µg/g.

Variation in carotenoid concentration in maize exists, allowing for selection through breeding techniques (Harjes et al. 2008). Carotenoids in maize are, from most abundant, lutein, zeaxanthin, beta-carotene, beta-cryptoxanthin, and alpha-carotene. Capturing natural variations in beta-carotene concentrations, provitamin A biofortified lines have been developed (Harjes et al. 2008).

Variability for biofortification related traits such as iron, zinc and vitamins are present among the rice, wheat and maize and selection for these traits among these crops are possible to initiate any genetic biofortification program.

6.5 Biofortified Cultivars Developed

Under the umbrella of HarvestPlus program of Consultative Group on International Agricultural Research (CGIAR) the initial phase of biofortification programs included six food crops, common bean, cassava, maize, rice, sweet potato, and wheat. The initial phase investment resulted in many success stories like orange sweet potato cultivars with high levels of β -carotene rich (over 200 mg/g) (Bouis and Islam, 2012), and beans with improved agronomic traits and grain type and 50–70 % more iron have been bred through conventional breeding (Nestel et al. 2006). Though conventional breeding still encompassing major part of HarvestPlus program research theme transgenic approaches are in some cases necessary and are being used. The most popular and earliest example of success story of transgenic biofortification research is development of Golden Rice or β -carotene rich rice. Golden rice transgenic lines have been under field trial in Philippines (transgenic of RC-28), Bangladesh (transgenic of BRRI Dhan-29) and will certainly helping to fight against iron deficiency. Recently, iron rich high yielding pearl millet cultivar, ICTP 8203-Fe has been launched as a result of collaborative effort between HarvestPlus program and Nirmal Seeds, a Hyderabad, India based seed company. The ongoing HarvestPlus phase included more food crops and a few more food legumes, especially lentil which is a regular component of everyday diet in major regions in South and South-east Asia. Present biofortification efforts in wheat

concentrate on use of higher source of iron and zinc, for example *Triticum spelta*, *T. diccon* × *Aegilops tauschii* derivatives, landraces (Velu et al. 2012). Biofortified wheat lines developed through backcrossing are being evaluated in field trials to observe their performance (Velu et al. 2012).

A few commercial cereal cultivars are developed so far using genetic biofortification technique and presently in almost all major cereals different biofortification programs for different micronutrient and vitamins are ongoing at national and international plant breeding institutes.

6.6 Challenges for the Breeder and Nutritionist

Iron and folate bioavailability of a staple food crop is mainly depends on the food matrix factors. The concentration of the promoter and inhibitor compounds in any food crop is influenced by both genetic and environmental factors. Before breeding for iron or any micronutrient the absorption and within plant transport of micronutrients is important and to be studied in more detail (Fig. 6.2). Modern plant breeding and molecular biology tools now make it possible to reduce antinutrients, such as phytic acid or increase the concentrations of promoter substances, such as beta-carotene, ascorbic acid and phytoferritin in plant foods. Promoters and inhibitors of Fe absorption within the food matrix must be considered with respect to the bioavailability of non-heme Fe in a food crop (Cook et al. 1972). Phytic acid (PA), nearly omnipresent in plants and used as the primary phosphorous (P) storage, inhibits absorption of Fe in the gut (Turnbull et al. 1962). Other inhibitors include fiber, heavy metals, and certain polyphenols and tannins (Glahn et al. 2002).

Enrichment with prebiotics, beta-carotene, ascorbic acid and phytoferritin has been shown to enhance the bioavailability of non-heme Fe in human plant-based diets (Welch 2002). Prebiotics improve Fe bioavailability as a result of biological fermentation of short chain polymers by natural microflora present in the colon (Yeung et al. 2005). Addition of vitamin A or beta-carotene can improve Fe bioavailability from plant-based foods (e.g., rice, wheat, corn) (Garcia-Carsal et al. 2000). For example, analysis of lentil (*Lens culinaris* Medicus) food matrix components, along with cell culture and preliminary human nutrition studies, revealed clear mineral absorption promoter and inhibitor roles in modulating the levels of mineral bioavailability. Lentils contain high levels of Fe absorption promoters, such as prebiotics and beta-carotene, and are low in antinutrients, such as phytic acid and polyphenols (Thavarajah and Thavarajah 2012). It is reported that molar ratios of phytic acid:Fe above ten lead to reduced human Fe bioavailability (Ariza-Nieto et al. 2007).

Increased micronutrient and vitamin content in biofortified cereal cultivars is the ultimate goal in any biofortification program. In recent time's bioavailability of nutrient becoming a more important issue along with increased concentration of nutrients in food. Analysis of food matrix factors in more details will surly convert future biofortification programs more precise and rewarding.

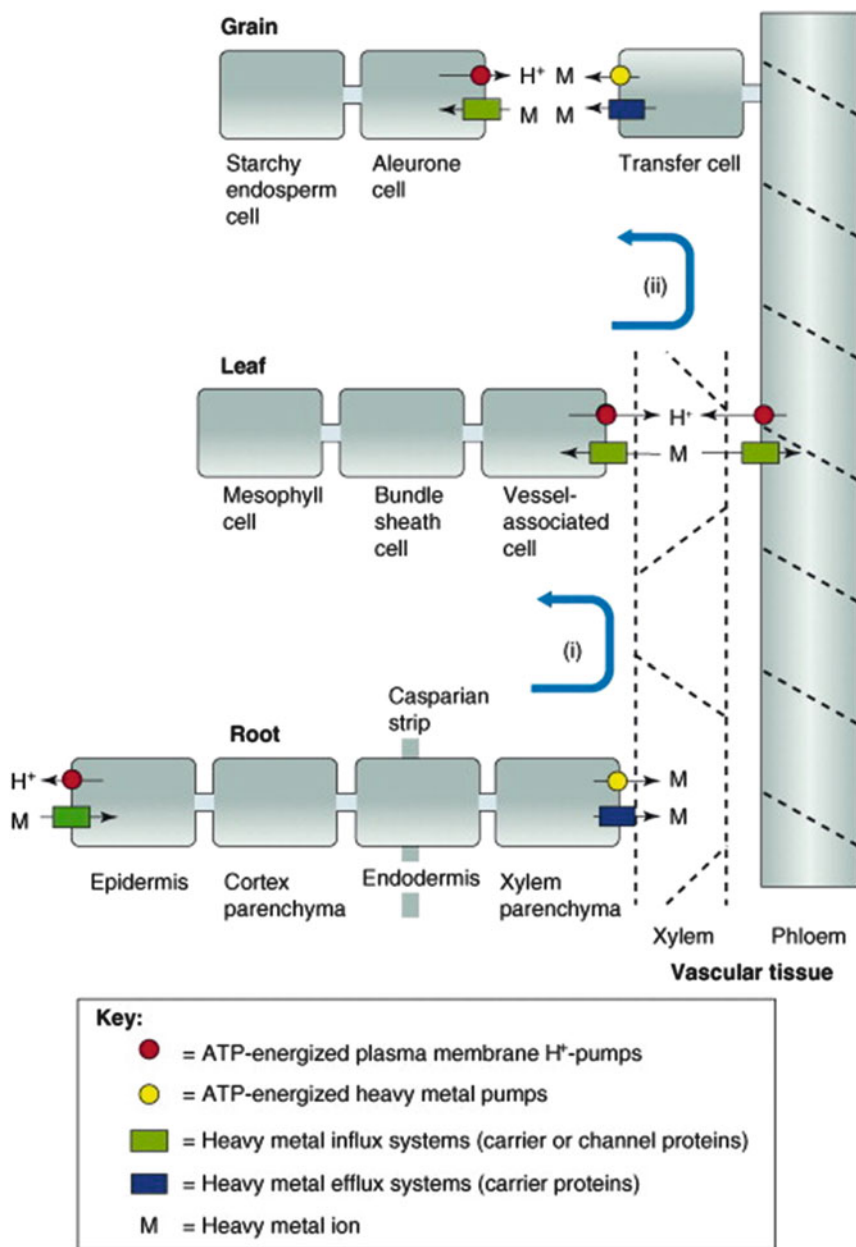


Fig. 6.2 Overview of the transport pathway of a metal ion from soil to grain. To reach the grain, nutrients enter and leave the symplastic continua of cells within the plant several times. For a divalent cation such as Zn, the bottlenecks that limit efficient transport seem to be the control points where Zn has to exit living cells. Plant roots contribute to making metal ions more available for uptake by two strategies. First, depending on the nutrient status of the soil, roots acidify the rhizosphere through

6.7 Conclusion

Since initial years of biofortification research have been concentrating on the major micronutrients but coming years would also include various secondary metabolites like vitamins and other important micronutrients, for example, Folic acid and Selenium (Se). Folic acid is the synthetic oxidized form of naturally occurring folates. It consists of a p-aminobenzoic molecule linked to a pteridine ring and one molecule of glutamic acid. Food folates, which exist in various forms, contain additional glutamate residues, making them polyglutamates (Bailey and Gregory 2006). Folate is a water soluble B vitamin, usually participates in numerous biochemical reactions involving one carbon transfer, for example, purine and pyrimidine synthesis as well as amino acid interconversions (Krumdieck 1990), prevention of chromosome breakage and hypomethylation of DNA (Fenech 2001). This role of folates reduces the risk factors leading to cancer and also plays critical role in regulating homocysteine status, an important risk factor for cardiovascular ailments (Pancharuniti et al. 1994). Lower levels of plasma folate are correlated with various health risks, neural tube defects is the prime one with a few other congenital defects (Berry et al. 2000). This is more important to mention that in many cases folate deficiency is also associated with macrocytic anemia (Boushey et al. 1995). There were reports of strong association of anemia due to folate deficiency along with iron deficiency (WHO 2007). The effective evaluation of plant materials for these many nutritional traits model cell culture or animal study would be a prerequisite as far as bioavailability or food matrix factors are concerned. Caco-2 cell culture or *Galus galus* or Pig model (*in vitro* and *in vivo* models) as well as various biotechnological tools would certainly contribute to our better understanding of the role of micronutrients and vitamins in human nutrition in coming days.

←
Fig. 6.2 (continued) plasma membrane H⁺-ATPases, proton pumps in the cell membrane (Palmgren 2001). An increased proton concentration in the soil results in cation exchange and release of divalent metal ions that are tightly bound to soil particles. Second, roots actively secrete low-molecular-weight compounds that can function as metal chelators in the soil. Depending on species, these include organic acids and phytosiderophores (Romheld and Marschner 1986; Takahashi et al. 1999). Secreted phytosiderophores are known to facilitate uptake of Fe by graminaceous plants, and recently, strong evidence has pointed to a similar role of these compounds in Zn uptake (Suzuki et al. 2008; Suzuki et al. 2006). (i) After metal ions have entered root cells by transport-protein-mediated processes, they migrate by diffusion to xylem parenchyma cells, from where they are actively transported out of the symplast into the dead xylem. In the shoot, they are again taken up by vessel-associated cells in the leaves. (ii) During the period of grain filling, metal ions are remobilized in leaves, from where they are exported and transported via the phloem to the fruit. Here they are exported by transfer cells from the mother plant and are subsequently taken up into the developing seed by specialized metal transport proteins (Reproduced with permission from Palmgren et al. (2008)). N.B. H⁺-ATPases are the proton (H⁺) translocating ATPases; required for uptake of most metabolites in plants, fungi and many protists. ATPases are the ATP hydrolyzing enzymes

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Chapter 7

Sorghum Fungal Diseases

Isha Sharma, Nilima Kumari, and Vinay Sharma

Abstract Sorghum is a vital life-sustaining food crop for humans and livestock. Sorghum is also a biofuel crop of growing importance. Moreover sorghum is drought and heat tolerant, and is an important crop in arid and semi-arid regions where major cereals fail to grow successfully. Sorghum thus constitutes a major staple foods for the world's poorest people. Sorghum is an important food crop within traditional low input, cereal-based farming systems in Africa, where 41 % of the world-wide area of this crop's production is located. However, the crop suffers from low yields due to a number of biotic stresses.

Sorghum is distinguished among other cereals by its unusually broad range of diseases which is one of the most important constraints to its production. The most destructive sorghum diseases reported are fungal diseases which are widespread globally and result in huge losses in yields both in terms of the quantity and quality of the grains. Anthracnose, turicum leaf blight, charcoal root rot and other fungal diseases are epidemics in many parts of the world. They cause severe crop losses depending upon the crop stage, susceptibility of cultivar and the prevailing environmental conditions. Food security issues have prompted many of the national as well as international programmes such as International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India, African programmes which actively encourage farmers to grow sorghum. Whilst traditional landrace materials showed some tolerance to many of these problems, often by escaping disease through late maturity associated with photoperiodicity, or a tall growth habit, the improved materials are frequently of short to intermediate height and early maturation and therefore more susceptible to disease problems, particularly fungal diseases.

Knowledge on disease epidemics and characterization of pathosystems is particularly important for long term disease management to achieve significant impact in reducing aspects of poverty and food and security in sorghum based semi arid cropping systems. This chapter reviews the current status of information on the most common fungal diseases of sorghum and discusses various aspects including disease significance and symptoms, genetics of resistance and host pathogen interaction and, management of disease. Besides the new approaches in disease

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control such as Quantitative Trait Loci mapping and transgenic approaches have also been discussed. Molecular markers and quantitative trait loci (QTL) analysis have been useful for locating the resistance gene(s) on the genome of sorghum and to carry out marker-assisted selection for some of the fungal diseases. It has provided foundation for fine-mapping and advancing the molecular breeding for others. Furthermore, the markers tagged to QTL regions can be used to enhance the sorghum breeding program for fungal resistance through marker-assisted selection and map-based cloning. As we have the whole genome sequence available of sorghum, it will be helpful to advance the molecular breeding by facilitating the positional cloning as well as marker-assisted selection. This will also be instrumental in determining the function of disease resistance genes available in the sorghum genome.

Keywords Sorghum bicolor • Macrophomina phaseolina • Colletotrichum graminicola • Exserohilum turcicum • Sorghum bicolor (L.) Moench

Abbreviations

AFLP	Amplified fragment length polymorphism
BSA	Bulk segregant analysis
<i>C. gloeosporioides</i>	<i>Colletotrichum gloeosporioides</i>
<i>C. sorghi</i>	<i>Claviceps sorghi</i>
<i>E. turcicum</i>	<i>Exserohilum turcicum</i>
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agricultural Organization Statistical Databases
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
ISAVN	International Sorghum Anthracnose Virulence Nursery
ITS	Internal transcribed spacer
MAS	Marker assisted selection
<i>P. sorghi</i>	<i>Peronosclerospora sorghi</i>
<i>P. sorghi</i>	<i>Peronosclerospora sorghi</i>
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PR	Pathogenesis Related
QTL	Quantitative trait loci
RAPD	Random amplified length polymorphism
RFLP	Restriction fragment length polymorphism
RIP	Ribosome-inactivating proteins
<i>S. cruenta</i>	<i>Sporisorium cruenta</i>
SCARs	Sequence-characterized amplified regions
sp	Species
TLB	Turcicum leaf blight
TLB	Turcicum leaf blight

7.1 Introduction

Plant diseases result from an interaction between the host plant and the pathogen. The genetics of such host-pathogen interactions are of considerable biological interest and of the greatest importance in developing disease control strategies through resistance breeding efforts. The variation in susceptibility to a pathogen among plant varieties is due to different kinds and numbers of genes for resistance that may be present in each variety. The effects of individual resistance genes vary from large to minute; depending on the importance of the functions they control (Agrios 1997). *Sorghum bicolor*, an important world crop, is used for food (as grain and in sorghum syrup or "sorghum molasses"), fodder, the production of alcoholic beverages, as well as biofuels. Sorghum is an important food crop in Africa, Central America, and South Asia and is the fifth most important cereal crop grown in the world (FAO 2011). It is drought and heat tolerant and thus an important crop in arid regions where major cereals do not produce sufficient yields. More than half of the world's sorghum is grown in semi-arid tropics of India and Africa, where it is a staple food for millions of poor people (Iqbal et al. 2010). However, the crop is vulnerable to fungal diseases resulting in decreased grain quality and yield loss.

Grain sorghum can be affected by a wide variety of diseases, which can cause serious losses of production and profitability. Some diseases occur across a wide area, at levels that do not cause extreme concern in any given field, but may add up to a considerable net loss due to their prevalence. Some diseases are readily detected due to easily identifiable symptoms that noticeably reduce yields. Plant diseases can mutate and new pathotypes or races can arise in a short time period. Due to the changing nature of plant diseases and yearly fluctuations in environmental conditions, sorghum production for plant disease incidence needs to be regularly monitored. This article has primarily reviewed the information on different aspects of fungal pathosystems of Sorghum and their current status.

7.2 Biology and Economic Importance of Sorghum

Sorghum {*Sorghum bicolor* (L.) Moench ($2n=2x=20$)} is a tropical C4 monocot plant belonging to family Gramineae (Fig. 7.1). This genus has many species and subspecies. There are several types of sorghum, including grain sorghums, sweet sorghums (for syrups), grass sorghums (for pasture and hay) and broomcorn. It is indigenous to Africa and Asia and is believed to have been domesticated in Sub-Saharan Africa particularly in the Nile basin from where it spread to other parts of the world (Kimber 2000). The species bicolor has wild and domesticated races which are *bicolor*, *caudatum*, *durra*, *guinea* and *kafir* (Doggett 1988). Sorghum is predominantly a self-pollinated crop with outcrossing rates between 3 and 15 %, depending on genotype and environment. Genetically, *Sorghum* is a functional diploid although there is evidence of tetraploid origin (Doggett 1988).

It is the fifth major cereal crop in the world after wheat, rice, maize and barley (Doggett 1988; FAOSTAT 2003). Also it is a subject of plant genomics research



Fig. 7.1 Different cultivars of Sorghum plant growing in the greenhouse (Image: courtesy ICRISAT, India)

based on its importance as one of the world's leading cereal crops, a bio-fuel crop of high and growing importance, a progenitor of one of the world's most noxious weeds, and as a botanical model for many tropical grasses with complex genomes (Paterson 2008).

World sorghum production is about 60 million tons annually from a cultivated area of 46 million ha. More than 35 % of sorghum is grown directly for human consumption and the rest is used primarily for animal feed and forage, alcohol production and industrial products (Awika and Rooney 2004). Sorghum performs relatively better than the other warm-season cereals in areas where the annual rainfall is in the range 500–700 mm per year. It is an important crop even in East Africa where the average annual rainfall is greater than 700 mm per year.

Thus, sorghum a tropical cereal domesticated along the Ethiopia as recently as 1,000 BC, is a source of energy, protein, vitamins and minerals for millions of poorest people. Sorghum production is important for human consumption and animal feed besides using as a biofuel. Furthermore it is a semi-subsistence enterprise that offers smaller returns than other investments such as livestock.

7.3 Production Constraints

Grain sorghum yields are especially low in Eastern Africa countries such as Uganda (15,106 Hg/Ha) as compared to yields in the United States (43,548 Hg/Ha) and well below the genetic potential (FAOSTAT 2009). These low yields of

Table 7.1 Estimated losses of grain sorghum production due to major plant diseases ('millions' mg yr⁻¹) in African countries

Country	Anthracnose	Charcoal Rot	Grain Mold	Late Blight	Mildew	Nematodes	Rust	Smut
Ethiopia	232	191	209	143	191	143	289	399
Kenya	15	8	20	20	6	1	15	13
Malawi	14	1	17	1	3	2	6	1
Mozambique	4	4	100	4	42	4	135	4
Rwanda	16	1	7	18	8	6	18	20
Tanzania	11	13	71	61	23	4	20	111
Uganda	33	9	36	13	10	5	21	47
Zambia	8	1	11	0	1	3	2	9
Zimbabwe	3	6	3	7	4	7	11	6
Total	333	233	471	266	288	173	515	546

Source: Intsormil.org/smscientificpubs/(Sorghum Atlas), 2006

sorghum are attributed to a number of biotic stress such as weeds e.g. *Striga* sp. (Ebiyau and Oryokot 2001), pests e.g. stem borers which include *Chilopartellus swinhoe*, *Busseolafusca fuller*, *Diatraea* sp., *Eldanasaccharina walker*, *Ostrinianubilalis hubner*, and *Sesamia* sp. (Sharma et al. 2007) and diseases e.g. *Turcicum* leaf blight (Nkonya et al. 1998; Tilahun et al. 2001). Diseases of sorghum, like those of other crops, vary in severity from year to year and from one field to another, depending upon environment, causal organisms, and the host plant's resistance.

The major diseases that affect sorghum include *Turcicum* leaf blight, (*Exserohilum turcicum* (Pass.) K.J. Leonard and E.G. Suggs {teliomorph: *Setosphaeria turcica* (Luttrell) Leonard and Suggs}); downy mildew {*Peronoscleropora sorghi* (Western & Uppal) Shaw}; anthracnose (*Colletotrichum sublineolum* Henn.) (De Vries and Toeniessen 2001) and sorghum smuts, including covered kernel smut (*Sporisorium sorghi* Ehrenberg (Link)); loose smut (*Sphacelotheca cruenta* (Kuhn), Langdon and Fullerton), long smuts (*Tolyposporium entrenbargii* (Kuhn) Pattouillard), charcoal rot (*Macrophomina phaseolina*) and leaf spot (*Gleocercospora sorghi*). Estimated losses of sorghum grain production due to major plant diseases in African countries are shown in Table 7.1.

7.4 The Sorghum Fungal Pathosystems

7.4.1 Anthracnose

Anthracnose persists as one of the most destructive diseases of sorghum. The pathogen, *Colletotrichum graminicola*, affects the foliage and inflorescence (including grain), as well as the stalks.

7.4.1.1 Significance and Epidemics of Anthracnose

Losses of up to 50 % are not uncommon. Sorghum anthracnose occurs worldwide, but is more typically observed in tropical and subtropical regions where frequent rainfalls and high humidity contribute to the development and spread of the disease (Pande et al. 1994; Thakur and Mathur 2000). Another species of *Colletotrichum* is *sublineolum* (Henn) which is responsible for sorghum anthracnose of stalks (Crouch et al. 2006), *graminicola* and *C. gloeosporioides* cause foliar and grain anthracnose of sorghum. Foliage infection is more widely prevalent, but at times, grain anthracnose occurs in severe form. To understand whether isolates infecting foliage (lamina and midrib) and that infecting grain are different, isolates from foliage and grain were compared for pathogenicity and vegetative compatibility (Thakur and Mathur 2000). The results suggest that although isolates from grain, leaf lamina and midrib can infect both foliage and grains, the organ specificity for infection does exist, and that resistance to grain and foliar infection is likely to be governed by different genetic factors.

Environmental factors play a major role in the development of *Colletotrichum* diseases. Lenné (1992) reported that the optimum conditions for the development of the most important species of *Colletotrichum* affecting legumes were temperatures of 18–28 °C with relative humidities of greater than 90 %. In general, the primary factor influencing the development of disease is the availability of water, as high humidity is essential for the sporulation of most *Colletotrichum* species. Other key factors such as temperature, often interact with factors such as leaf surface wetness, humidity, light or competitive microflora (Rotem et al. 1978; Royle and Butler 1986). *Colletotrichum graminicola*, *C. sublineolum* and *C. falcatum* overwinter in soil in decaying plant residues as mycelium, acervuli, melanized hyphopodia, sclerotia and microsclerotia (Crouch and Beirn 2009; Sukno et al. 2008).

7.4.1.2 Symptoms and Etiology

Symptoms include a foliar phase, stalk rot, and colonization of the panicle including the grain. Foliar anthracnose can be recognized by a range of symptoms including an "oval leaf spot," diffuse or patchy foliar colonization, and midrib infection. The range in foliar symptoms may be caused by variation in the pathogen, host resistance (Pastor-Corrales and Frederikson 1980), or physiologic status of the host following infection. Weakened, chlorotic, stressed, or senescent leaves of susceptible cultivars are rapidly colonized by the pathogen. Infection and colonization of the panicle frequently result in losses in both quality and quantity of grain (Reyes et al. 1969). Anthracnose stalk rot, occasionally referred to as red rot, develops after the other phases and ultimately results in lodging. Symptoms of stalk rot can be diagnosed by their irregularly mottled or marbled pattern of colonization (Le Beau and Coleman 1950). Variation in pigment and rate of colonization are related to host-plant color and susceptibility to anthracnose.

Typical symptoms may be noted as absence of mycelium in the pale areas surrounded by pigmented, sparsely colonized areas (Fig. 7.2).

7.4.1.3 Physiological Races

Colletotrichum graminicola is a highly variable species. Evidence for races exists from observations made in the United States and from other regions of the world (Harris and Johnson 1967; Pastor-Corrales and Frederikson 1980; Nakamura 1982). These and other reports clearly demonstrate differences in pathogenicity of isolates between and within locations. Uniquely different populations of *C. graminicola* are suggested by the differential reaction of sorghum entries in the International Sorghum Anthracnose Virulence Nursery (ISAVN) from Nigeria, Brazil, and the USA (King and Frederiksen 1976). Nakamura (1982) identified five races of *C. graminicola* using five differential cultivars of sorghum obtained from single spore isolates gathered from a number of diseased plants throughout Brazil. Nakamura's work confirmed that several physiological forms of *C. graminicola* are present not only within an area, but between locations as well. Races of *C. graminicola* have been reported in the United States too (Ali and Warren 1987). These observations suggest that the species is dynamic and affected by directional selection pressure by host resistance genes, and that, profoundly different races exist in different regions

Fig. 7.2 Anthracnose disease infestations on sorghum foliage showing patchy colonization (Image courtesy: ICRISAT, India)



of the world. These facts present challenging problems when using host resistance as the sole measure of control in areas with severe anthracnose. In India, Pande et al. (1991) characterized the pathogenicity of nine sorghum isolates of *C. graminicola* from different locations in 30 sorghum cultivars and concluded that the nine isolates were distinct.

7.4.1.4 Genetics of Resistance to *Colletotrichum* in Sorghum

Sources of resistance to anthracnose in sorghum have been reported by many workers (Harris and Johnson 1967; Rosenow and Frederiksen 1982). These resistant sorghums have been used as parents in breeding programs or as replacements for susceptible cultivars. Coleman and Stokes (1954) determined that separate but linked genes conditioned resistances to stalk rot (LsLs) and foliar anthracnose (LL) in the sweet sorghum cultivar Sart. The appearance of new races of *C. graminicola* attacking some of these originally resistant sorghums suggests that other alleles must be involved. Jones (1979) found that resistance to anthracnose was conditioned by one dominant gene for one parent and perhaps as many as three dominant genes for another. Further it has been argued that environment plays a major role in the interpretation of the genetics of resistance (Jones 1979; Pastor-Corrales and Frederikson 1980).

In rice (*Oryza sativa*) and sorghum (*Sorghum bicolor*), flavonoid compounds have been shown to act as phytoalexins against *Magnaporthe grisea* and *Colletotrichum* sp., respectively (Snyder and Nicholson 1990; Kodama et al. 1992). The contribution of flavonoid phytoalexins to resistance against *C. sublineolum* in sorghum has been investigated by comparing the response of several sorghum cultivars that differentially produce 3-deoxyanthocyanidins (Wharton and Julian 1996; Lo et al. 1999; Basavaraju et al. 2009). These studies indicated that phytoalexin production in the resistant cultivars was not only more rapid but also more intense than in the susceptible lines. In addition, phytoalexin accumulation was associated with the distortion of fungal hyphae and restriction of fungal proliferation in the resistant cultivars and extensive fungal colonization of the tissue on the leaf blades of susceptible sorghum plants (Nicholson et al. 1987; Tenkuano et al. 1998; Lo et al. 1999).

Genetic transformation can be used to confer tolerance to plant diseases such as anthracnose. The tolerance can be developed by introducing genes encoding proteins such as chitinases and chitosanases that hydrolyse fungal cell wall. Particle bombardment was used to genetically transform a sorghum genotype, KAT 412, with *chitinase* (*harchit*) and *chitosanase* (*harcho*) genes isolated from *Trichoderma harzianum* (Kosambo-Ayoo et al. 2011). Microspectrophotometry was performed on intact, pigmented vesicle-like inclusions within living sorghum cells that were accumulating phytoalexins as a response to attempted fungal infection. The amounts of luteolinidin and apigeninidin in cells involved in the phytoalexin response at individual infection sites were also determined. The results of both analyses confirmed that at the infection site the deoxyanthocyani-

dins accumulate to levels in substantial excess of those required for inhibition of the fungus *C. graminicola* (Shih et al. 2007). Two cultivars which differed in their reaction to *C. sublineolum*, were examined for their phytoalexin response and expression of defense-related genes. In the incompatible interaction, fungal development in the host was contained during early stages of pathogenesis. When compared to the susceptible cultivar, defense responses in the resistant cultivar were characterized by a greater and faster accumulation of phytoalexins and an earlier induction of defense-related genes encoding chalcone synthase and pathogenesis-related protein PR-10. In the compatible interaction, the pathogen was able to colonize the host with the proliferation of primary and secondary hyphae. Moreover the resistant cultivar accumulated a complex phytoalexin mixture, including luteolinidin and 5-methoxyluteolinidin, neither of which was produced by the susceptible cultivar following fungal inoculation (Lo and Nicholson 1998). In another study, it has been shown that sorghum yellow seed1 (*y1*) encodes a MYB transcription factor that regulates phlobaphene biosynthesis. The phlobaphene act as the precursors of 3- deoxyanthocyanidins, Based on cosegregation analysis, functional *y1* gene has been genetically linked with resistance to *C. sublineolum* (Ibraheem et al. 2010).

7.4.1.5 Disease Management

Sorghum anthracnose is a disease of worldwide importance and host-plant resistance is the most practical method of disease management (Erpelding 2011). Resistance is available for both the leaf blight and stalk rot phases. However, hybrids showing resistance to anthracnose stalk rot often do not show resistance to stalk rots caused by other fungi. Seed protectant fungicide, thiram is labeled for control of seedling blight on sorghum (nu-distance.unl.edu/homer/disease/agron/sorghum/sranthrac.html).

As discussed in this section, anthracnose is a deleterious problem contributing upto 50 % losses or more causing leaf blight and stalk rot. By characterizing the genetic inheritance of resistance in sorghum and using gene transformation technologies for introducing the gene encoding PR proteins into sorghum imparts tolerance against anthracnose.

7.4.2 *Turcicum* Leaf Blight

Turcicum leaf blight is highly destructive and drastically affects sorghum grain and fodder yield as well as fodder quality (Ogliaril et al. 2007). It is a fungal disease that thrives under humid conditions (Frederikson 2000; Mohan et al. 2009) and is distributed all across the globe (Fig. 7.3). Estimated sorghum losses caused by TLB in Africa, Asia, and the Americas is up to or greater than 50 % in most susceptible sorghum varieties (Mittal and Boora 2005).

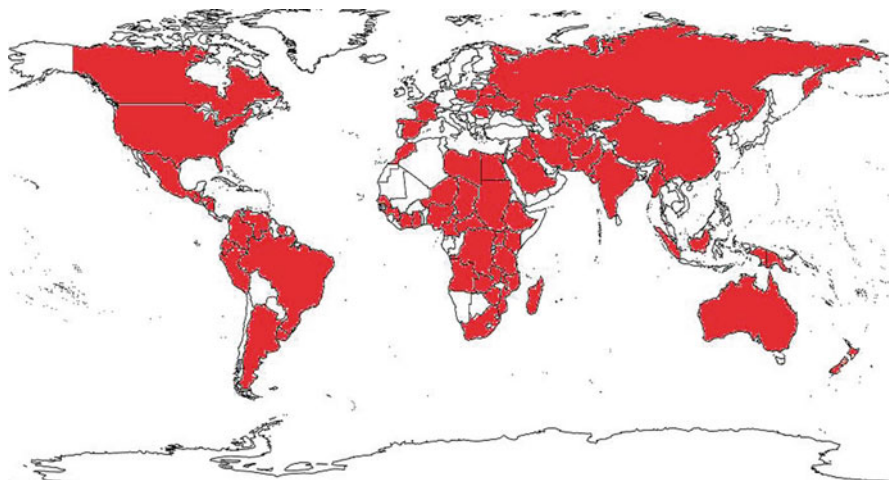


Fig. 7.3 Distribution of Turcicum leaf blight in the world

7.4.2.1 Significance and Epidemics of Leaf Blight

Turcicum leaf blight, caused by the ascomycete fungus *Exserohilum turcicum* (Pass) K.J. Leonard and E.G. Suggs, is one of the most destructive foliar diseases of sorghum (Carson 1995; Ogljaril et al. 2007). This pathogen has three major groups of hosts: maize (*Zea mays* L.) (Welz and Geiger 2000), sorghum (*Sorghum bicolor*) (Ngugi et al. 2000) and wild relatives of sorghum or maize including Johnson grass (*Sorghum halapense*), teosinte and other grass species (Esele 1995). *Turcicum* leaf blight causes yield losses of up to or greater than 50 % in most susceptible sorghum varieties (Mittal and Boora 2005) and is favoured by mild temperatures and humid weather with heavy dews (Narro et al. 1992). Pathogenic fitness and environmental conditions are important factors in determining disease development and epidemics of *E. turcicum* depends on its ability to infect, grow and sporulate (Leonard et al. 1989). Spore production and lesion development occur between 10 and 34.4 °C but optimum minimum daily temperatures for disease development are near or slightly below 16.1 °C with average daily temperature near 22.2 °C. This fungus can also produce thick walled spores called chlamydospores, which lengthen the survival time of the organism in soil. *Exserohilum turcicum* can survive from season to season as mycelia, sclerotia or chlamydospores on infected crop debris or in the soil (Casela et al. 1993). Disease epidemics are favoured by high rainfall and relative humidity, moderate temperatures, and the presence of large amounts of inoculum (Hennessy et al. 1990). Previous evidences on the epidemiology of the disease have indicated that leaf blight is often most severe on younger plants (Julian et al. 1994). However there is a little definitive information on mechanisms underlying these observations (Ngugi et al. 2000).



Fig. 7.4 Early symptoms of *Exerohilum turcicum* infection comprising slightly oval lesions and necrotic wild-type elongated lesions on sorghum plants (Image: courtesy ICRISAT, India)

7.4.2.2 Symptoms and Etiology

The most commonly observed symptoms of *Exerohilum turcicum* infection are long elliptical tan lesions that develop first on the lower leaves and then progress upwards. Symptoms can range from small cigar-shaped lesions to complete destruction of the foliage (Welz and Geiger 2000). The earliest symptoms of infection are slightly oval, water-soaked, small spots on the leaves that grow into elongated, spindle-shaped necrotic lesions. They may appear first on lower leaves and increase in number as the plant develops and can lead to complete blighting of the foliage (Richards and Kucharek 2006). Typical lesions are gray-green, elliptical or cigar-shaped and are typically 12 mm wide and 3–15 cm long with yellow to gray centres and red margins. Spore production causes the lesions to appear dark gray, olive or black (King and Mukuru 1994). Sometimes lesions are multiple-pointed at the tips. A lesion may or may not be surrounded by a dark reddish-brown purple border or a narrow band of water soaking (Degefu 1990; Kucharek 2000) (Fig. 7.4).

7.4.2.3 *Exerohilum turcicum* Physiological Races

The classification of *Exerohilum turcicum* into races is based on differential reactions of isolates to specific *Ht* loci for resistance to *Helminthosporium turcicum* (Bergquist and Masias 1974; Leonard et al. 1989). Five naturally occurring races of *E. turcicum* have been reported to overcome specific *Ht* resistant genes in the United States (Windes and Pederson 1991) and others have been reported from crosses of races in the laboratory (Fallah and Pataky 1994). In Uganda, mating type analysis revealed the occurrence of *MAT 1*, *MAT 2* and *MAT 1, 2* on sorghum (Ramathani

2010). Both the mating type and race differential study suggested a great potential of having more virulent races of *E. turcicum* in the future (Ramathani 2010). Earlier studies have suggested existence of races or isolates of differing pathotypes of *E. turcicum* on sorghum (Tarumoto et al. 1977).

7.4.2.4 Genetics of Resistance to *Exserohilum turcicum* in sorghum

In the *Turcicum* leaf blight pathosystem, resistance is controlled by mono- and polygenes (Lipps et al. 1997). In sorghum resistance to infection or damage is often characterised by pigmentation (Torres-Montalvo et al. 1992). The accumulation of phytoalexins (flavonoids) in sorghum affects the response to pathogen infection (Nicholson et al. 1987). It has been suggested that the type and quantity of anthocyanin flavonoids, 3-deoxyanthocyanidins (Aguero et al. 2002) produced in response to pathogen attack in sorghum may vary. There may be a common biosynthetic pathway for the production of the type of flavonoids required for plant colour and for those involved in hypersensitivity (Klein et al. 2001).

Changes in environmental conditions from season to season and the probable occurrence of strains of *E. turcicum* are important external variables that affect resistance scoring (Tarumoto et al. 1977). Inheritance of resistance to leaf blight was found to be controlled by a single dominant gene (Boora et al. 1999; Mittal and Boora 2005) with field inoculation being one of the satisfactory methods for such studies (Tarumoto et al. 1977). Studies were initiated to identify cultivars with good combining abilities that could provide promising crosses for 16 breeding programs showed heritability values for both double-crosses and diallel crosses in Uganda, indicating that progenic resistance was highly heritable (Olujong et al. 1996).

7.4.2.5 Disease Management

The disease is controlled by the use of resistant cultivars and by rotation. However, rotation is made less effective if infected grasses persist in fields or in field margins. High yielding and resistant cultivars and hybrids have been identified and are available to growers. The most appropriate and economical strategy to manage turcicum leaf blight of sorghum is through exploitation of host resistance, which is not only environmentally friendly but convenient to adapt at farmers' level. Screening of sorghum inbred lines against *E. turcicum* and evaluation of sorghum genotypes against *E. turcicum* is an effective strategy. Disease management through host plant resistance is the most effective strategy, but this requires information on population biology of the pathogen and the effect of environment (Thakur et al. 2007).

In summary, turcicum leaf blight affects several cultivated hybrids and reduces the grain yield by 70 %. The disease has attained economic status around the world and continued to be a major constraint in sorghum production. Hence, understanding the epidemiology and population structure of turcicum leaf blight in fungal sorghum pathosystem is the area mainly to be focused.

7.4.3 Charcoal Rot

Charcoal rot, caused by *Macrophomina phaseolina* (Tassi) Goid, is the most common and probably also the most important root and stalk rot disease of sorghum prevalent in Asia, Africa and Mediterranean Europe (Tarr 1962). Dhingra and Sinclair (1977, 1978) have provided comprehensive information on the biology of *M. phaseolina* and the epidemiology and control of the diseases it causes in many plant species. *M. phaseolina* has been reported on four continents with a wide host range affecting more than 500 cultivated and wild plant species (Mihail and Taylor 1995) that include economically important crops such as soybean, cotton, maize, sorghum (Cloud and Rupe 1991; Su et al. 2001). Charcoal rot is frequently associated with periods of drought, reducing crop productivity and incomes (Perez-Brandán et al. 2012). Severe epidemics of charcoal rot of sorghum in Karnataka (India) were reported by various workers (Anahosur and Rao 1977; Hiremath and Palakshappa 1994).

7.4.3.1 Significance and Epidemics of Charcoal Rot

Macrophomina phaseolina is an emerging and devastating fungal pathogen that causes significant losses in crop production under high temperatures and drought stress. An increasing number of disease incidence reports highlight the wide prevalence of the pathogen around the world and its contribution toward crop yield suppression (Muchero et al. 2011).

7.4.3.2 Symptoms and Etiology

A variety of symptoms are associated with charcoal rot. These includes root rot, soft stalks, lodging of plants, premature drying stalks and poorly developed panicles with small and inferior quality grains (His 1956). Lodging of the plants (Fig. 7.5) the most striking and common indication of the disease is caused by the disintegration of pith and cortex by the pathogen and the vascular bundles are profusely covered with tiny black sclerotia of pathogen, giving a charcoal rot appearance. Generally the plants senescence and lodge due to stem breaking at or just above ground level (Reed et al. 1983). When inoculum is present, the occurrence of charcoal rot in a particular area is greatly influenced, like most plant diseases, by environmental conditions. It may be widespread in some years and localized or even absent in others. Diversity in *M. phaseolina* is due to the heterokaryotic condition of mycelium as well as the presence of two asexual sub-phases, one saprophytic (*Rhizoctonia bataticola*) where microsclerotia and mycelia are mainly produced and another pathogenic (Beas-Fernández et al. 2006) where microsclerotia, mycelia and pycnidia are produced in host tissues.

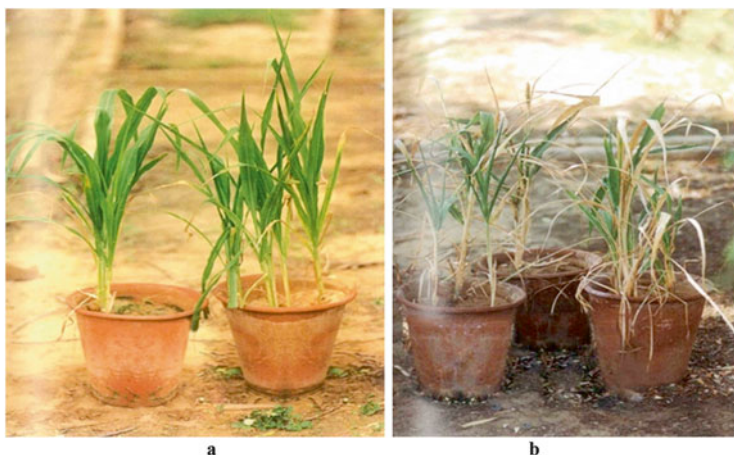


Fig. 7.5 One month old plants of *Sorghum bicolor*; (a) control and (b) infected by *M. phaseolina* showing lodging of the plant

7.4.3.3 Physiological Races

Macrophomina phaseolina is highly variable in pathogenicity and mycological characteristics. The fungus, a plurivorous pathogen, can affect 75 different plant families and about 400 plant species (Dhingra and Sinclair 1977).

Pathogen variation and physiological and specialization of *M. phaseolina* isolates are not known in charcoal rot of sorghum. However adaptations to other methods to assess pathogenicity or reaction to charcoal rot isolates have been published. For example, good correlations among seed of sorghum (Manici et al. 1995) or seedling inoculation and detached-leaf method have been found (Bañuelos-Baladrán and Mayek-Pérez 2008) as well as the use of toothpicks for assessing disease under controlled drought-stressed conditions or soil infestations by using colonized PDA or microsclerotia (De la Peña-Devesa et al. 2009). Plant pathogenic isolates of *M. phaseolina* have shown variation in morphological and physiological characteristics (Pearson et al. 1986) as well as in the pathogenicity or host specificity (Reyes-Franco et al. 2006 and Arias et al. 2011). Genetic variation has also been detected among *M. phaseolina* isolates using random amplified polymorphic DNA (RAPD) which identified an association between *M. phaseolina* and the plant-host origin of the isolates (Purkayastha et al. 2006; Rajkumar and Kuruvinashetti 2007).

7.4.3.4 Genetics of Resistance to *Macrophomina phaseolina* in Sorghum

Though resistance to this disease has been indicated to be of qualitative nature (Rosenow and Frederiksen 1982), polygenic nature of resistance has also been reported. The genetics of charcoal rot resistance in sorghum as indicated by

non-lodging of inoculated plants was investigated through combining ability analysis (Jahagirdar 2007). The results of the study indicated that charcoal rot inherited as recessive character and is controlled by a set of five pairs of gene exhibiting trigenic ratio depending on the parents involved in the cross. Jahagirdar (2007) have studied the role of sugars and phenols in charcoal rot resistance of sorghum. High level of sugar and phenols in stalk of the sorghum genotypes may be attributed to the resistance mechanism against charcoal rot and may be taken up as supporting criteria in identifying sources of resistance in addition to other character. Sharma et al. 2014 have studied the response to *Macrophomina phaseolina* attack, in sorghum plant and production of antifungal genes was induced. Based on this information, we can make genetically modified varieties of sorghum capable of constitutive production of defense related genes in plants to reduce the risk of damages and losses in plants.

7.4.3.5 Disease Management

The ideal and most effective control strategy for charcoal rot is to prevent drought stress from predisposing plants to infection. This can be done by proper management of the soil-plant-water system. Cultural practices that reduce pathogen inoculum in soil and that increase water availability and use by plants (e.g., plant density, rate of nitrogen fertilization, use of varieties with different rooting characteristics, and crop rotation) have been suggested as possible measures of reducing drought-stress-related diseases (Cook and Papendick 1972). Such measures have been successful in controlling fusarium foot rot of wheat (Cook 1980). Drought resistance as an indirect method of charcoal rot control raises the obvious and important question: will genotypes that resist drought also resist charcoal rot? Presently, there is insufficient knowledge of the interactions of drought stress, the charcoal rot pathogen, and the host.

To recap, charcoal rot is a major disease in the dryer regions of the world and appears to be especially destructive on high yielding cultivars under moisture stress and other pathogens. To develop high yielding and durable charcoal rot resistant varieties a better understanding of the life cycle of fungus, its interaction with the sorghum at ecological, genetic and physiological level, that is regulation of the expression of disease related genes is fundamental issue for the sorghum improvement.

7.4.4 Other Diseases

7.4.4.1 Zonate Leaf Spot

Zonate leaf spot, caused by the fungus *Gleocercospora sorghi* (Bain & Edgerton ex Deighton) is an important foliar disease of sorghum (Thind 2005) and commonly occurs in all humid and hot regions of the world. Zonate leaf spot is common

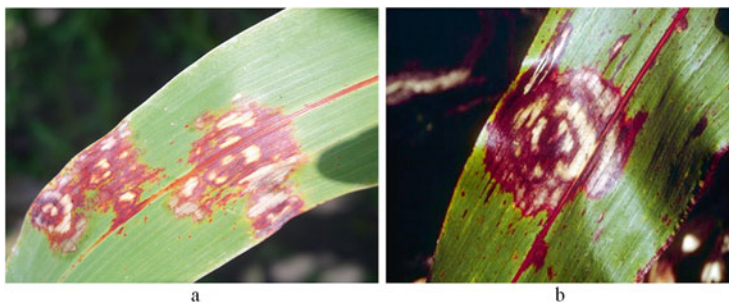


Fig. 7.6 Development of zonate leaf spot on sorghum; (a) spot enlargement and (b) mature symptoms becomes circular on the interior of the leaf (Image: courtesy ICRISAT, India)

throughout the sorghum producing areas of the USA occurring in high rainfall years or periods of high rainfall. Severe disease on sorghum seedlings may result in defoliation and even death of affected plants. Abundant spotting on leaves of older plants may result in poorly filled grain as a result of foliage destruction.

The disease spreads through spores produced in sporodochia. Black sclerotia are formed in necrotic lesions, which play a major role in the survival and initial spread of the pathogen. The secondary infection is caused by conidia. The disease zonate leaf spot are of the major constraints for the production of forage sorghum as they adversely affect the fodder yield and quality (Ghongale et al. 2003). Other host plants for the fungus that causes zonate leaf spot include corn, sudangrass, sorghum- Sudangrass hybrids, johnsongrass, broomcorn, napiergrass, sugarcane, bentgrass, bermudagrass, and possibly other grasses.

The first visible symptoms are the appearance of small non-diagnostic lesions on the lower leaves. As the lesions mature they become circular or target shaped on the interior of the leaf and semicircular on the leaf margins. Lesions elongate, run together, and whole leaves may be blighted. When the weather is favorable the disease progresses up the plant and lesions may occur on all leaves of the plant (Kutama et al. 2010) (Fig. 7.6).

Generation mean analysis was carried out for ten crosses between two resistant and two susceptible parents to find the genetic basic of resistance to zonate leaf spot disease in forage sorghum (Grewal 1988). Duplicate type epistasis was detected for the inheritance of this disease. Resistance to this disease revealed overdominance. Appropriate breeding plans were suggested to exploit the disease resistance (Grewal 1988). Nonadditive gene action was important for resistance to anthracnose and zonate leaf spot (Sharma et al. 2000). High phenol content was reported in resistant genotype of sorghum for resistance against *Gloeocercospora sorghi* (Ghongale et al. 2003).

Due to cost, fungicide applications for the management of zonate leaf spot are not feasible in grain production. Residue management by crop rotation is the most feasible disease management option. Crop rotation, deep tillage, and clean cultivation are recommended to destroy residues of susceptible weed hosts and reduce the losses from this disease (Picardo 2007).

7.4.4.2 Downy Mildew

Sorghum downy mildew induced by *Peronosclerospora sorghi* (Weston and Uppal) Shaw 1978 is a serious disease of sorghum and maize and causes heavy losses in the grain yield in many parts of the semi-arid tropics where sorghum is staple for human and other animal consumption (Kamala et al. 2002). Sorghum is also attacked by another downy mildew pathogen, viz; *Sclerophthora macrospora* (Sacc.) causing crazy top (Spencer-Phillips et al. 2003).

The disease causes economically significant losses in sorghum production in many parts of the world Jegera et al. (1998) demonstrated that the epidemics of *P. sorghi* in Africa appears to be more sporadic than *Sclerospora graminicola* causing downy mildew in pearl millet. Sorghum downy mildew is a devastating disease on both sorghum and maize, both in the tropics and sub-tropics (Frederiksen et al. 1969). *Peronosclerospora sorghi* produces two forms of inoculum, oospores and conidia. Oospores are the sexual resting structure of the fungus. They reside in the soil and allow the pathogen to survive from one season to another in the absence of a host. Oospores germinate by a germ tube in response to host root exudate (Pratt 1978), which then infects the sorghum seedling to give rise to systemic infection. Conidia disseminated from systemically-infected plants infect leaves and produce local lesions. Conidial infections of very young seedlings may induce systemic infection (Yeh and Frederiksen 1980).

Downy mildew is a systemic infection that affects both young and mature plants. Young plants may be infected through the shoots by conidia or through roots by soil infesting oospores (Bock 1995). In regions with distinct dry season and no collateral host, the primary systemic infection will arise from soilborne oospores (Jegera et al. 1998).

In cool, humid weather the abaxial surfaces of chlorotic leaves become covered with a white, downy growth consisting of the conidiophores and conidia of the fungus. Later stages of the disease result in death of interveinal tissue of the leaves which eventually shred. Diseased plants are usually barren or partially barren. Localized infections appear as brown necrotic lesions on leaf blades (Esele 1995).

Craig and Frederiksen (1980) identified two pathotypes (P1 and P2) of *P. sorghi* in Texas by differential pathogenicity on sorghum. Among them, P2 caused higher percentage of mildew in differential sorghum cultivars than did P1. Subsequently, the existence of three pathotypes of *P. sorghi* (P1, P2 and P3) was demonstrated in Texas (Craig and Frederiksen 1983; Craig 1986). Pawar (1986) reported that the pathotypes from Africa and India had a much wider range of virulence than did pathotypes from the Americas. Isakeit and Jaster (2005) reported the existence of a new pathotype of *P. sorghi* in Texas which showed metalaxyl resistance. Perumal et al. (2006) analyzed the genetic variability among the 14 isolates of *P. sorghi* including metalaxyl resistant and susceptible as well as common pathotypes 1 and 3 and reported that approximately 25 % of the bands were polymorphic across the isolates tested with the majority of differences specific to the pathotype P1. Mathiyazhagan et al. (2006) reported the genetic variability between the isolates from sorghum and corn using restriction fragment length polymorphism (RFLP)

Fig. 7.7 Shredded sorghum leaves due to death of interveinal tissue (With kind permission from Dr. Rob Williams, CAB International, U.K.)



analysis of the polymerase chain reaction (PCR)-amplified internal transcribed spacer (ITS) region of ribosomal DNA (Fig. 7.7).

Host-plant resistance is an effective and economical means of controlling downy mildew diseases in maize (*Zea mays* L.). Genetic mapping of resistance genes for sorghum downy mildew in maize revealed multiple locus inheritance. A combination of AFLP (amplified fragment length polymorphism) technique with bulked segregant analysis (BSA) was applied to map the genes involved in the resistance to sorghum in a recombinant inbred population. Further, sequence-characterized amplified regions (SCARs) were produced and used to assess the presence of Sorghum Downy Mildew resistance genes and characterize specific genotypes (Agrama et al. 2002).

General approach towards disease management is primarily to use resistant varieties. Other precautions such as removing infected crop debris, treating seeds or fungicide, using seeds with low moisture content (<9 %) before planting and sowing when soil temp is below 20 °C which is unfavorable for oospore germination are also recommended (Nallathambi et al. 2010).

7.4.4.3 Ergot

Sorghum ergot is a disease (caused by a fungus *Claviceps africana* and *C. sorghi*) that infects the ovaries of sorghum flowers and often converts them into a white, fungal mass (sphacelia). Following infection of the flowers, seed production is much reduced and instead of producing seed, the sorghum heads exude a sugary substance known as honeydew. In addition to reduced seed set, honeydew can become encrusted on harvest machinery and cause problems in harvesting the sorghum grain. Ergot can be an economically devastating problem. Yield losses of 10–80 % occurred in hybrid seed production in India and regular annual losses of 12–25 % were recorded in Zimbabwe. In USA the annual production cost increased by \$5 million due to ergot in USA (Bandyopadhyay et al. 1998). However, *C. sorghi* has limited economic impact because of its narrow distribution.

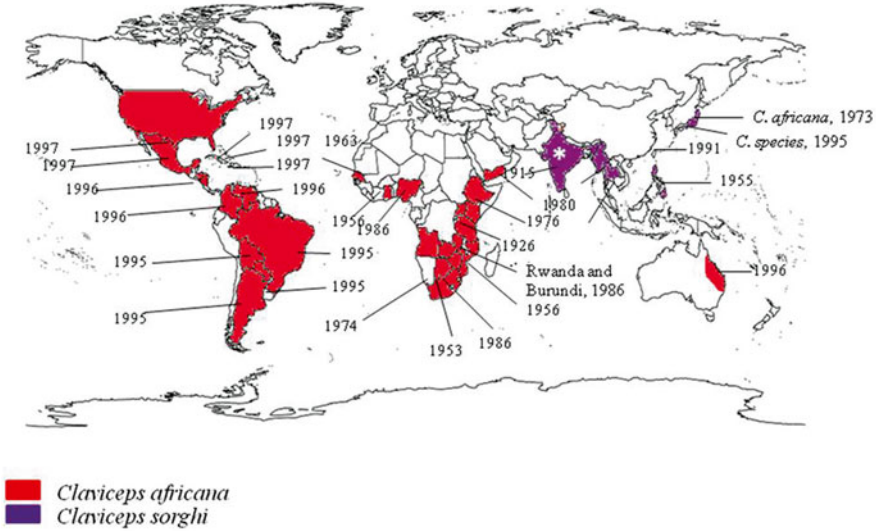


Fig. 7.8 Distribution of sorghum ergot and its pathogens worldwide with the year of first report of the pathogens in each country. The asterisk (*) indicates the country (India) where the disease was first reported

Although yield losses associated with ergot infection can be significant, indirect losses may also be important. For example harvesting grain from ergot-infected fields can be difficult. Ergot contamination reduces grain quality and limits its use as a feedstock. Epidemics of sorghum ergot disease were reported from different parts of India in 1999 and 2000 (Navi et al. 2002). Ergot disease is primarily an economic problem in F1 hybrid seed production. It is particularly severe in male-sterile lines (A-lines) when either nonsynchronous flowering of A-line and restorer lines (R-lines) or adverse environmental conditions result in lack of viable pollen and delayed seed set (Bandyopadhyay et al. 1998). Losses of 10–80 % have been reported in hybrid seed production fields in India (Fig. 7.8).

Due to infection, individual ovaries between the glumes of some or all florets are colonized by the parasite. Infected florets become evident when sticky, orange-brown liquid droplets of spore-bearing honeydew are exuded. The soft, white, narrow and elongate growth of mycelium forming the sphacelium becomes evident soon after, appearing from between the glume tips; the glumes are barely distorted laterally. The sphacelium grows at the inner end and may achieve a length of 14 mm (Frederickson and Mantle 1988). Honeydew droplets may coat panicles, seeds, leaves and the stalk. In the case of colonization of the honeydew and sphacelia by the hyperparasitic fungus *Cerebella andropogonis*, black, spherical, convoluted growths are seen at floret tips (Mohan and Jeyarajan 1991; Bandyopadhyay et al. 1998). Upon dissection, a discoloured sphacelium of reduced size is found underneath. Other moulds may also grow on the honeydew. Under favorable conditions long (1–2 cm) straight or curved, cream to light brown, hard sclerotia developed (Williams et al. 1978).

There is currently no source of resistance to any species of sorghum ergot for deployment in the field in A-lines, although many workers have devised screening methodologies for *C. sorghi* and report variable success in finding 'resistant' lines (Chandrasekaran et al. 1985; Lakshmanan et al. 1989; Hiremath and Lakshman 1990). In trying to evaluate resistance to *C. africana*, McLaren (1992) concluded that simple comparisons of severity data from tests of genotypes from different localities, following either natural infection or artificial inoculation, is meaningless, and this will also be the case for *C. sorghi* resistance. Susceptibility to ergot is extremely sensitive to environmental factors at flowering and a few weeks before (McLaren and Wehner 1990; McLaren 1997). Cool nights of <12 °C at 2–3 weeks before anthesis result in pollen sterility and increased ergot severity. Therefore, tolerance of low, pre-flowering minimum temperatures is important for disease avoidance (McLaren 1997). Similarly, the mean maximum temperature 1–4 days after pollen shed affects fertilization and thus affects ergot incidence.

Careful screening and selection for floral characteristics that reduce disease may prove to be one useful strategy. In the USA, many A-line sorghums have a protracted stigma receptivity period, enhancing ergot susceptibility (Odvody 1997) and disease reduction may possibly be achieved by decreasing that ergot-susceptible period of the A-line stigma and possibly also by advantageous modifications might include reducing the floret gaping period, selecting for more rapid post-fertilization changes in the A-line, breeding for cold-temperature tolerance in R-line pollen production and extending the pollen production period. Information on the genetics of ergot resistance is lacking in graminaceous crops, except pearl millet. Resistance in pearl millet is controlled by polygenic recessive genes (Thakur et al. 1989), implying that to breed ergot resistant hybrids, resistance should be incorporated into both male-sterile and pollen parents (Rai and Thakur 1995).

Soaking seeds with 5 % salt solution aid to remove ergot infested seeds, as ergot infested seeds float in the salt solution. Control of ergot with fungicides such as Propiconazole or Tebuconazole has proved to be cost effective in seed production plots (Bandopadhyay et al. 1998). Multiple sprayings at 5–7 days interval from flag leaf stage till end of anthesis are recommended to accommodate the variation in flowering within a field.

In India, early sowing reduced the incidence of *C. sorghi* (Anahosur and Patil 1982). Field practices aimed at reducing the risk or severity of all ergot pathogens include the removal of infected panicles at harvest, crop rotations and deep ploughing of crop residues. However, these methods have limited impact (Frederickson and Leuschner 1997).

7.4.4.4 Covered Smut

Covered kernel smut of sorghum is caused by *Sphacelotheca sorghi* (Link) Clinton and *S. cruenta* (Kuhn) Potter. The disease has been reported to be endemic in many sorghum fields from year to year and has side distribution in all sorghum growing areas (Mengistu 1982). The disease destroys all kernels in a head and replaces them

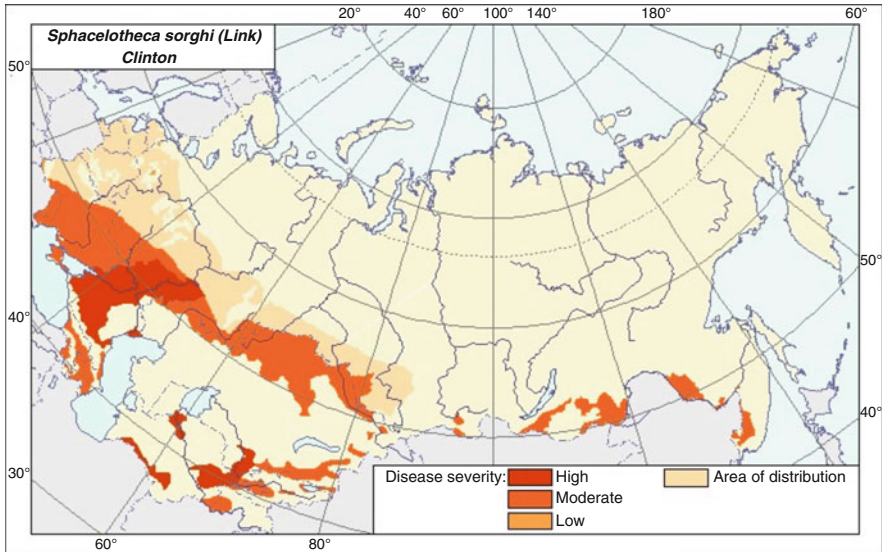


Fig. 7.9 Area of distribution and severity zones of the covered smut of sorghum (With kind permission from Dr. AI Silaev and Prof. Andrei Nikolaevich Frolov, Russia)

with a cone-shaped gall or may affect only portions of a panicle. At harvest time, these galls are broken and spores contaminate the outer surface of other kernels.

The pathogen of the disease has a suppressive action on the growth and development of affected plants. Losses of corn yield correspond to the percent of disease distribution in crops. Protective measures are cultivation of resistant varieties and hybrids, presowing seed treatment. The widespread use of seed treatments has now virtually eliminated the disease from commercial hybrid grain sorghum. Covered smut can still be a serious disease when seed treatments are not used, for example on small-scale farms (De Milliano et al. 1992). The disease can be effectively controlled by thiram (Wall and Meckenstock 1992) (Fig. 7.9).

The pathogen of the disease in its development cycle forms teliospores (wintering stage of the fungus) and sporidia. The first signs of affection of sorghum with the Covered Smut are noticeable after ejection of inflorescences, when smut sori start to develop instead of the flower elements, reaching 0.5–1.0 cm in size. More often all ovaries of the inflorescences are affected, but occasionally part of them remains unaffected, and normal caryopses are formed. The sori are covered with a dense light capsule which collapses during harvest, thus releasing teliospores and polluting grain. The source of infection is the infected seeds. Contamination of the plants occurs only during the sprouting stage (Silaev 2005). The fungus *S. sorghi* can grow and develop at temperatures from 10 to 32 °C. The soil temperature 18–23 °C and soil humidity 15–20 % during the period of seed germination are optimal for the contamination of plants. Two species of the kernel smuts of sorghum, viz *Sphacelotheca sorghi* and *S. cruenta* are known to consist of pathogenically and otherwise distinct physiologic forms.



Fig. 7.10 Covered kernel smut on sorghum (a) grains of sorghum replaced by smut sori and (b) sorghum covered with honeydew, clear transparent oval shaped sori on the spikelet (Image: courtesy ICRISAT, India)

Management of this disease can be done by developing plant resistant hybrids and by the use of certified disease-free seed (Fig. 7.10).

In summary, zonate leaf spot, ergot, downy mildew and covered smut are among the most serious diseases of sorghum that cause considerable reduction in the yield as well as quality of this crop in hot and humid climatic conditions, making threshing difficult, reduce germination and predispose seedlings to other diseases. So, it is essential to understand the genetic basis of resistance to these diseases to formulate an effective breeding program in sorghum. Host-plant resistance is an effective and economical means of controlling the diseases in sorghum along with chemical control and cultural methods, the use of certified disease-free seed and by developing plant resistant hybrids.

7.5 New Approaches in Developing Disease Resistance

The use of molecular probe primers is allowing us to gain new insights into fungal plant pathogens and to host responses to potential pathogens. Currently scientists (Prom et al. 2012) are developing real-time PCR primers for two downy mildews

that are considered to be threat to sorghum production along with PCR-based tags genes for resistance to head smut, anthracnose, downy mildew and grain mold. These molecular tags will be useful for breeding cultivars with more durable resistance and for cloning specific resistance genes. They have also used PCR to clone segments of the sorghum equivalents of genes that function in known host defense pathways. These clones are being used to compare the rate and timing of induction of each gene in resistant and susceptible lines following inoculation with a pathogen. Through the use of DNA fingerprinting technology, it is now possible to identify and track the spread and origin of new pathogen races, and to identify multiple, unique resistance genes that can be combined to provide more stable resistance and to identify and tag new genes for resistance to anthracnose, downy mildew and head smut. Genomic approaches are beginning to revolutionize our understanding of plant disease resistance. Large-scale sequencing will reveal the detailed organization of resistance-gene clusters and the genetic mechanisms involved in generating new resistance specificities. Global functional analyses will elucidate the complex regulatory networks and the diversity of proteins involved in resistance and susceptibility (Michelmore 2000). Sorghum is attacked by a number of viral, bacterial and fungal pathogens and also by insect and nematode pests. Fungal pathogens of sorghum cause severe crop damages by infecting the spikes, leaves and roots. Introgression of genes from the wild relatives exhibiting resistance to pests and pathogens has been successfully utilized over the years for generation of resistant varieties in sorghum. However, development of resistant hybrids through classical plant breeding has been slow because of the unavailability of disease resistant sources and because artificial inoculation is difficult (Han et al. 2002). The integration of transgenic approaches with classical breeding techniques offers a potential chemical-free and environment-friendly solution for controlling pests and pathogens. Moreover the availability of novel transgenes encoding highly potential anti-microbial peptides, defense-related proteins and enzymes for the production of anti-microbial compounds in crop plants, have been greatly enhancing the possibility of engineering crop plants for resistance to pests and pathogens (Sharma et al. 2000). Structural and biochemical responses against fungal attack include plant cell wall reinforcement, phytoalexins accumulation with microbial toxicity, ribosome-inactivating proteins (RIP) that inhibit protein synthesis, antimicrobial peptides and synthesis of other PR proteins (Hassanein et al. 2009). Genetic engineering allows the expression of foreign genes from distant unrelated species as well as the modification of the usual pattern of expression of an already present gene. Quantitative trait loci (QTL) mapping is an effective approach for studying genetically complex forms of disease resistance, where effects of specific resistance loci can be determined and interactions between resistance genes, plant development, and the environment can be analyzed (Semagn et al. 2010). QTL mapping also provides a framework for Marker Assisted Selection (MAS) of complex characters and the positional cloning of genes with partial resistance MAS is potentially an important tool for use in crop improvement (Lin et al. 2008). Despite decades of conventional breeding and selection, there are still a large number of fungal diseases that make sorghum production challenging in various parts of the world. Current advances in

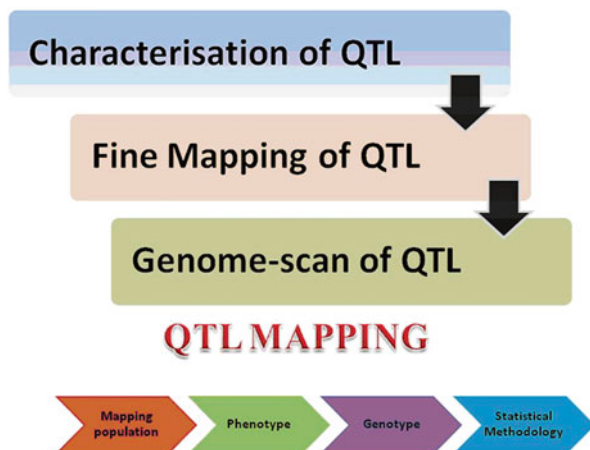


Fig. 7.11 The phases of Quantitative Trait Loci mapping. All phases of QTL mapping involve analyses of quantitative traits that have a complex genetic background and are influenced by environmental factors. Therefore, in addition to the need for genetic marker information, powerful analyses require good phenotypic records from a large number of animals and the use of suitable quantitative statistical methods

plant genomics, including structural and functional genomics and biotechnology provide important tools for sorghum improvement. These tools are being used for genetic analysis and crop improvement.

Quantitative trait loci (QTL) mapping, an effective approach for studying genetically complex forms of disease resistance, where effects of specific resistance loci can be determined and interactions between resistance genes, plant development, and the environment can be analyzed. The integration of transgenic approaches with classical breeding techniques offers a solution for controlling pests and pathogens. Moreover the availability of novel transgenes encoding highly potential anti-microbial peptides, defense-related proteins and enzymes for the production of anti-microbial compounds in crop plants, have been greatly enhancing the possibility of engineering crop plants for resistance to pests and pathogens and the use of molecular based probes for developing the resistance in the plants against diseases (Fig. 7.11).

7.6 Conclusion

Every fungal pathogen has different requirements of temperature and soil moisture conditions for disease establishment and development. However, diseases caused by these pathogens are induced if stress conditions occur at anthesis or post-flowering. Complex traits are involved in the genetics of resistance to the pathogens, while selection procedures are troublesome and the results sometimes inconsistent.

Effective and faster improvements of these traits may be done by applying molecular technologies. Attempts should be made to tag the different genes associated with the resistance to sorghum diseases and to use linked RFLPs, for instance, to pyramid these genes into a single line. Resistant gene combinations may contribute to the durability of resistance. Conventional breeding and the molecular biological approaches provide an approach to understand the biology of host-pathogen interaction so that the disease can be managed and crop loss prevented. Complexity of disease resistance mechanism will be dissected relatively more easily as we decipher the function of genes involved in such pathways. Connecting the dots of current efforts in genomics, metabolomics and proteomics with the whole genome sequence is expected take our understanding to a novel height. Hence, it can be concluded that a comprehensive management of sorghum diseases due to fungal pathogens will require multi-pronged approaches which will include development of transgenics though incorporation of resistance gene clusters, identifying and transferring genes for phytoalexins especially 3-deoxyanthocyanidins and their overexpression, transfer of PR proteins genes such as chitinase and β 1–3-glucanases as well as testing the potential of the antimicrobial peptides in effective control of countering the pathogens.

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Chapter 8

Sorghum for Health and Business

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Abstract Sorghum is the fifth most important cereal crop cultivated globally on an area of about 40.51 million ha. Grain sorghum is vital food crop for millions especially for the people in the arid and semi-arid parts of Africa and Asia. In developed countries, the crop is majorly used as animal or poultry feed. With continuous increase in the human population and decreasing agricultural resources especially irrigation water, sorghum represents important crop for future as it can produce optimum yield under drought and other stress conditions. The major challenges facing sorghum research and development is to deliver technologies that will enable the agricultural sector to affect transformation of “subsistence farming” to sustainable and market oriented. This can be achieved through reassessment of crop research in terms of current and future demand, resolving specific production constraints by improving productivity, development of post-harvest processing and value-addition technologies, creation of demand, marketing strategies and policies that would result in additional farm income and employment without sacrificing overall goal of attaining sustainable food and nutritional security.

This chapter focuses on the nutritive composition of sorghum grain, consumption pattern in Africa and Asia, grain attributes affecting end-use quality, health benefits of sorghum, major constraints as food and suggestive measures for sustainable production. A value chain model followed at Directorate of Sorghum Research, India for sustainable use of sorghum as food is discussed. Sorghum is comparable with fine cereals like rice and wheat for most of the grain nutritional composition including starch, protein, fat, vitamins and minerals etc. The quality of sorghum grain used for preparation of various food products is an important parameter that determines its end use. In general, bold, round lustrous and intermediate endosperm textured grains are preferred for variety of food preparations. Protein quality in

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sorghum is also a major aspect discussed by scientists across world. The protein quality is considered to be slightly inferior compared to other fine cereals. Research efforts are in progress for development of lines with improved protein quality. However supplementation of sorghum foods with other pulses would circumvent the problems associated with sorghum protein. Sorghum grain possesses unique grain nutritional properties having significant and positive role in promoting nutrition and health of human beings. Particularly the slow release carbohydrate property of sorghum increases satiety and has role in controlling diabetes. Gluten is absent in sorghum and is considered safe for people suffering for celiac disease. In developed countries there is a growing demand for gluten free food especially sorghum due to the intolerance to wheat. Recently farmers in US have started producing sorghum hybrids that are a white grain, known as “food-grade” sorghum.

Keywords Sorghum • Grain • Composition • Nutritional and health benefits • Protein digestibility • Phytochemicals • Antioxidants • Diabetes • Obesity • Celiac disease • Cancer prevention • Food processing • Value addition • Commercialization • Sustainability

8.1 Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop across the world mostly cultivated in the arid and semi-arid tropics for its better adaptation to various stresses, including drought, heat and salinity. The high grain yield potential coupled with nutritional superiority makes sorghum ideal food crop for millions in the arid and semi-arid tropics of the world. Under the context of global climate change and limiting water resources, it is foreseen as an important crop for future. Globally sorghum is cultivated in 40.51 mha with maximum area in India (7.67 mha) followed by Sudan (5.61 mha), Nigeria (4.74 mha), Niger (3.32 mha) and other countries in (Rakshit et al. 2012). Sorghum is believed to be dietary staple for more than 300 millions of people in the developing countries (Dicko et al. 2006). The crop is one of the most efficient cereal grains in terms of conversion of solar energy and water to food/feed energy. The major sorghum producers are USA, India, Mexico, Nigeria, Sudan and Ethiopia. Other sorghum producing countries include Australia, Brazil, Argentina, China, Burkina Faso, Mali, Egypt, Niger, Tanzania, Chad and Cameroon. The sorghum grain is mainly used as food (55 %), in the form of leavened, unleavened breads (*rotis*) and porridges (thick or thin) in Asia and Africa, and as feed (33 %) in the America. The sorghum stover obtained after harvest of the grains is excellent source of dry fodder for livestock, especially in India. In the context of rising input costs especially irrigation being one of the scarce and costly input in agriculture, sorghum is gaining more

importance for sustaining livelihoods of dry land farmers. In recent years, a temporary setback was observed in popularity of sorghum as food mainly due to the availability of fine cereals like rice and wheat through government policy and programs. The sorghum grain holds promise as an important cereal grain in the twenty-first century mainly due to its drought tolerant nature as well as its nutritional superiority. Nutritional superiority of sorghum grains include slow release carbohydrates, high dietary fiber, minerals rich, gluten free and presence of antioxidants in coloured grain varieties. The health benefits associated with sorghum make it as most ideal food for urban population suffering from lifestyle diseases such as obesity, diabetes and celiac disease. Till recently, sorghum is mainly consumed by rural masses and to lesser extent consumed by the urban people largely due to non-availability of ready to cook form of sorghum products. This can be overcome by technological interventions such as development of ready to eat/ ready to cook sorghum foods such as semolina, flakes, pops and biscuits. Apart from serving as nutritional/health food among the consumers, this will also generate more demand for sorghum thereby increasing the income and sustaining livelihood of sorghum farmers of the world.



Photo 8.1 The Director, Directorate of Sorghum Research, explaining to the Deputy Directorate General (Crop Science) of Indian council of Agricultural research about the improved post-rainy cultivar (Phule Revati) possessing superior grain yield, fodder yield and quality

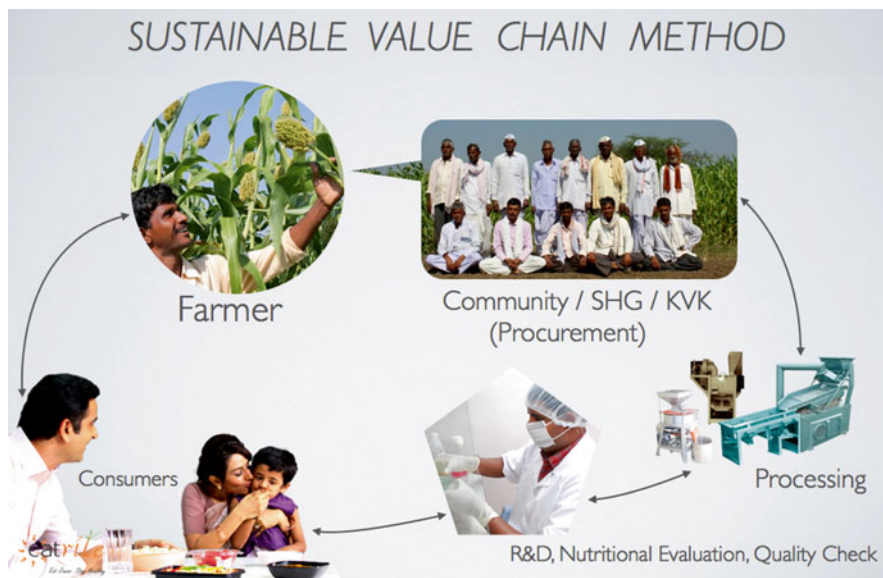


Photo 8.2 Sustainable value chain approach for production, popularization and commercialization of sorghum food products

8.2 Sorghum Grain Composition

Sorghum has been, for centuries one of the important staple foods for millions of poor people in the semi-arid tropics of Africa. Most of the regions in the parts of Africa sorghum remain a principal source of energy, protein, vitamins and minerals. The sorghum grain composition and its nutritional facts have been reviewed in detail by Dicko et al. (2006). Sorghum, like other cereals such as rice and wheat is rich in starch which makes up about 60–80 % of normal kernels depending on the cultivars (Udachan et al. 2012). Starch plays an important role in physical, chemical and nutritive attributes of the finished foods. Normal sorghum also known as non-waxy types contain 70–80 % amylopectin and 20–25 % amylose, while the waxy varieties contain 5 % or even lesser amylose.

The significant advantage of sorghum starch is due to substantial proportion of the resistant starch present in the grains which has positive role in digestibility. Sorghum is also a good source of crude fibre (2–3 %) of which the major being the insoluble fibre (Table 8.1). The fibre component is generally located in the pericarp regions of the sorghum grains and they are less likely to be lost when consumed as whole grain. The dietary insoluble fiber are said to prevent gastrointestinal problems and helps in constipation. The much talked is the sorghum protein content and quality. Like other cereals, the proteins in sorghum range from 7 to 15 % (Beta et al. 1995). The majority of sorghum proteins are found within the endosperm of the kernel, distributed within both protein bodies and the endosperm's protein matrix. Based on solubility, sorghum proteins have been classified as albumins, globulins, kafirins, cross-linked kafirins and glutelins. The kafirins form the major part of sor-

Table 8.1 Nutrient composition of sorghum, millets and other cereals (per 100 g edible portion)

Crop	Protein (g)	Fat (g)	Ash (g)	Crude fibre (g)	Carbohydrate (g)	Ca (mg)	Fe (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)
Sorghum	10.4	3.1	1.6	2.0	70.7	25	5.4	0.38	0.15	4.3
Finger millet	7.7	1.5	2.6	3.6	72.6	350	3.9	0.42	0.19	1.1
Pearl millet	11.8	4.8	2.2	2.3	67.0	42	11.0	0.38	0.21	2.8
Foxtail millet	11.2	4.0	3.3	6.7	63.2	31	2.8	0.59	0.11	3.2
Rice (brown)	7.9	2.7	1.3	1.0	76.0	33	1.8	0.41	0.04	4.3
Wheat	11.6	2.0	1.6	2.0	71.0	30	3.5	0.41	0.10	5.1
Maize	9.2	4.6	1.2	2.8	73.0	26	2.7	0.38	0.20	3.6

Source: www.fao.org

ghum proteins (50–70 %) (Hamaker et al. 1995; Oria et al. 1995; Duodu et al. 2003) and classified as alpha, beta and gamma depending on the nature of the proteins. Alpha- Kafirins make up about 80 % of the total kafirins and are considered the principal storage proteins of sorghum, whereas beta-kafirins and gamma-kafirins comprise about 5 and 15 % of total kafirins respectively.

The concern is about the sorghum protein quality in which the amino acids lysine and methionine are deficient. Sorghum protein is said to be less digestible as compared to other cereals especially when cooked. A highly protein digestible sorghum line derived from a high-lysine chemical mutant (P721) Opaque, also known as P721Q (Weaver et al. 1998) is reported to have 10–15 % more protein digestibility when uncooked and 25 % higher digestibility when cooked. More specifically, the digestibility of alpha-kafirins increased as high as 90–95 % when analyzed using pepsin digestion. Crude fat in sorghum grain averages to about 2–3 %, slightly higher than that of wheat and rice. The fat in sorghum grain are mainly present in the germ and are rich in polyunsaturated fatty acids (Glew et al. 1997). The fatty acid composition of sorghum fat (linoleic acid 49 %, oleic 31 %, palmitic 14 %, linolenic 2.7 %, stearic 2.1 %, etc.) is similar in content to that of corn fat, but it is more unsaturated (Afify et al. 2012). The oil content of sorghum varieties are reported to decrease on germination, cooking and fermentation. The decrease in the oil content could be due to increased activities of the lipolytic enzymes during germination and fermentation, which hydrolyses the oils to fatty acids and glycerol (Raham and Aal 1986). Sorghum is also good source of B vitamins such as thiamin, riboflavin, pyridoxine and the fat soluble vitamins such as A, D, E and K. Sorghum is also an important source of minerals as compared to wheat and other cereals. Sorghum is also rich in phosphorus, potassium, iron, zinc (Glew et al. 1997; Anglani 1998) and when compared to wheat, it has higher proportion of zinc in its grains (Hopkins et al. 1998).

Nutritionally sorghum is comparable with other fine cereals like rice and maize in terms of starch, protein, vitamin, mineral and most of other nutritional compositions. However, sorghum is superior with respect to the carbohydrates present in sorghum is unique and are referred to slow release carbohydrates. Apart from this, sorghum is rich source of various minerals especially magnesium. Moreover the dietary fiber present in sorghum grain promotes healthy digestion and combats disease of the digestive tract. The protein in sorghum is slightly inferior especially they lack essential amino acids. Combination of sorghum with other protein sources like pulses would compensate the deficiency of certain amino acids such as lysine. All these positive benefits of sorghum may be exploited on commercial scale for promoting sorghum for health and well being.

8.3 Traditional Pattern of Sorghum Food Consumption in Africa and Asia

Sorghum is grown and utilized in United States, Australia and other developed nations mainly for animal feed while in Asia and Africa, the grain is one of the major energy source for millions of poor people and the biomass left out after

harvest of grains is an important source of fodder. In India, the crop is traditionally consumed in the form of *rotis/bhakris*, a variety of unleavened bread usually made from sorghum, is the staple diet in many parts of India, such as Maharashtra state and northern Karnataka state. In Rayalaseema area of Andhra Pradesh, *jonna rotte* is widely consumed and it is generally prepared from yellow sorghum grains. The post-rainy sorghum forms a major part of the food grain sorghum production in India. The dry weather conditions during the maturity phase are more ideal for obtaining quality produce. The post rainy sorghum is characterized by lustrous, pearly white, attractive grains and is largely preferred for human consumption by the rural households.

Maldandi, a popular landrace is the highly preferred cultivar over decades for its superior grain quality. The landrace is still largely cultivated on a wider scale in Maharashtra, India. The Maldandi landrace has evolved over decades and its drought tolerance mechanism is a major factor for large scale adoption by the farmers. With regard to *roti* making, the most important parameters affecting the quality include grain colour, grain weight, endosperm texture, milling yield, water absorption, breaking strength, kneading quality, rolling quality and *roti* colour, taste, texture, aroma and keeping quality. Subsequently large number of landraces were identified having vernacular connection with Maldandi and are believed to have originated from the original Maldandi landrace. These landraces are also morphological and molecularly diverse from the original Maldandi land race and a few of them were noticed to have superior *roti* making qualities. Apart from its use for *roti* making, processed food products such as popped sorghum, *hurda*, *annam*, *pops* are being consumed on localized scales and are likely to be significant avenues for diversifying utilization trends. Sorghum is also consumed as steamed products such as *idli* and *dosa* (leavened) in parts of Tamil Nadu, India. The dried products include *papad* and have a long shelf life of even upto 6 months. The newly developed cultivars from Indian sorghum breeding programmes viz., CSV 18R, CSV 22R, Phule Vasudha, Phule Yashoda and Phule Revathi possess superior grain quality and suited for diverse food uses.

In Africa, sorghum is processed into a very wide variety of nutritious traditional foods, such as porridges (fermented and non-fermented) semi-leavened bread, cous-cous and dumplings. Porridge, a type of food preparation from sorghum is widely consumed sorghum food of Africa. The porridges are classified by the pasting intensity and this depends on the grain variety used. Porridge are broadly classified as thick or thin porridge, which are further sub-classified based on their pH, as neutral, acid, alkaline and fermented porridges. The thin porridges are being consumed as weaning food because of high energy present in them (Traore et al. 2004). The thick porridges are consumed with sauces and soups. The acid form of thick porridge is prepared from tamarind water. Fermented porridge is also majorly prepared and consumed in several parts of Africa. The shelf life, palatability and the texture of the fermented porridges are reported to have improved after fermentation. *Ogi* is a popular fermented porridge in Nigeria and usually prepared using combination of sorghum, millet and maize. The best quality acid porridge was prepared from West African local cultivar with white and corneous endosperm grain characteristics (Rooney and Murthy 1982). Alkali form of porridge is prepared by mixing with sorghum flour with alkali and are consumed in Mali and Nigeria.

Injera is another form of sorghum consumption in Ethiopia. *Injera* is a yeast-risen flatbread with a unique, slightly spongy texture traditionally made out of teff flour. Soft endosperm types with white or red pericarp are said to produce good quality *injera* (Rooney and Murthy 1982). Couscous is another steamed cooked and granulated traditional African food produced in North Africa. It is prepared by mixing flour with water to obtain agglomerated flour-water mixtures and put on the top of a special pot called couscoussiere. White colored grain sorghum varieties from tan type plants yielded the best couscous product (Galiba et al. 1988). Couscous quality depends upon the size uniformity, color, stickiness and mouth-feel of the product (Aboubacar and Hamaker 1999).

Apart from using sorghum majorly as source of food in different countries, it is also used for making beers and is consumed locally in Africa. The traditional sorghum beers of Africa are generally brewed with sorghum varieties having colored grains (red or brown). The white varieties are also mixed with red sorghum to obtain colored beers. Colored beers are highly preferred by consumers and are believed to be healthy (Kayode et al. 2005). The primary quality criterion of selection of sorghum varieties for beer is their potential to produce malt based on amylase properties (Verbruggen 1996).

African traditional sorghum beer is consumed while it is still fermenting, and the drink contains large amounts of fragments of insoluble materials (Rooney and Serna-Saldivar 1991). However, African traditional sorghum beers vary in their production processes depending on the geographic localization. *Ikigage* or *amarwa* is a traditional alcoholic beverage produced in Rwanda. *Merissa* is a traditionally prepared alcoholic beverage in Sudan using malted red sorghum. The traditional sorghum beer of Zimbabwe is known as *doro*, *chibuku*, *hwahwa*, *mhamba* or *uthwala* depending on regions (Chamunorwa et al. 2002). *Dolo* is a popular traditional alcoholic beverage manufactured in Burkina Faso, and is generally made from red sorghum malt. In Nigeria, the alcoholic beverages are called as *Pito* and *burukutu* which are brewed with red or white sorghum malt. *Amgba*, well known by the name *bili bili*, is a very popular traditional alcoholic beverage in Cameroon. *Tchoukoutou* or *chakpalo* is a traditional alcoholic beverage produced in Benin and Togo principally using sorghum malt (red and brown varieties) (Lyumugabe et al. 2012). Sorghum is also consumed in Mexico and Central America in the form of unleavened bread known as tortillas. Sorghum kernels with white and thick pericarp and yellow endosperm are reported to produce excellent quality tortillas.

For millions of years, sorghum is an important food staple and source of livelihood for semiarid tropics especially in Asia and Africa. From the above it is clear that the traditional pattern of consumption varied among countries/regions. However, compared to fine cereals like rice and wheat, processing in sorghum and millets is not well established and requires great deal of efforts. Sorghum food products are not consumed on a commercial scale due to difficulties associated with preparation of some of the food products. Focused fundamental and applied research is essential to unleash technologies which can make more acceptable food products while maintaining the nutritional benefits intact.

8.4 Physical Grain Attributes Affecting Milling and End Use Quality

Sorghum grain quality are differentiated both at physical and biochemical characteristics. The physical grain quality fetches more market price to the produce and also plays a major role in milling while, the biochemical properties have more influence on nutritional properties as well as its end product specificity. The most important physical grain quality traits are grain colour, grain size and shape, pericarp and endosperm texture and grain lustre. Majority of the food items especially in India are prepared with white/yellow white pericarp grains and are referred to as food grade sorghums (Table 8.2). Yellow-white colored pearly grains fetches high market price. Although white or yellow white grain colour is highly preferred for food purposes over colored sorghum. Products made from colored sorghum are reported not to affect significantly the acceptance of most of the products for taste, texture, and keeping quality. In some parts of Africa porridges made from red and brown sorghums are consumed.

For seed coat/pericarp colour of sorghum grains, wide variability is observed ranging from white, black, brown, red, orange and yellow types. Grains with pigmented testa and not the coloured pericarp are associated with tannin content. Red grain types are largely utilized for brewing industry. With regard to size and shape, bold and round grains are more preferred. Most of the post-rainy cultivars in India grown for food purposes possess bold and round grains but grains with round with oval shape are said to have processing advantages during milling. Bold grains are positively correlated with higher grain weight. Test weights of 3.5–4.0 g/100 seed are acceptable for food uses. Higher test weights are associated with floury and softer endosperm textures and are more susceptible to grain moulds and storage pests. With reference to seed coat thickness, thin seed coat/pericarp are more preferred over thick pericarp types. In Africa, thick pericarp types with harder endosperm are found advantageous for traditional dehulling.

Endosperm texture is an important grain attribute that determines the end-use quality. Those grains possessing a thick pericarp and highly corneous endosperm produced the maximum quantity of decorticated grain without breakage with minimum effort and time. Grains with thin pericarp and corneous endosperm are acceptable for machine dehulling. Endosperm texture relates to the proportion of the floury to corneous endosperm in the kernel and are broadly classified as vitreous (hard), intermediate and floury (soft) types. Hard endosperm textured grains are generally associated with smaller seed size, contain higher proportion of corneous endosperm having densely packed starch granules and are resistant to milling, contain more protein content but less protein digestibility. Hard endosperm textured grains are preferred during rainy season as it is one of the associate trait for grain mold tolerance. Floury endosperms have lower seed weight and thereby have low grain yield potential.

Most of the cultivars grown for food purposes during the post-rainy season are the intermediate endosperm textured. The porridges consumed in African countries

Table 8.2 Physical and chemical attributes of food grade sorghum cultivars

Cultivars	Grain color	Size of mark of germ	Endosperm texture	Grain lustre	Grain shape (dorsal)	Grain shape (ventral)	1,000 seed weight	Grain hardness (sec)	Amylose (%)	Protein (%)	Fat (%)	Crude fibre (%)	Ash (%)	Acid insoluble ash (%)
CSV 18R	Yellow white	Large	¾ farinaceous	Lustrous	Circular	Elliptic	42.15	16.5	8.04	11.1	3.06	3.03	1.75	0.0006
Phule Vasudha	Yellow white	Large	½ vitreous	Lustrous	Circular	Elliptic	38.83	24.5	4.61	10.12	4.74	2.11	1.92	0.0008
DSV 4	Yellow white	Very large	¾ farinaceous	Lustrous	Circular	Elliptic	40.69	20.0	5.04	9.62	3.27	3.32	4.28	0.0006
CSV 22R	Yellow white	Medium	¾ farinaceous	Lustrous	Elliptic	Elliptic	37.64	15.5	8.94	11.95	3.22	2.71	1.85	0.0045
M 35-1	Yellow white	Medium	½ vitreous	Lustrous	Circular	Elliptic	42.1	23.5	6.81	10.02	2.74	2.35	2.63	0.0004

Source: Directorate of Sorghum Research, Hyderabad, India

are made out of hard endosperm textured grains. The cultivar M 35-1 developed during 1960s in India is known for its best roti making quality and possess medium endosperm texture. Semolina is a semi-processed food widely consumed in India. The semolina recovery during processing was observed to be high in hard endosperm types compared to floury types. Floury endosperm types recorded more flour yield and generated good colored flour as compared to hard types. Yellow endosperm textured grains do exist and are consumed in some parts of the Andhra Pradesh, India. Yellow endosperm textured grains are believed to be nutritionally superior, possessing medicinal properties but reports claiming the medicinal properties are poorly documented.

Bold lustrous round grains with moderate endosperm texture are desired for preparation of most of the food products. In India, particularly, post-rainy cultivars belonging to the durra race possess bold round and lustrous grains and are desired for making unleavened *rotis*. These cultivars fetch more market prices for the produce as well as highly acceptance by the consumers.

8.5 Protein Digestibility in Sorghum

Considering nutritional point of view, protein quality is one of the major focuses for improvement among the researchers. The various aspects of protein quality, protein digestibility are one among the important aspect that hinders the nutritional quality of sorghum. Studies have shown protein digestibility of cooked sorghum is 46 %, whereas values of 81, 73, and 66 % were reported for cooked wheat, maize, and rice, respectively (Axtell et al. 1981). Sorghum lines with high-lysine content are reported to have high protein digestibility. Large efforts made by the research groups led to identification of germplasm and mutant lines with high lysine content and thereby superior protein digestibility. High lysine population derived from the mutant P721Q that have approximately 10–15 % higher uncooked and 25 % higher cooked protein digestibilities (Weaver et al. 1998). High digestible sorghum are also said to show little reduction in digestibility after cooking, compared to the large reduction of normal sorghum cultivars. Singh and Axtell (1973) reported lines IS 151167 and IS 11758 containing high protein in the range of 15.7 and 17.2 % and the lysine content in these cultivars were 3.44 and 3.13 g/100 g proteins respectively.

Sufficient variability was observed among the breeding lines for grain protein digestibility. Two improved lines SPV 1775 and SPV 1758 were found to possess high protein digestibility of about 70 % (Ganapathy et al. 2010). Up to now no commercial cultivar has been released combining quality protein as that of maize wherein many Quality protein maize cultivars were developed and released with superior protein quality. Among the breeders combining high protein digestibility with superior yield has been always a challenge. The endosperm texture of high protein digestible lines were noticed to be floury, have low grain hardness, low seed weight and thereby low yielding potential. Grain hardness is the most desired traits for developing lines with high yielding potential and also it is an important trait for grain mold tolerance in sorghum. Tesso et al. (2006) identified sorghum grain phe-

notype with a unique modified endosperm texture that has near-normal hardness and consisted of superior nutritional quality traits of high digestibility and superior lysine content. This has been identified among F_6 families developed from crosses involving hard endosperm, normal nutritional quality sorghum lines, and improved HPD/*hl* sorghum mutant P721Q-derived lines. The novel vitreous endosperm formations were reported to have originated in the central portion of the kernel endosperm with opaque portions present both centrally and peripherally surrounding the vitreous portion. The Kernels showing modification differed for their vitreous content from a slight interior section to one that filled out to the kernel periphery.

In general, the post rainy cultivars in India have been reported to have high protein digestibility. The endosperm textures of most of these cultivars are towards floury and are preferred for preparation of unleavened breads such as *rotis*. Therefore, evident quality characteristics deserve an important consideration in a breeding programme, and efforts need to be made to screen for characteristics that might be associated with nutritional and quality of food products.

Grains of most cereals lack amino acids such as lysine and tryptophan in required quantities are said to be limiting amino acids. In Maize, breeding efforts led to development and release of high lysine cultivars, however such cultivars are not available in sorghum. For development of new lines with improved protein quality, breeding efforts should aim at identification of germplasm with superior amino acid composition which can subsequently used for introgression into elite breeding lines for development of cultivars with improved protein quality.

8.6 Sorghum Grain for Alternate Uses

Sorghum grain besides majorly being used as a food crop in Africa and Asia is also finding potential place for alternative uses such as livestock and poultry feed, potable alcohol, starch and ethanol production (Kleih et al. 2000). Starch and protein quality of the grain are the two important characters that enhances the value of sorghum as an industrial crop. Several researchers have investigated the digestibility of sorghum starch (Rooney and Pflugfelder 1986; Wong et al. 2009) and sorghum protein for its use as food or feed. In India, the rainy season produce is most commonly used for industrial purposes due to inferior quality grains infected by molds. Mold affected grains are generally not used for food purposes but recent efforts are being made to remove mold affected parts by decortication or pearling techniques and are being tested for its feasibility for food uses. The sorghum produce obtained from the post rainy season are too expensive to be used for industrial purposes and highly valued for food uses (Aruna et al. 2012). Hardly there is any surplus production to be used for other alternate purposes.

In USA and Australia, sorghum is one of the major livestock feed and its performance is compared to that of corn. Several studies have shown sorghum can be a good alternative to maize for poultry industry (Etuk et al. 2012). The demand of sorghum for poultry feed largely depends on the maize production and its price

which is presently the largest energy source for poultry. For sorghum to compete with maize in poultry sector, its price should be at least 15–20 % lower than maize. In African countries, substantial portion of the grain is used for making traditional beers. The red colored grains are more preferred ones for making traditional beers. African traditional sorghum beers are reported to be consumed while it is still fermenting, and the drink contains large amounts of fragments of insoluble materials (Lyumugabe et al. 2012). Suitability of grain to produce good quality potable alcohol may persist as long as it continues to be a competitive raw material. In recent years, the demand for sorghum by the ethanol industry has been growing in the United States (Sarath et al. 2008). Sorghum is considered as the number 2 crop for grain-based ethanol production. About 30–35 % of domestic sorghum production is utilized in the grain-to-ethanol market (Kubecka 2011). The total starch content and the proportion of amylose to amylopectin are important starch qualities that impact the ethanol and starch industry. Wu et al. (2007) reported that low amylose content in sorghum grain may be associated with increased ethanol conversion efficiency. Waxy sorghums are more preferred in brewing industry, since they gelatinize more rapidly. The future of sorghum remains promising for various alternate uses especially could make a larger contribution to the ethanol requirements of the world.

Till date, most of breeding efforts were aimed at developing lines with high yield and agronomic superiority. For sustainable cultivation of sorghum, more emphasis should be made to identify and develop lines which are suited to specific end uses like starch or poultry industry. This will create more demand as well as fetch more market price to the farmers for their produce.

8.7 Health Benefits of Sorghum

8.7.1 Sorghum in Cancer Prevention

Sorghum grain contains higher levels of phytochemicals such as tannins, phenolic acids, anthocyanins, phytosterols and policosanols which have potential benefits on human health (Awika and Rooney 2004; Dykes and Rooney 2006). The phytochemicals and their associated health benefits are present in greater quantity in the coloured sorghum varieties compared to white varieties. Earlier there was a myth that colored sorghum varieties do contain higher levels of tannic acids which are toxic to animals and humans (Dykes and Rooney 2006), but now it understood that the tannins present in sorghum are the condensed tannins which are non-toxic and have beneficial properties to human health. It was also believed that all sorghum varieties with pigmented pericarp do contain tannins but it is the pigmented testa rather than the pericarp colour is the true indicator of tannin content in sorghum (Dykes and Rooney 2006). The phenols present in sorghum grains predominantly are the phenolic acids and flavonoids. The phenolic acids are benzoic or cinnamic acid derivatives whereas the flavonoids include tannins and anthocyanins as the most important constituents. Sorghum phytosterols contain mostly free sterols or stanols and their fatty acid/ferulate esters (Awika and Rooney 2004; Dykes and Rooney 2006).

A recent study at University of Georgia reported greater antioxidant and anti-inflammatory properties in selected sorghum varieties than blue berries and pomegranates. They have polyphenols compounds in high tannin varieties were estimated in the range of 23–62 mg/g while the blue berries contains 5 mg/g and pomegranates contain 2–3.5 mg/g. Foods rich in antioxidant phytochemicals play a greater role in prevention of diseases related to oxidative stress such as heart disease and cancer. Cancer cells are said to exist under oxidative stress. The survival potential of cancer cells are said to get increased due to oxidative stress by the way of inducing mutation in cancer cells. Mild levels of reactive oxygen species (ROS) in food resulted in increased proliferation of cancer cells (Devi et al. 2011). The antioxidant phytochemicals present in sorghum grain could be best utilized for prevention of diseases such as cancer and heart diseases. Sorghum grains rich in antioxidants can be used as direct food such as cookies, bread or can be used as a substitute with other foods. As an alternate, the active compounds can be extracted by decortication and can be used for various dietary supplements. Moreover the antioxidants such as anthocyanin reported to be stable can be used as natural colorants having functional properties.

8.7.2 Sorghum and Diabetes

Diabetes is a condition with higher levels of glucose and inability of the body to breakdown for energy subsequently leading to organ damage. The protein and starch in sorghum grain are more slowly digested compared to other cereals, and slower rates of digestibility are particularly beneficial for people with diabetes. The tannins present in sorghum binds to the proteins and carbohydrates forming insoluble complexes and making it less digestible. Studies on supplementation of sorghum foods for reducing diabetics in humans showed better control of blood glucose and glycosylated haemoglobin.

Park et al. (2012) in their study observed that sorghum extract has anti-diabetic effects exerted its therapeutic effects through peroxisome proliferator-activated receptor gamma overexpression in mice fed with a high fat diet. Administration of sorghum extract both 0.5 and 1 % significantly reduced serum glucose levels, and reported that only the 1 % sorghum extract treatment significantly lowered serum insulin level. Kim and Park (2012), reported that the hypoglycemic effect of sorghum extract was related to hepatic gluconeogenesis and not the glucose uptake of skeletal muscle, and the effect was similar to that of anti-diabetic medication.

8.7.3 Sorghum Is Safe for People with Celiac Disease

Celiac disease is a condition in which genetically susceptible people have an autoimmune reaction to gluten proteins found in all wheat types and closely related cereals such as barley and rye. This reaction results in the formation of auto

antibodies and the destruction of the villi in the small intestine, which results in malabsorption of nutrients and other gluten-induced autoimmune diseases (Ciacci et al. 2007). Sorghum starch is gluten-free and a good alternative to wheat flour for individuals suffering from celiac disease. Up to 1 % of the U.S. population (and about 0.5 % worldwide) is believed to have celiac disease, an autoimmune reaction to gluten proteins found in wheat, barley and rye. Sorghum-derived products did not show toxicity for celiac patients in both in vitro and in vivo challenge and considered safe for people with celiac disease (Ciacci et al. 2007).

8.7.4 Sorghum and Obesity

Sorghum containing tannins are reported to reduce the weight gain due to low caloric availability and therefore this property is potentially useful in helping to reduce obesity in humans (Dykes and Rooney 2006). Tannins also directly bind to digestive enzymes including sucrase, amylases, trypsin, chymotrypsin and lipases thus inhibiting their activity (Mathanghi 2012). Foods prepared from high tannin sorghums varieties have a longer passage in the stomach (Awika and Rooney 2004) and also said to have lower digestibility rate. Presence of slowly digestible starch in sorghum food products are said to exhibit a low glycemic index and increase satiety (Shin et al. 2004) which in turn helps in managing obesity.

To use tannin sorghums for commercial purposes to help fight obesity, further confirmation of results of potential health benefits in humans, the levels of tannins required for desired effects and potential side effects if any on the availability of other essential micronutrients needs adequate research in this direction. Sorghum is an excellent source of dietary fibre and this fibre helps to curb food intake by sense of stomach fullness leading to increased satiety feeling thereby leading to decrease in food intakes.



Photo 8.3 Mobile van in Hyderabad, India displaying the nutritional and health benefits of sorghum

8.7.5 *Other Advantages of Sorghum*

In some parts of Africa, where maize has replaced sorghum there are reports from University of Witwatersrand medical school in South Africa who believe that “the change of the staple diet of black South Africans from sorghum to maize (corn) is the cause of the epidemic of squamous carcinoma of the esophagus. They relate the cancer to *Fusarium* fungi that grow freely on maize and less noticed on sorghum and further substantiated that in Africa where the staple food is sorghum, they observed a low incidence of squamous carcinoma of the esophagus (Isaacson 2005).

8.8 **Need for Value Addition and Alternate Food Products from Sorghum**

Sorghum is much better compared to other cereal crop such as rice for many of the nutritional parameters. The diversified uses of sorghum as food, feed and industrial uses are expected to increase the demand of sorghum and this demand can be fulfilled by demand driven farm production for specific processed/value added products. Recently, it is observed that there is a drastic reduction in the consumption of sorghum especially in urban areas (Gali and Rao 2012). The major factors affecting are the changes in food habits, urbanization, time consuming and cumbersome procedures of food preparation. Despite the fact that the, consumption of sorghum as direct food is low in urban areas, the demand for processed sorghum food is increasing in the urban areas (Dayakar et al. 2010).

There is increasing acceptability of sorghum foods when available in ready-to-cook or ready-to-eat form of convenient foods as awareness of sorghum as health food is gaining importance. Under this context, value addition and processing interventions has acquired great importance in creating demand and sustaining the livelihood of the sorghum farmers (Dayakar et al. 2010). Different value added foods such as flakes, pops, vermicelli, pasta, bread and biscuits are now available in organized markets. Sorghum can be puffed, popped, shredded and flaked to produce ready-to-eat breakfast cereals. Sorghum alone is not considered as a bread making cereal because of the lack of gluten, but addition of 20–50 % sorghum flour to wheat flour produces excellent bread (Dicko et al. 2006). Among interesting features of sorghum utilization is biscuits and other cooked products. In the USA and Japan, sorghum utilization as human food is increasing because of its use in snacks and cookies (Rooney and Waniska 2004). Sorghum has been intentionally introduced in China for food needs and it is becoming one of the most important crops in this country (Kangama and Rumei 2005). The future promise of sorghum in the developed world is substitution of wheat for people who are allergic to gluten (Fenster 2003). Pre-cooked sorghum flours mixed with vitamins and exogenous sources of

proteins (peanuts or soybeans) are commercially available in many African countries for the preparation of instant soft porridge for infants.

This can be achieved through reassessment of crop research in terms of current and future demand, resolving specific production constraints by improving productivity, development of post-harvest processing and value-addition technologies, creation of demand, marketing strategies and policies that would result in additional farm income and employment without sacrificing overall goal of attaining sustainable food and nutritional security.

8.9 A Model Approach for Value Addition and Commercialization of Sorghum Foods

The area under rainy season sorghum in India is 2.89 million hectares with a production of 3.05 million tons, while post-rainy sorghum is grown on 4.88 million hectares producing 4.18 million tons (Rakshit et al. 2012). Of late, sorghum production and consumption in India has come down over the past three decades, primarily due to the disincentives resulting from the public distribution system for fine cereals, change in consumer food habits, tastes, and inconveniences attached with the preparation of recipes. Therefore it has now become imperative to augment reorientation of research and development efforts of sorghum through value-addition and development of processed foods and industrial products.

At Directorate of Sorghum Research (DSR), Hyderabad, India, National Agricultural Innovation Project (NAIP) under Indian council of Agricultural Research (ICAR) emerged out with a value chain model on production and commercialization of sorghum food products. Renewed efforts were made to create demand for sorghum through processing interventions by diversifying its food uses; integrating all functions from on-farm production to consumption in a 'production to consumption' value-chain (Fig. 8.1). All the stake holders were linked for synergizing their incomes. To develop different nutritional and value added ready to eat/ready to cook sorghum food products (Table 8.3) such as multigrain flour, biscuits, flakes, semolina, pops, vermicelli and pasta were produced using various processing interventions were undertaken on a pilot scale. Thus consumers were given wide range choices in the form of ready-to-eat items, and all sorts of convenient foods of sorghum were made available in the market with reference to other cereal products such as wheat, rice and maize. The study also aimed at testing the nutritional superiority of sorghum based food products. Nutritional studies were conducted by active collaboration with National Institute of Nutrition (NIN), Hyderabad, India. Initial clinical trials on school children and diabetic groups were promising. Further confirmation of the results on larger scale will greatly help to popularize sorghum among the target groups.

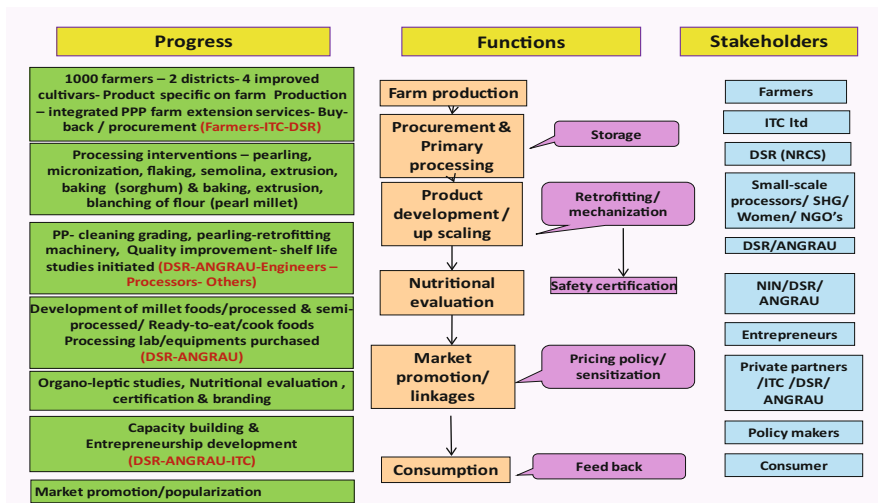


Fig. 8.1 Value-chain model on production and commercialization sorghum foods. *ITC* private company with diversified business including Agri-business, *DSR* Directorate of Sorghum Research, *ANGRAU* Acharya NG Ranga Agricultural University, *NGO* non-governmental organization, *SHG* self help group, *NIN* National Institute of Nutrition

The primary function of the value-chain project was to develop end-product specific on-farm production using specific cultivars which were initiated both during the rainy and post rainy seasons in the major sorghum growing regions of the country. Around 400 ha of land were brought under this pilot study covering about 500 farmers during each sorghum growing season. The beneficiary farmers were technologically supported by Directorate of Sorghum Research through supply of seeds of improved cultivars suitable for product-specific on-farm production on intensive scale. Integrated farm extension services are provided to the participating farmers in private-public-partnership mode through by our private partner Imperial Tobacco Company of India (ITC) who have diversified business including agri-business as one of the segments. The farmers were given buy-back assurance by the private partner and they procured and bulked the identity preserved grain of fair average quality from the participating farmers and in turn supplied to the small-scale processors at prevailing market price. Thus the value-chain was integrated with all the stakeholders. Simultaneously, research and development efforts on identification of product-specific cultivars and shelf-life studies on flour and other products were undertaken at Directorate of Sorghum Research, Hyderabad and Acharya N G Ranga Agricultural University (ANGRAU), Hyderabad, India. Six different sorghum based food products viz., multigrain flour, biscuits, flakes, pops, vermicelli and pasta were packed, labeled and promoted under Directorate of Sorghum Research registered brand “*Eatrite*” DSR, Hyderabad.

Table 8.3 Nutritional parameters of sorghum flour and sorghum based food products

Name of product	Moisture (g)	Protein (g)	Fat (g)	Total dietary fibre (g)	Insoluble (g)	Soluble (g)	Carbohydrates (g)	Calcium (mg)	Magnesium (mg)	Iron (mg)	Zinc (mg)
Flour	8.25	8.96	3.24	8.69	7.1	1.59	68.7	15.4	140.2	3.52	1.69
Vermicelli	8.72	9.79	2.87	9.23	7.92	1.31	68.5	8.81	79.6	1.84	0.96
Pasta	8.68	9.5	1.22	5.56	4.82	0.74	74.4	16.99	54.3	2.9	0.98
Flakes	10.55	7.23	1.79	5.97	5.43	0.54	73.8	10.94	68.9	3.44	0.88
Biscuits	2.76	6.7	23.72	5.27	3.54	1.73	59.3	68.8	92.2	2.02	1.69
Soya blend	7.89	11.9	2.62	12.71	9.77	2.94	63.2	25.4	62.9	3.03	1.06

Source: Directorate of Sorghum Research, Hyderabad, India



Photo 8.4 Ready-to-cook and Ready-to-eat sorghum food products being developed and popularised at Directorate of Sorghum Research, Hyderabad, India

Under different business models for processing, packing, marketing & distribution of sorghum foods, Directorate of Sorghum Research entered franchise agreement with about ten different private entrepreneurs for production and commercialization of sorghum food products. In turn they would pay a royalty on franchisee of our brand. Entering agreements with various private entrepreneurs plays major role in the sustainability of value chain. Subsequently up scaling and fine-tuning of different food products was carried out to satisfy the changing consumer tastes/preferences. Entrepreneurship development and popularization was also an integral part of commercializing sorghum value-added products. Social networking websites like Orkut, Twitter were used in combination with news and print media to generate interest among health conscious urban consumers. Road shows were conducted in mall and public parks to promote by showcase diverse sorghum processed foods, offering a range of choices to the consumers. Thus event managers are outsourced to create awareness on health and nutritional characters of sorghum foods. The spin-offs are huge, as the response has been overwhelming both from consumers and private entrepreneurs who came forward to link themselves up with the value-chain.

Meanwhile, efforts were made to sensitize the government departments through “Brainstorming sessions of National seminar on Millets” to introduce sorghum in mid-day meals scheme of school children and public distribution systems. Department of Agriculture and Co-operation under Ministry of Agriculture, India recognized the pilot effort as a model for backward integration and are contemplating to extend it to other commodity groups. Farmers were benefited by intensive cultivation and market assurance for their produce while consumers were benefitted by the choice of Sorghum products available for ensuring their nutritional security. The successful implementation of the project has helped the farmers as well as the policy makers for rolling out a new program “Initiative for nutritional security through intensive millets promotion program on a larger scale with huge budget for promotion of millets in the country.



Photo 8.5 Various sorghum based food products being developed and commercialized using the Directorate of Sorghum Registered brand “Eatrile”

8.10 Major Constraints of Sorghum for Food Related Uses

The major problem for food uses is the time consuming and cumbersome procedures of food preparation. There is also substantial problem with regard to shelf life of the sorghum products as the case with other millet food products. There is also drudgery of processing and technical knowledge on value addition. Other concerns include lack of availability of specific end product cultivars, lack of supply chain and regulated markets for procuring superior quality grains and also lack of better storage facilities.

8.11 Suggestive Measures for Sustainable Sorghum Production as Health Food

1. Evaluation of germplasm/landraces for different end-product specific uses.
2. Breeding cultivars with major attention on improving the nutritional quality and shelf life.
3. Development of healthy/specialty foods from sorghum such as diabetic foods, high fibre foods, weaning foods, functional foods, quick cooking cereals, etc. Branding of sorghum products as “health food” with proper certification may play a major role in increasing the demand of sorghum.
4. Development of low-cost machinery for preparation of value added processed food products and making it available in sorghum growing regions.
5. Up gradation of the existing food products for improving the quality and consumer acceptability.
6. Continuous supply of sorghum chain should be ensured for large scale promotion of sorghum value added food products.
7. Creating awareness among the urban and rural masses about the nutritional superiority of sorghum and popularization of value added sorghum food products as health foods.

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Chapter 9

Sorghum and Millet Seed Systems in Southern Africa

Medson Chisi

Abstract Sorghum and millet are major traditional cereal crops in southern Africa for food security in marginal areas. They are largely grown by smallholder farmers. Most national agricultural research systems (NARS) in the region have developed sorghum and millet varieties that have found wide appeal to farmers. Impact studies conducted in the region, however, show that adoption rates are largely low. Some of the reasons cited for this are lack of improved seed, largely due to poor seed delivery systems. Smallholder farmers, as a result, have limited access to responsive, high-yielding, locally adapted varieties of their staple food crops.

Both the formal and informal seed systems reviewed in this article do not entirely address this problem. Initiatives to improve access to sorghum and millet varieties released in the region must primarily target the informal sector and integrate it with formal sector to efficiently provide seed. This involves establishing local seed producers who can then supply their community members with seed. Four models were proposed at the local level and are currently in use:

1. On-farm seed production by the community for local distribution.
2. Smallholder farmer seed production on contract by seed companies
3. Production and packaging in small pack
4. Rural primary schools as centers for multiplication and distribution

This article, through the above approaches, presents some practical examples of an integrated approach to multiply and distribute improved seed of sorghum and millet to farmers in Tanzania, Zambia and Zimbabwe.

Keywords Smallholder • Food security • Private sector • Seed systems • Sorghum • Millet • Seed policy • Seed quality declared seed • Germplasm

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9.1 Introduction

Seed is an integral part of human civilization and food security is heavily dependent on seed security. This resource and the knowledge learnt from it have been passed on from one generation to the next. With increased knowledge about the science of agriculture, crop yield and quality have been significantly increased by procedures such as selection and crossbreeding and time to develop varieties reduced by novel approaches such as biotechnology. While the release of these varieties to farmers by breeders is commendable, the whole process is rendered useless when seed of these varieties is not accessible to farmers (Chisi 2002). Viable seed supply systems to multiply and distribute the seeds or plant materials that have been developed or preserved are critical for the success of food security programmes. In developing countries, this process cannot be left to the formal sector alone (Sperling et al. 1992; Almekinders et al. 1994; Almekinders 2000). The linkages among stakeholders in the seed industry need a serious examination to improve the situation and drastic changes to the national seed policies to allow the informal sector to have a role. Opportunities exist for the participation of public research institutions, non-governmental organizations and research trusts in seed production of small grains for smallholder farmers (Chisi et al. 1997).

9.2 Sorghum and Millet

Sorghum and millet are important traditional cereal crops in Africa. The crops are important in marginal areas of southern Africa where other cereal crops such as maize would normally fail (National Academy Press 1996). These areas are characterised by high surface and soil temperatures, low and poorly distributed rainfall. The fertility of the soil is generally poor. Some sorghum in southern Africa and finger millet are grown in high rainfall areas. In general local landraces of sorghum and millet are characterised by lateness to maturity, poor harvest index, variable plant height, head and seed characteristics as well as being susceptible to diseases and insect pests like downy mildew, blast, smut, anthracnose, shootfly and armoured cricket (Chisi 2002).

The crops are largely grown at subsistence level and by small holder farmers. In South Africa sorghum is grown on a commercial level (Pretorius et al. 1994). On a comparative basis Tanzania and South Africa utilise sorghum on a wider scale than other countries in the region (Rao et al. 1989; Anandajayasekeram et al. 1995). There is no doubt that sorghum and millet are essential for the attainment of food security and for the establishment of sustainable production systems in the semi-arid regions of southern Africa.

9.3 Crop Improvement in Southern Africa

In the beginning most of the breeding programmes placed emphasis on introductions, evaluating it and then selecting adapted lines. Later programmes embarked on hybridisation followed by evaluation and selection in segregating populations. Whatever method that was employed the main focus was on earliness, increasing grain yield and disease and pest resistance. Varieties such as Framida and Red Swazi were released with wide appeal from these initial efforts (Anandajayasekeram et al. 1995; Obilana et al. 1996). However, without exception now, all the countries in southern Africa have a breeding programme that is attempting to address problems affecting production by farmers. The programmes have also shifted the focus from wide to specific adaptation. It is also true that these programmes are at varying levels of advancement in terms of funding and staffing and consequently effectiveness (Obilana et al. 1996; Chisi 2002).

In a concerted effort to improve the production and productivity of these crops, governments in southern Africa under the auspices of Southern Africa Development Community (SADC) pooled their resources together and established Sorghum and Millet Improvement Programme (SMIP) in the early eighties (Rao et al. 1989; Anandajayasekeram et al. 1995). The programme was managed by ICRISAT. In early 1983 significant efforts were made through the regional programme SMIP to acquire new and enhanced germplasm from all possible sources. About 10,200 exotic germplasm of sorghum and 6,163 of pearl millet and close to 3,000 finger millet lines were acquired and evaluated at the regional centre at Matopos (Rao et al. 1989). In addition, the local collections from within the region formed the foundation of national breeding programmes.

In sorghum and finger millet, crop improvement activities have largely used the pedigree and backcross method to advance the breeding material. In pearl millet population improvement methods have been employed. These strategies need to be identified and built into technologies designed to improve production and productivity of these crops.

In southern Africa, except South Africa, both at national agricultural research systems (NARS) and regional level scientists are largely working independently as opposed to collaboratively (Chisi 2002; Mgonja et al. 2005a). Plant breeders, entomologists, pathologists, biochemists and indeed food technologists rarely collaborate in sorghum and millet research. This scenario is partly due to poor structural arrangements and poor staffing in some of these countries. This result in delays in meeting set objectives and proves expensive in the long run. The net effect of all this is to slow down the identification of suitable lines for crosses and later selection for desirable traits for farmers.

9.4 Varieties Released in the Region

Through collaborative ventures among NARS, SMIP and INTSORMIL, most countries in the region have released a number of varieties to farmers with varying degrees of successes. Some of the varieties were released through NARS own efforts (Tables 9.1 and 9.2). However, because the NARS had access to the germ-plasm distributed by SMIP, multiple variety releases in which some genotypes were released in more than one country but at different times. A good example is a variety SDS 3220 that was released as Macia in 1989 (Mozambique, Zimbabwe, Namibia, Tanzania), Phofu (Botswana) (Mgonja et al. 2005a, b). The fact that multiple releases could be had independently in the region by NARS has shifted the thinking from nationalisation to regionalisation of testing in an environment of shrinking investment into research programmes.

Table 9.1 Sorghum varieties released by NARS in southern Africa

Country	Variety name	Hybrid/variety	Year of release
Botswana	Phofu	V	1994
	Mahube	V	1994
	Mmabaitse	V	1994
	BSH1	H	1994
Malawi	Pirira 1	V	
	Pirira 2	V	
Mozambique	Macia	V	
	Mamonhe	V	
	Chokwe	V	
Tanzania	Tegemeo	V	1983
	Macia	V	1999
Zambia	Kuyuma	V	1989
	ZSV-15	V	1998
	Sima	V	1989
	WP-13	V	1996
	ZSV-12	V	1996
	MMSH-375	H	1992
	MMSH-413	H	1992
	MMSH-1324	H	1998
MMSH-1257	H	1998	
Zimbabwe	SV-1	V	1987
	SV-2	V	1987
	Macia	V	1998

H hybrid, *V* open pollinated variety

Table 9.2 Pearl and finger millet varieties released by NARS in southern Africa

Country	Variety name	Year of release
Malawi	Tupatupa	1996
Namibia	Okashana 1	1989
	Okashana 2	
Mozambique	Kuphanjala	1999
	Kuphanjala	1999
	Changara	1999
Tanzania	Okoa	1994
	Shibe	1994
Zambia	Kaufela	1989
	Lubasi	1993
	Kuomboka	1999
	Sepo	1998
	Tuso	1998
Zimbabwe	PMV-1	1987
	PMV-2	1992
	PMV-3	1998
Finger millet		
Zambia	Lima	–
	Nyika	–
	Senga	–

9.5 Impact Studies

A number of studies that have been taken in the region to study the impact of sorghum and millet research have shown that the results have been modest. Specific studies conducted in Botswana, Zimbabwe, Namibia, and Zambia show modest internal rates of return (Anandajayasekeram et al. 1995; Chisi et al. 1997). For example, the estimated internal rate of return ranged from 12 to 19% in Zambia depending on the future adoption path (Chisi et al. 1997). Some of the reasons cited for the marginal returns are lack of improved seed, lack of information, marketing and utilisation, poor grain quality and fertility management issues. In addition, a general perception that sorghum and pearl millet are crops of resource poor farmers in terms of production and consumption has not helped the promotion of the crops.

9.6 Agricultural Policies

As stated above, most countries in the region recognize the importance of agriculture in the economy and have placed the development of the agriculture sector as one of the top priorities. In general, the policy environment for agriculture has focused on economic liberalization and market-oriented reforms in which the private sector takes a major lead (Chilonda and Minde 2007). Private sector

involvement was permitted following the economic liberalization policies that swept through the continent in the late 1980s and early 1990s. In most of the SADC countries policies have consistently been amended to meet the dictates of the increasing demand of seed and the involvement of private seed companies (CALR 2012). The involvement of the private sector has worked well with agro-inputs such as fertilizer, pesticides and insecticides, but it has met with mixed success as regards seeds. Most of the seed companies have a well articulated programme on major cereals such as maize and wheat. However, small grains such as sorghum and millets have almost been ignored by these private seed companies.

It should be said that the seed sector in southern Africa is regulated with stringent measures put in place. The model followed is not very different from that proposed by Harris and Cortes, 2009. It is also understood the regulations are meant to protect the consumers and ensure high quality seed is sold to farmers (Minot 2007; Harries and Cortes 2009). However, it should be understood that in most of the countries farmers remain seed insecure among other reasons because of conflicting seed laws and regulations and procedures. There have been a number of studies done in the region that indicate the regulations and laws could be waived to get the seed to the intended target, the farmers within and outside the country in the region. In a related study, Mgonja et al. (2005a, b) showed that a regionalised breeding approach can enhance efficiency in agricultural research (Fig. 9.1). The strategy had a lot of implications on variety registration, access to seed and seed trade in the region. It is these kinds of results that drove the push towards harmonisation of seed policies in the region.

It should be noted that a lot of progress has been made in the process and the MOU for the implementation of SADC regulatory system was developed and signed. So far eleven countries have signed it and it came into force on 7 July 2013. With the MOU in place it is hoped that varieties will be made available to farmers with ease in more than one country. It should also be easier for investors and traders to enter markets and seed movement should be efficient.

The SADC Harmonized Seed Regulatory System consists of rules, standards, procedures and supporting measures necessary to facilitate the movement of seed between countries in the region. It establishes commonly agreed regulatory standards, rules and procedures related to: Seed Variety Release; Seed Certification and Quality Assurance; and Quarantine and Phytosanitary Measures for Seed. The rationale for this System is premised on the need to facilitate enhanced seed trade in the region and to increase the availability of high quality seed to farmers through rationalizing and removing national regulatory barriers for the movement of seed across borders. This is expected to promote economies of scale in seed supply and to reduce market uncertainty (Rohrbach et al. 2003; Mgonja et al. 2005a).

9.7 The Problem

As stated above remarkable progress has been made in identifying improved germplasm and a number of varieties have been released by national programmes in the region. Almost all the countries in the SADC region can boast of an improved

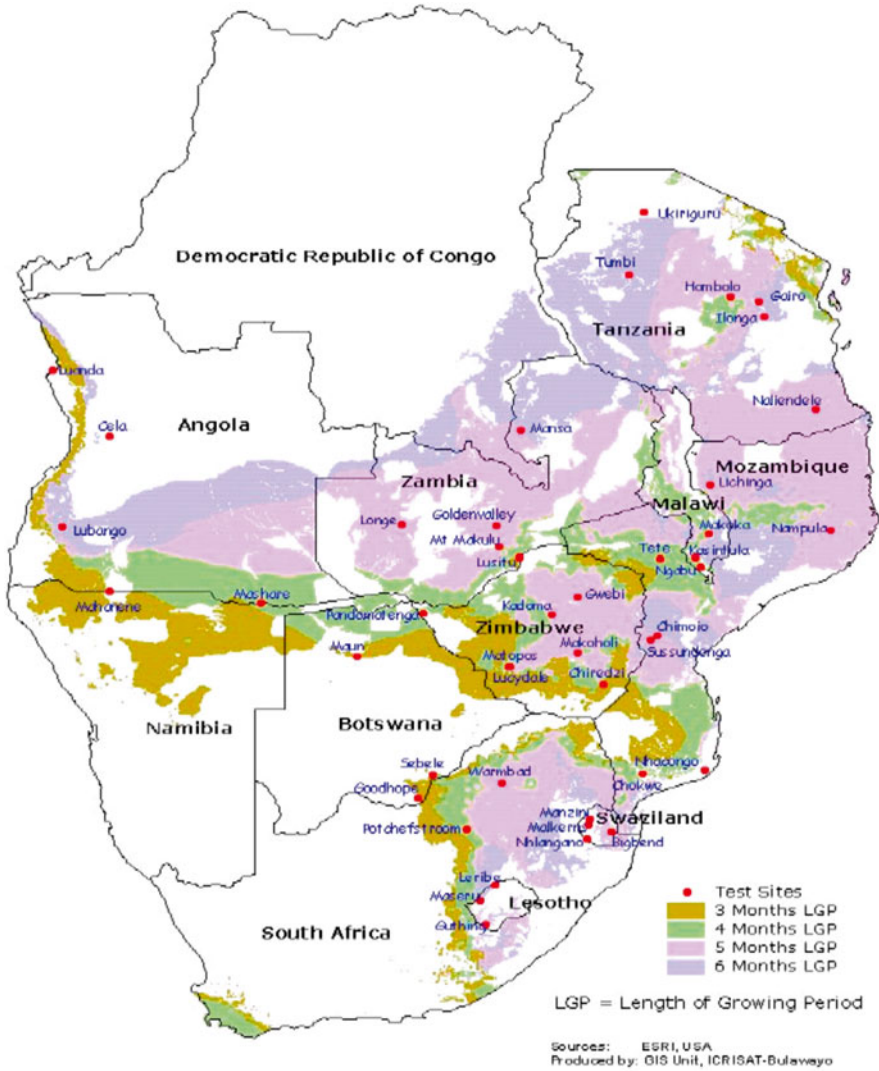


Fig. 9.1 Overlay of test sites and lengths of growing period showing similar test sites in the Southern Africa Development Community (SADC) countries (From Mgonja et al. 2005a, b)

variety of either sorghum or millet (Tables 9.1 and 9.2). Coming from an era of state controlled enterprises, it has taken long for the private sector in most of the SADC countries to absorb the concept of liberalisation in the various sectors of the economy including the seed sector. It should be stated that seed production is a specialized and essential industry in agriculture. It is made up of several components that include research, production, quality control and marketing (Baird 1985). Both the private and public sector are involved and their roles may be complementary. While

the private sector's interest in commercial crops such as maize is obvious, it is very low in the small grain crops to warrant investment in these open-pollinated varieties. Hybrid varieties for major cereals have proved popular with all the categories of farmers. This is yet to be seen with sorghum and millet hybrids.

The development and release of improved sorghum and millet varieties by NARS programmes can be viewed as a major achievement, yet seed of these is not readily available on the market (Anandajayasekera et al. 1995; Chisi et al. 1997). Private seed companies are reluctant to produce seed of these small grains citing low demand and the high cost of distributing the seed in far flung areas. This has resulted in a critical shortage of improved sorghum and millet seed on the market. The farmers have to rely on low-quality seed that has been saved and reused, degenerating over the course of time. There is need therefore for research programmes and other stakeholders to intervene and find sustainable measures to get improved seed to farmers.

9.8 Quality Seed

Quality seed is one of the primary requirements for establishing food security to farmers. Guaranteeing farmers access to quality seed can only be achieved if there is a viable seed supply system to multiply and distribute the seeds that have been produced or preserved and if mechanisms to assist farmers in emergency situations have been established (Sperling et al. 1992; Zerbe 2001). Agricultural policies aimed at securing ongoing food supplies in a country must emphasize seed system strategies that will ensure the availability of quality and locally appropriate seed varieties to farmers in a timely and affordable fashion (Baird 1985; Setimela et al. 2004; Harries and Cortes 2009).

9.9 Seed Delivery Systems

Maredia et al. (1999) and Minot (2007) define “seed system” as a set of public and private institutions which participate in the production, processing, storage, and distribution of seed. There are many variations of seed systems found in southern Africa and can be described simply as formal and informal seed systems (Setimela et al. 2004; Rubyogo et al. 2007a; Nakaponda 2012). Wekundah (2012) elaborates and describes three systems including a third one integrated seed system a community based system.

The improved varieties and hybrids have been released the seed of these is not readily available from the various seed producers. Except in South Africa, seed companies that were tasked to be conduits of government released varieties have failed to deliver citing low demand and the high cost of distributing the seed in far flung areas (David and Sperling 1999; Setimela et al. 2004). The private sector's interest in these crops is not sufficiently high to warrant investment in

open-pollinated varieties but hybrids. Hybrid varieties have proved to be popular with all the categories of farmers. The idea of purchasing seed of hybrid varieties can be a hindrance to small holder farmers who are generally resource poor. This has contributed to the low adoption of these varieties. There is need to move beyond foundation seed and deliver seed to farmers.

9.10 Formal Seed Sector

In southern Africa, in general, the seed supply sector can be classified into the formal and informal sector. The formal seed supply systems include public sector institutions such as seed agencies providing seed certification and quality control. The formal seed sector is an official or private control of seed monitored through the entire process of breeding, multiplication, processing and storage, leading to the final product. The private sector has a huge stake taking up the role of increasing, distributing and marketing the seed (Baird 1985; Zerbe 2001). The central premise of the formal system is that there is a clear distinction between ‘seed’ and ‘grain’ (Sperling and Cooper 2003).

The formal seed sector was set-up and organised with the principal goal to diffuse quality seed of improved varieties developed by the formal breeding programmes. Typically, the private seed sector supplies seed for cross-pollinated cash and food crops like maize, vegetables, cotton, and providing a fair share of the seed needed for the total area planted. In many cases, this refers to the category of crops for which farmers can pay inputs and that are characterised by a breeding system or other seed technology for which specialisation is economically profitable (Almekinders 2000; Setimela et al. 2004). The advantages of specializations are often related with the use of hybrid seed, specialised storage or seed health problems. Following the wave of economic liberalization that affected the region in the early nineties, a number of private seed companies were formed to conduct research and develop varieties (Chisi et al. 1997; Rohrbach and Kiala 2007; Zerbe 2001; SADC 2008). Most of the companies have concentrated on high value crops such as maize and vegetables (Rubyogo et al. 2007a, b). However, for most self-pollinating food crops such as cereals and legumes, the public sector is the major seed source.

Whereas the advantages of improved varieties such as sorghum and millet developed by national programmes are known, seed of the same is not available for purchase or otherwise. The crop yields of improved varieties were at least two to four times higher than the local varieties (Chisi et al. 1997). Most of the seed companies are not able to produce seed of these crops citing low demand and higher distribution costs. Remoteness and accessibility of agricultural production areas further increased the difficulties for timely supply of quality seed from the formal seed sector. In addition, the diversity of varieties, the variability in seed demands are difficult to cope with by large-scale, centralised formal breeding and seed programmes. Because the crops are self pollinated, chances are higher that the farmers will retain the seed from the previous crop thereby reducing seed sales.

9.11 Informal Seed Sector

The informal seed sector, sometimes described as traditional, is the opposite of the formal seed sector but it is all production activities of smallholder farmers. In southern Africa, it is estimated that about 90% of smallholder farmers' seed requirements are met through these sources (Monyo et al. 2003). A clear-cut distinction between the informal and formal seed system does not exist in the situations where public or private institutions are engaged in the production of uncertified, unlabeled or registered seed lots (David and Sperling 1999; Almekinders 2000).

The informal seed sector is simply the farmers themselves that provide each other and themselves with seed for sowing. The seed supply systems are comprised of indigenous strategies to improve the quality and quantity of seed used by farmers (Almekinders 2000). In this system, the farmer saves part of the harvest or exchanges seeds with farmers in the community. Although the informal seed sector provides a dynamic and flexible system of seed supply, usage, handling, trade and exchange, continuous use of untested seed inevitably leads to a degeneration of the seed quality – genetically, physiologically and seed health wise. For sorghum and millet, it is generally recommended that farmers obtain fresh certified seed after three or four cycles of retention (Chisi et al. 1997). Farmers depend on their own seed for sowing, not only because of the inadequate access to seeds from the formal seed sector, but also because the formal seed sector more often provides seeds of a limited range of cultivars and varieties, which not always fulfill the needs of the farmers to diversify the production. Although on-farm growing and maintenance of locally adapted landraces, cultivars and wild species help the farmer decrease the impact of a series of production constraints like drought, flooding, heat, cold, pests and diseases.

In the region, farmers' produced, selected and stored seed is still the predominant source of seed. Farmers' seed production is in most cases integrated in the normal crop production. Seed is selected from the crop production after harvest or before planting. Practices such as selection of heads or ears from the field before harvest, separate storage of seed, etc., reflect more specialised seed production practices. Farmers' practices of seed production, selection and storage are at the same time the basic components of local crop development and variety maintenance (Sperling et al. 1992; Sperling and Cooper 2003) (Table 9.3).

Table 9.3 Source of seed for communal farmers in Zimbabwe (% of farmers)

Source	Maize	Sorghum	Groundnuts
Farm saved	2	56	72
Community seed system	13	14	18
Formal seed sector	85	25	10

Source: Zimbabwe 1989 Seed Survey, cited in Cromwell (1996) p 95

9.11.1 Collaborations/Partnerships

Collaborations with agriculture institutions such as universities, extension and NGOs is one of the many avenues that should be pursued to get improved seed to farmers. As stated above, many NGOs are highly involved in the production of both foundation and certified seed that has benefited smallholder farmers and in agricultural extension. Extension services are fundamental to the success of agricultural development including seed production. It is the responsibility of extension services to provide advice to farmers concerning seed selection, treatment and storage, and to share information regarding the release of new varieties and demonstrate their characteristics. Without an effective extension system responsive to farmers' needs, it would be difficult to develop a seed supply system, particularly on-farm improved seed production systems. Extension also serves as a feedback mechanism to provide research with farmers' observations on varieties or technology offered.

9.12 On-Farm Seed Production

Generally most NARS programme have embarked on on-farm seed production as a way of getting the seed to farmers. Smallholder farmers are provided with enough seed to cover a quarter to a half hectare. The crop is monitored and supervised by extension officers recruited by an NGO. At harvest the farmer pays back in kind the same quantity and retains the rest. The seed from the seed-bank is given to another farmer and the process continues till the fourth year when fresh seed is acquired. This has met with some success with smallholder farmers in a number of countries in alleviating the problem of lack of improved seed (David 2003; Rubyogo et al. 2007a, b). However there is need to train smallholder farmers in ensuring that standards are kept high in seed production.

As stated above, there are several approaches that could be used to address the issue of seed delivery to farmers at a local level. A simplified model in the informal sector involves public research institutions (NARS), working in collaboration with NGOs, extension and farmer groups (Fig. 9.2).

It has been estimated that the bulk of the small seed grains production in region is still in the hands of the informal seed supply system (FAO/World Bank 2001; Setimela et al. 2004; FAO 2006). Monyo et al. 2003 proposed three other innovative community supply seed models.

9.12.1 Smallholder Farmer Seed Production on Contract for Sale to Commercial Seed Companies

In this particular case a partnership between a research entity (source of seed) and a private seed company (offers market) produce and distribute seed widely. The research organisation offers training and supervises the seed production.

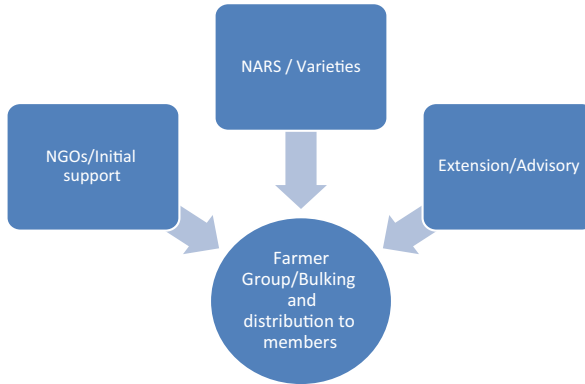


Fig. 9.2 Simplified model of seed delivery to smallholder farmers – farmers are supported by researchers, extension and NGOs to produce and distribute it to others

9.12.2 Model II. Promotion of Improved Seed Through Sale of Small Packets

The strategy for Model II was a practical step in addressing the seed needs of the rural resource poor farmers. The strategy entailed packing seed of popular varieties in small packs of 500 g, 1 kg, 2 kg and 5 kg. The prices of the packs reflected the full cost of packing and distribution. Monyo et al. 2003 reported an estimated 80 % of the seed placed in urban shops was sold while 55 % was sold in rural shops. The different seed packs allowed farmers with less money to purchase what they could afford.

9.12.3 Model III. Rural Primary Schools as Centers for Production and Dissemination of Improved Seeds

Basically the strategy uses schools in the rural areas as multiplication and distribution centers. The selected schools are carefully selected to be representative of the community and are given enough seed to plant 1 ha and serve about 500–700 farmers.

Smallholder seed production, in terms of certification, in a number of countries in the region falls under the “quality declared seed” system. Standards are established or adjusted according to countries. The quality of seed is controlled by the producer. The producer declares the quality of the seed, while the governmental local seed controlling agencies only control a part of the seed production with random control like spot-checking and field inspections. Farmers clearly recognize the difference between grain and seed, even in the context of free seed transactions between households (FAO/World Bank 2001).

9.13 Conclusion

A functioning seed industry is a key component of modern agriculture. It is generally agreed that advances have been made in germplasm enhancement in the region. High crop yields are obtained from seed varieties with high yield potential with improved crop management. The challenges for the formal seed sector for high valued cash crops as outlined above are minimal and the private sector's involvement is high. However, the informal seed sector is full of challenges and the smallholder farmers in marginal areas are affected the most. Therefore initiatives to improve access to improved seed varieties must primarily target the informal sector and integrate it with formal sector to efficiently provide seed. This involves establishing and offering some kind of training to local seed producers who can then supply their community members with seed.

The community based seed systems have some components of both the formal and informal systems. The involvement of local seed producers who can then supply their community members with seed is one of the solutions to lack of improved seed. In addition, public breeders in collaboration with research trusts and NGOs have a major role in producing breeder seed and increasing improved seed for the benefit of smallholder farmers.

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