Mansour Ghorbanpour
Ajit Varma *Editors*

Medicinal Plants and Environmental Challenges

Springer

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Contents

Importance of Medicinal and Aromatic Plants in Human Life

Mansour Ghorbanpour, Javad Hadian, Shahab Nikabadi and Ajit Varma

Abstract The plant kingdom includes a high number of species, producing a diversity of bioactive molecules with different chemical scaffolds. Over the centuries, the use of medicinal and aromatic plants has become an important part of daily life despite the progress in modern medical and pharmaceutical industry. They are now being progressively cosmetics, foods and teas, as well as alternative medicines. The growing interest in herbs and their ability to offer economical uses is a part of the movement towards greener economics and life styles. This movement is based on the belief that the plants have a vast potential for their use as a curative medicine. Medicinal and aromatic plants will also maintain their importance in the search for new, valuable sources of drugs and lead compounds. In view of the steadily rising demands on these important natural resources, attention should be paid to the sustainable forms of production and utilization.

Keywords Medicinal and aromatic plants · Phytochemicals · Traditional-/modern medicine

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Introduction

With the emergence of human civilization, plants have been the main source to cure, heal and alleviate various diseases. Medicinal plants belong to a big plant group with a great interest due to its pharmaceutical, cosmetic and nutritional values (Abdul Rasool Hassan 2012). The synthesize compounds of these plants are useful to preserve the health in humans and animals. These include aromatic substances, such as phenols or their oxygen-substituted derivatives which is called tannins. Plants have an almost limitless ability to synthesize aromatic substances mainly secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. These compounds are usually served as the molecules of plant defense against predation by microorganisms, insects, and herbivores.

Many of the herbs and spices used by humans to season food yield useful medicinal compounds. The demand for medicinal plants is currently increasing in both developed and developing countries for various reasons.

In some, it would be the growing recognition that natural products have fewer or even no side effects; for others, however it would be their accessibility and affordable costs that would tip the scales. Medicinal and aromatic plants can be processed to become essential oils through distillation, however, their cut flower marketing and the plants parts are in demand.

They are used in pharmacy, cosmetology, perfumes and the food industry among others. With increased demands for the available resources, a number of important plant species have become scarce in areas where they were previously abundant. When the collection and use is not regulated, some species may become threatened with extinction. In recent years, the use of medicinal and aromatic plants has increased greatly in western countries, India and China. In Europe, at least 2000 medicinal and aromatic plants species are traded commercially.

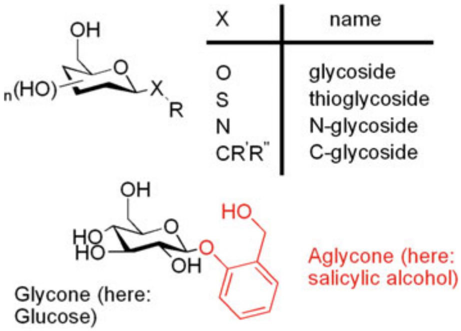
Medicinal plants have a promising future because there are about half million plants around the world, and most of their pharmaceutical capability have not investigated yet, which should be in demand of the present and future studies.

Therapeutic Importance of Phytochemicals

Plants have evolved to synthesize an extremely diverse range of chemical compounds known as secondary metabolites. These secondary metabolites have no apparent role in primary plant growth and development processes, so they are unique to plants from a single species and increase during times of high stress such as drought, temperature and bacterial infection (Taiz and Zeiger 2006). Many of these compounds exhibit anti-microbial, anti-oxidant, cytotoxic and other medicinally useful properties (Taiz and Zeiger 2006). These activities can be attributed to the presence of a variety of phytochemical constituents, which can be divided into three main chemically distinct groups: terpenes, phenolics and nitrogen containing compounds (alkaloids). The following is a brief presentation of the main chemical compositions of medicinal plants.

Glycosides

The glycosides are the products of secondary metabolism including various categories of secondary metabolites which bounded to mono or oligosaccharide and to uronic aid. The saccharide or uronic acid part is called glycone, and the other part is aglycone. The aglycon may be a terpene, flavonoid, coumarine or any other natural products. The main groups of glycosides (based on chemical nature of aglycon and pharmacological activity) are cardiac glycosides, cyanogenic glycosides, glucosinolates, saponins and anthraquinone glycosides. However, flavonoids are frequently occurred as glycosides.



The cardiac glycosides or cardenolides are a family of steroids that bind and inhibit the intracellular Na^+/K^+-ATPase pumps in the membrane of cells with high selectivity and affinity. These intracellular pumps are critical for the cardiac cells' function and their effects are really remarkable on the heart which leads to contractility increase and rate reduction.

Thus, cardio glycosidic plants are commonly used in the treatment of heart failure and cardiac arrhythmia. Cardioactive glycosides are found in a diverse group of plant species including *Digitalis purpura* and *D. lanata* (Scrophulariaceae), *Nerium oleander* (Apocynaceae) and *Convallaria majalis* (Convallariaceae) (Oerther 2011; Bernhoft 2010).

The cyanogenic glycosides or cyanoglycosides have aglycones derived from amino acids. These compounds are usually interfered with the iodine utilization and result in hypothyroidism. Cyanogenic glycosides can become very toxic and lethal in high dosage, when hydrogen cyanide (HCN) release. Cyanogenic glycosides with at least 2500 taxa are relatively id in plant kingdom. Most of the taxa belong to families Fabaceae, Rosaceae (in particular in *Prunus* spp.), Linaceae, Compositae and others (Vetter 2000).

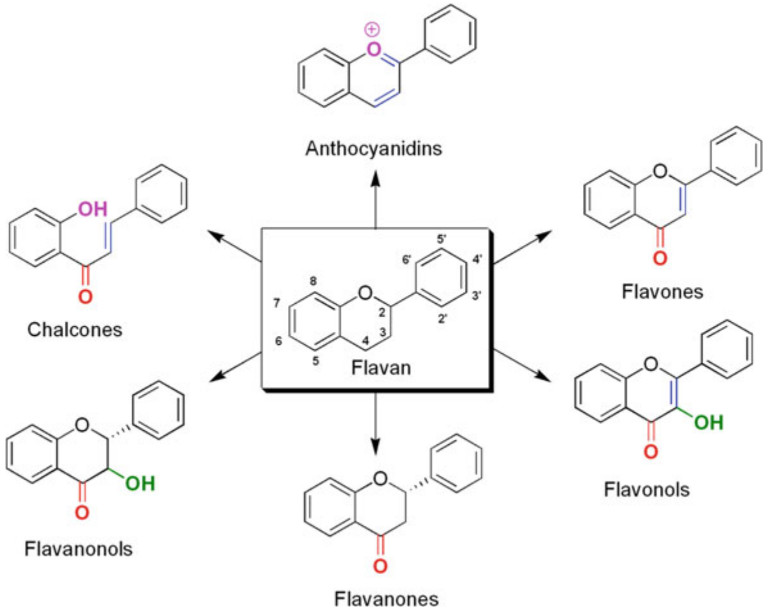
The glucosinolates (*S*-glucopyranosylthiohydroximates) contain sulphur-containing, pungent amino acid-derived a glycones. They are enzymatically hydrolyzed to produce sulfate ions, D-glucose, and characteristic degradation products such as isothiocyanates (Ishida et al. 2014). The compounds show a complex set of effects on cytochrome P450 isoforms in various cells and tend to decrease hepatic bioactivation of environmental procarcinogens. The glucosinolates cause skin irritation and also induce hypothyroidism and goitre. The Brassicaceae, Capparidaceae and Resedaceae are the main family associated with glucosinolate production.

The saponin glycosides are large molecules with a hydrophilic glycone and a hydrophobic aglycone, which give emulsifying properties and can be used as detergents. In addition, saponins exert a wide range of pharmacological activities including expectorant, anti inflammatory, vasoprotective, hypocholesterolemic, immunomodulatory, hypoglycemic, molluscicidal, antifungal, antiparasitic and many others (Sparg et al. 2004; Sahu et al. 2008). Plants rich in saponins, like *Panax ginseng* or *Glycyrrhiza glabra*, have been used for medicinal purposes since ancient times (Fiore et al. 2005) and to date continue to play a significant role not only in medicine but also in food and cosmetic industry, where they are utilized as emulsifiers or sweeteners (Guçlu-Ustundag and Mazza 2007). Saponins are also use as adjuvants in the production of vaccines (Sun et al. 2009). Aglycone component of anthraquinone glycosides is a polyhydroxyanthraquinone derivative.

Anthraquinone glycosides are generally orange, red, or brown-red compounds found in fairly limited distribution within the plant kingdom. In Polygonaceae (dock family) for instance, they could be found in *Rumex crispus* (curly dock) and *Rheum* spp. (rhubarbs). Their primary effect is induction of water and electrolyte secretion as well as peristalsis in colon.

Flavonoids and Proanthocyanidins

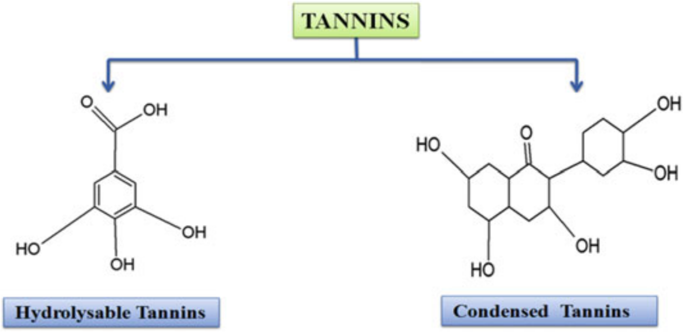
Flavonoids consist of a large group of polyphenolic compounds with a benzo-γ-pyrone structure, which are ubiquitously observed in plants. Proanthocyanidins are oligomers of flavonoids. Both groups of compounds can occur as glycosides and methylated derivatives. Flavonoids are the most common and widely distributed group of plant phenolic compounds, occurring virtually in all plant parts, particularly the photosynthesis plant cells (Kumar and Pandey 2013). All compounds contain phenol-groups involved in an effect as general antioxidant. Other actions are diverse-several structures reduce inflammation or carcinogenicity. The group isoflavones are primarily known as phytoestrogens. Flavonoids and proanthocyanidins are all pigments occurring in a long range of plant families. Isoflavones are produced by species of Fabaeceae (bean family).



Tannins

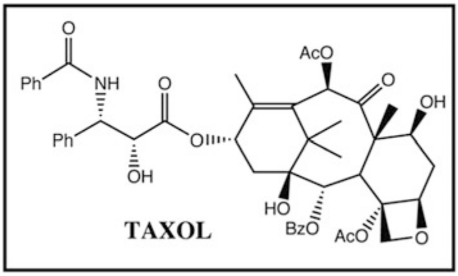
Tannins are distinctive group of polyphenolic polymers found widely in the plant kingdom. They are usually classified into two categories (on the basis of their structure and reactivity).

The first group is condensed tannins which have no carbohydrate core and comprise a group of polyhydroxy-flavan-3-ol oligomers and polymers linked by carbon-carbon bonds between flavanol subunits. The second group are hydrolysable tannins which are polyesters of gallic acid (gallo tannins) and hexahydroxy-diphenic acid (ellagitannins) with a central polyols such as sugars/glucose and phenolic such as catechin. The two types of tannins have most properties in common, but hydrolysable tannins are less stable and have greater potential to cause toxicity. The water solubility is restricted and decrease in general with the size of the tannin molecule. Tannins indiscriminately bind to proteins and larger tannins are used as astringents in cases of diarrhoea, skin bleedings and transudates. Condensed tannins are the most widely distributed tannins in vascular plants. However, hydrolyzable tannins are restricted to the dicotyledons (Bernays et al. 1989). Examples of plant families associated with presence of tannins are Fagaceae (beech family) and Polygonaceae (knotweed family).



Terpenoids and Phenylpropanoids

Terpenoids, also referred to as isoprenoids or isopentenoids, represent the largest and most diverse category of chemicals among the myriad compounds produced by plants (Tholl 2015). The terpenoids are synthesized via the five-carbon building block isoprene. Based on the number of the building blocks, terpenoids are commonly classified as hemiterpenes (C_5), monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), sesterpenes (C_{25}), triterpenes (C_{30}), tetraterpenes (C_{40}), polyterpenes ($>C_{40}$) (Ashour et al. 2010; Martin et al. 2003). The less diverse phenylpropanoid are based on a nine carbon skeleton and are synthesized via another pathway. Plant-based terpenoids are very lipophilic and tend to have strong flavors, which traditionally have been used by humans in the food, pharmaceutical, and chemical industries, and more recently exploited for development of biofuel products. Among these terpenoids, taxol (diterpene) of *Taxus baccata* and artemisinin (sesquiterpene lactone) from *Artemisia annua* are well known antineoplastic and antimalarial agents (Croteau et al. 2006; Pollier et al. 2011).



Resins

The resins are complex lipid-soluble mixtures-usually both non-volatile and volatile compounds (Langenheim 2003). The non-volatile fraction may consist of diterpenoid and triterpenoid compounds, and mono- and sequiterpenoids predominate in the volatile fraction. Resins are poorly defined chemically, however, isoprene (C_5H_8) units are the fundamental building blocks of all true resins. Most typical resins are secreted by a number of plants, especially pines and other conifers and are a major non-wood product of forests, but resins are also present in herbaceous plants (mainly in seed bearing plants). They are all sticky and the fluidity depends on their contents of volatile compounds. The plant resins are used widespread as adhesives, ingredients of cosmetic preparations, as fragrances in daily rituals and religious ceremonies, as coating materials and as remedies in folk medicine (Langenheim 2003; Bernhoft 2010). Most resins are antimicrobial and wound healing, but their actions depend on the composition of the chemical mixture. Today resins are used in the manufacture of a wide range of products including paints and lacquers, rubber, soaps, linoleum, essential oils, furniture polishes and pesticides.

Lignans

Lignans are a class of secondary plant metabolites produced by oxidative dimerization of two phenylpropanoid units at the β and β′ carbon atoms. Although their molecular backbone consists only of two phenylpropanoid (C6-C3) units, lignans show an enormous structural diversity (Saleem et al. 2005). They are derived from the shikimic acid biosynthetic pathway (Imai et al. 2006). Lignans are present at highest concentrations in oil seeds (e.g., flax, soy, rapeseed and sesame), but are also found in other parts of a long range of plants of different families. Several lignans show clinical activity as phytoestrogenic, cathartic or antineoplastic effects. The plant lignans most commonly detected in foods are lariciresinol, matairesinol, pinoresinol and secoisolariciresinol (Thompson et al. 2006).

Alkaloids

The alkaloids are a diverse group of low-molecular-weight, nitrogen-containing compounds, usually with potent activity and bitter taste found in about 20% of flowering plants, and are especially common in some families such as Fabaceae, Liliaceae, Solanaceae and Ranunculaceae. Compared with other classes of natural compounds, alkaloids are characterized by a great structural diversity and there is no uniform classification of alkaloids. The various groups of alkaloids have diverse biological and pharmacological properties.

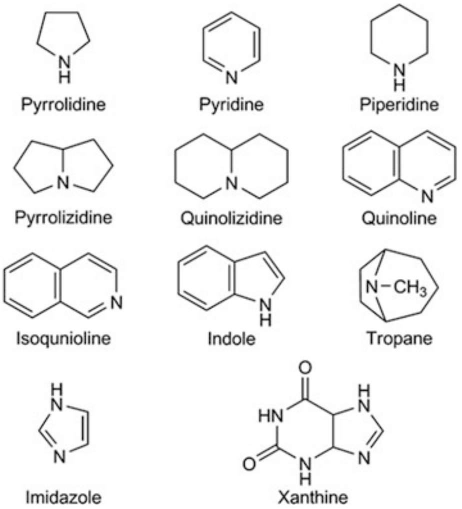
Tropane alkaloids consist of over 200 known compounds with a tropane ring in their structures, such as the anticholinergic drugs atropine and scopolamine and the stimulant cocaine (Lounasmaa and Tamminen 1993). Poisonous Solanaceae family plants, presently classified as genera: Atropa, Brugmansia, Datura, Duboisia, Hyoscyamus and Scopolia, with many alkaloid-containing species (Griffin and Lin 2000; Ghorbanpour et al. 2013), were well known already in ancient times, and records of their employment in folk medicine of various ethnic groups are abundant (Sneader 2005a, b). The compounds have anticholinergic activity (muscarine receptor antagonists) and are used medically to reduce smooth muscle spasms, hypersecretion and pain.

Pyrrolizidine alkaloids are considered to be important secondary metabolites largely on account of their biological activities, which include acute hepatotoxic, mutagenic, carcinogenic, teratogenic, anticancer properties and neuroactive properties (Asres et al. 2004). Pyrrolizidine alkaloids are produced in Asteraceae (daisy family), particularly in *Senecio* spp. (Ragworts) and in Boraginaceae (borage family).

The isoquinoline alkaloids are a large class of medicinally active alkaloids whose properties are variable. Their properties include being antispasmodic, antimicrobial, antitumour, antifungal, anti-inflammatory, cholagogue, hepatoprotective, antiviral, amoebicidal, anti-oxidant and can act as enzyme inhibitors. This class notably includes morphine and codeine. They are typically found in the Papaveraceae, Berberidaceae and Ranunculaceae families. They are derived from the amino acids phenylalanine or tyrosine.

Compared with other plant alkaloids, purine alkaloids are distributed widely throughout the plant kingdom although accumulation of high concentrations is restricted to a limited number of species, including *Coffea arabica* (coffee), *Camellia sinensis* (tea) and *Theobroma cacao*(cacao) (Ashihara and Crozier 1999).

Compounds called pseudoalkaloids most often have all of the chemical characteristics of the true alkaloids, however, they are not derived from amino acids. They are produced by species in Apiaceae (carrot family) for instance *Cicuta virosa* (cowbane), *Conium maculatum* (hemlock), and in Taxaceae (yew family) for instance *Taxus baccata* (yew). The pseudoalkaloids in *Cicutavirosa* and *Conium maculatum* have effects on the central nervous system and taxine in yews like *T. baccata* inhibits the ion transport of the hearth.



Important Plant Families Having Given Molecules/Drugs of Importance

Plants of commercial importance (e.g. Apiaceae, Apocynaceae, Araliaceae, Araceae, Asphodelaceae, Asteraceae, Ginkgoaceae, Hypericaceae, Lamiaceae, Papaveraceae, Piperaceae, Rhamnaceae, Rubiaceae, Rutaceae, Solanaceae and Zingiberaceae) serve as main sources of pharmaceutically active compounds (Gurib-Fakim 2006). In this chapter, the pharmaceutically most important families are highlighted, especially those that have yielded many, or very important, botanical drugs.

Apiaceae (Umbelliferae)

The flowering plant family Apiaceae (Umbelliferae) comprises 300–455 genera and some 3000–3750 species (Pimenov and Leonov 1993). It is cosmopolitan, being particularly abundant in the northern hemisphere. Members of this family are often rich in essential oil, which is one of the main reasons for the pharmaceutical importance for many of the apiaceous drugs.

Important medicinal plants in the family and their uses are the following (Gurib-Fakim 2006):

- *Carum carvi* L. (caraway), a carminative and also important as a spice.
- *Coriandrum sativum* L. (coriander), a carminative and also important as a spice.
- *Foeniculum vulgare* Miller (fennel), a mild carminative.
- *Levisticum officinale* Koch (lovage), a carminative and antidyspeptic.
- *Pimpinella anisum* L. (anise-fruit), an expectorant, spasmolytic and carminative.

Apocynaceae

Apocynaceae is a family of flowering plants that includes trees, shrubs, herbs, stem succulents, and vines, commonly called the dogbane family (Endress and Bruyns 2000). This family has wide distribution both in temperate and tropical regions of the world. Among the world famous species is the Rosy Periwinkle (*Catharanthus roseus*). Members of this family are well known for their alkaloidal contents with potent pharmacological activity.

Araliaceae

Araliaceae include 43 genera and almost 1400 species. Majority of them are prone to be grown in the tropical zones of both hemisphere, and are rarely observed in temperate climates.

Important medicinal plants from the family:

- *Hedera helix* L. [(common) ivy], used as a cough remedy. The *H. helix* leaves contain saponins, flavonoids, phenolic acids, emetine alkaloid, amino acids, sterols, proteins, vitamins, polyacetylenes, etc. (Parvu et al. 2015).
- *Panax ginseng* C.A. Meyer (ginseng), used as an adaptogene (a very ill-defined category) to combat mental and physical stress [and sometimes replaced by *Eleutherococcus* (*Acanthopanax*) *senticosus* (Rupr and Maxim) Maxim from the same family]. The triterpenoids (ginsengosides) are implicated in the pharmacological effects of *P. ginseng*, while saponins (hederasaponins) are used for the secretolytic effect of *H. helix*.

Palmaceae (Arecaceae)

The palms which comprises of approximately 2700 species are almost exclusively woody and is an important family as it includes many species widely used as food, and over the past years at least one of its members has become medicinally important.

The *Serenoa repens* (Sawpalmetto) is now being used for difficulty in micturition in benign prostate hyperplasia in the early stages.

The accumulation of polyphenols, some relatively simple alkaloids (especially pyridine derivatives), steroidal saponins, fatty acids [coconut (*Cocos nucifera* L.) and oil palm (*Elaeis guineensis* Jacq.)] is typical. The pharmaceutical use of the Sawpalmetto seems to be due to the presence of a relatively large amount of the triterpenoid-β-sitosterol (Gurib-Fakim 2006).

Asphodelaceae

This family are often included in the Liliaceae (lily family). This family, with about 600 species, are widely distributed in South Africa and some species occur in the Mediterranean regions (Gurib-Fakim 2006). The best-known members in this family are *Aloe vera* (L.) Burman f. (syn. *Aloe barbadensis*, Barbardos aloe) and *A. ferox* Miller (Cape aloe), both strong purgatives. The genus Aloe is characterized by the presence of polysaccharides accumulating in the leaves as well as anthranoids and anthraglycosides (aloe-emodin), which are responsible for the species' laxative effects.

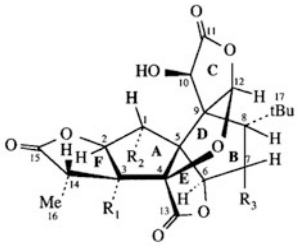
Asteraceae (Compositae)

This large family comprises of some 25,000 species and 1400 genera and is distributed and well represented in most ecosystems except for Antarctica (Gurib-Fakim 2006). A typical chemical trait of this family is the presence of polyfructanes (especially inulin) as storage carbohydrates (instead of polysaccharides) in perennial taxa. Important medicinal plants from the family are the following:

- *Arnica Montana* L. (arnica), used topically, especially for bruises.
- *Artemisia absinthum* L. (wormwood or absinthium), used as a bitter tonic and choleretic.
- *Calendula officinalis* L. (marigold), used topically, especially for some skin afflictions.
- *Cnicus benedictus* L. (cnicus), used as a cholagogue (a bitter aromatic stimulant).
- *Cynara scolymus* L. (artichoke), used in the treatment of liver and gallbladder complaints and several other conditions.
- *Echinacea angustifolia* DC., *E. pallid* Nuttall and *E. purpurea* (L.) Moench (Cone flower), now commonly used as an immunostimulant.
- *Matricaria recutita* L. (chamomille/camomille; several botanical synonyms are also commonly used, including *Chamomilla recutita* and *Matricaria chamomilla*).
- *Tussilago farfara* L. (coltsfoot), a now little used expectorant and demulcent.

Ginkgoaceae

This is one of the most ancient families of the seed bearing plants and had been widely distributed during the Mesozoic (180 million years ago). Only one species namely *Ginkgo biloba* L. has survived phytochemically, this plant is characterized by the presence of the gingkolides, which are unusual two-ringed diterpenoids with three lactone functions. Biflavones and glycosylated flavonoids are other groups of typical natural products.

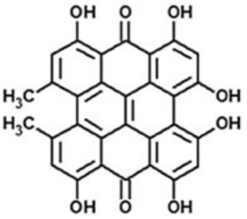


	R1 (C3)	R2 (C1)	R3 (C7)
Ginkgolide A	OH	H	H
Ginkgolide B	OH	OH	H
Ginkgolide C	OH	OH	OH
Ginkgolide J	OH	H	OH

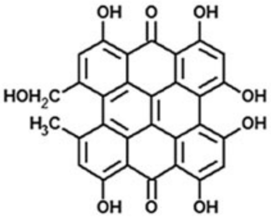
Hypericaceae

This family of some 900 species, are pharmaceutical importance because of St. John's wort (*Hypericum perforatum* L.), which in the last decade of the 20th century became one of the most important medicinal plants in modern medicine.

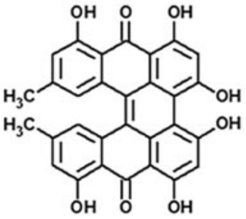
The former name Guttiferae is an important indicator of a characteristic chemical feature: the presence of resins, balsam and other glands containing excretory products. For example, the hypericin glands, with red colour, are present especially in the flowers and contain naphthodianthrones, including hypericin (a naphthodianthrone) and pseudohypericin, which are characteristic for some sections of the genus. Typical for the family in general are also xanthones (found nearly exclusively in this family and in the Gentianaceae). The genus is known to accumulate flavonoids and their glycosides (rutoside, hyperoside), as well as hyperforin (a lipophilic phloroglucinol) and its derivatives (Barnes et al. 2001), which are derived from the terpenoid pathway.



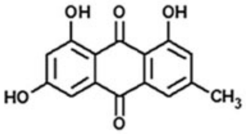
Hypericin



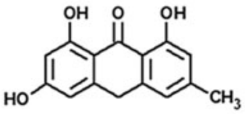
Pseudohypericin



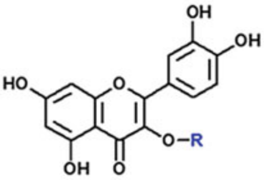
Protohypericin



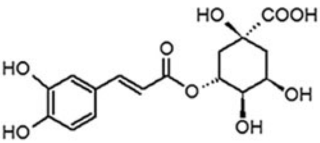
Emodin



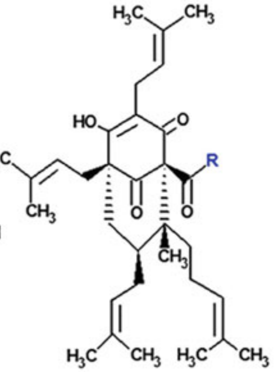
Emodin anthrone



Quercetin: R = H
Quercitrin: R = Rhamnose
Hyperoside: R = Galactose
Rutin: R = Rutinose



Chlorogenic acid



Hyperforin: R = $CH(CH_3)_2$

Lamiaceae

The Lamiaceae is a family yielding a high number of medicinal taxa (over 5000 species), especially due to their high content of essential oil. Important medicinal plants from the family are as below:

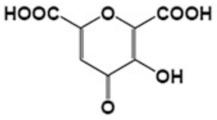
- *Lavandula angustifolia* Miller (lavender), a mild carminative and spasmolytic.
- *Melissa officinalis* L. (balm), a mild sedative, carminative and spasmolytic.
- *Mentha arvensis* L. var. *piperascens* Malinvaud (Japanese mint), yields a commonly used essential oil (e.g. for respiratory problems).
- *Mentha piperita* L. (peppermint), a commonly used carminative and spasmolytic and a hybrid between *M. spicata* L. and *M. aquatic* L.
- *Mentha spicata* L. (spearmint), commonly used in toothpaste and chewing gum, with mild carminative effects.
- *Rosmarinus officinalis L.* (rosemary), a carminative and spasmolytic.
- *Salvia officinalis* L. (sage), used as a topical antiseptic (gargling) and orally as a carminative and spasmolytic.
- *Thymus vulgaris* L. (thyme), a carminative and spasmolytic.

Some segments of the family are known to accumulate monoterpenoid glycosides. Many species also accumulate rosmarinic acid (and other derivatives of caffeic acid. Besides the antioxidant potential, rosmarinic acid has been reported to present a multitude of pharmacological and biological activities (Amoah et al. 2016).

Papaveraceae

The Papaveraceae are an economically important family of about 42 genera and approximately 775 known species, has produced a multitude of pharmacologically or toxicologically important genera (e.g. chelidonium, glaucium, papaver). This family is particularly rich in the isoquinoline alkaloids including morphine, papaverine, codeine, thebaine and noscapine. Other alkaloids present include the benzylisoquinoline such as papaverine (vasodilator and smooth muscle relaxant) and noscapine (antitussive and antitumorogenic) (Dewick 2002; Schmeller and Wink 1998). The two most important medicinal plants from the family are:

- *Chelidonium majus* L. (greater celandine), which yields the alkaloid chelidonine, employed as a cholagogue.
- *Papaver somniferum* L. [(opium) poppy], which yields a multitude of pharmacologically active alkaloids and is a well-known and dangerous narcotic.



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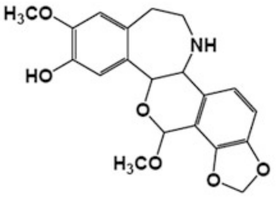

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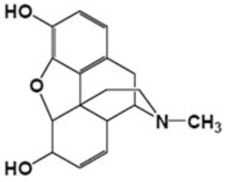

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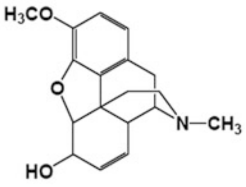

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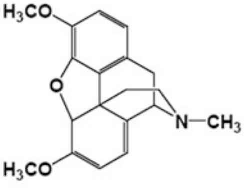

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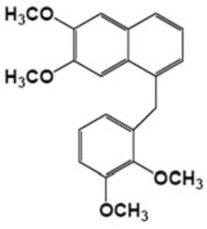
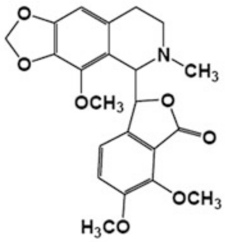


Papaverine



Noscapine

Piperaceae

The Pepper family consists of herbs and shrubs comprise some 2000 species is mostly restricted in the tropics.

Important medicinal plants from the family are the following:

- *Piper methysticum* Forster f. (kava-kava), traditionally used as a mild stimulant in Oceania and now used for conditions of nervous anxiety; recent reports of liver toxicity has resulted in withdrawal in many countries.
- *Piper nigrum* L. (black and white pepper), occasionally used in rubefacient preparations and as a spice.

Some of the isolated molecules from the family are:

Pungent acidic amides, such as piperine, are known from several members of this family, and sometimes essential oil is present. The α-pyrone derivatives (e.g. kavain) from *P. methysticum* are another group of commonly found compounds known from species of Piper.

Rhamnaceae

This family consists of 50 genera and more than 900 species. This family has a worldwide distribution but is more common in the tropical and subtropical regions of the world. The family is best known pharmaceutically because some taxa accumulate anthraquinones. Also alkaloids of the benzylisoquinoline type and the cyclo-peptide type are known from many taxa.

Important medicinal plants from the family are:

- *Rhamnus purshiana* DC. (American cascara) and *R. frangula* L. (syn. *Frangula alnus*, European alder, buckthorn), both used as strong purgatives (Gurib-Fakim 2006).

Rubiaceae

This large family of over 10,000 species has yielded one of the most important stimulants, coffee (*Coffea arabica* L. and *C. canephora* Pierre ex Froehner) and one of the first and most important medicinal plants brought over from the 'New World', cinchona bark.

The family is known for a large diversity of classes of natural products, including iridoids (a group of monoterpenoids), alkaloids (including indole alkaloids such asquinine from *Cinchona* spp.), methylxanthines such as caffeine, theobromine and theophylline, and anthranoidsin some taxa (e.g. the now obsolete medicinal plant *Rubia tinctorum*, which was withdrawn because of its genotoxic effect).

Important medicinal plants from the family are:

- *Cinchona succirubra* Weddell, *C. calisaya* Weddell and *Cinchona* spp. (cinchona, Peruvian bark), used as a bitter tonic, febrifuge and against malaria.

Rutaceae

This family comprises of 1700 species distributed throughout the world however the tropics are particularly rich in them (Gurib-Fakim 2006). The family includes some of the most important fruit bearing plants known: the genus Citrus with orange, lemon, lime, mandarin, grapefruit, etc.

Essential oil is common in many taxa (Citrus, Ruta) and can be found in lysigenous secretory cavities in the parenchyma and pericarp. Alkaloids are also frequently found, especially benzyl tetrahydroisoquinoline, acridone and imidazole types (pilocarpine). The acridone alkaloids have so far only been reported from the Rutaceae. Other groups of natural products typically encountered are furano- and pyranocoumarins (e.g. bergapten from *Citrus aurantium* sub sp. *bergamia*, used to flavour Earl Grey tea), as well as simple coumarins.

Important medicinal plants from the family are:

- *Pilocarpus jaborandi* Holmes and *Pilocarpus* spp. (pilocarpus), for the isolation of pilocarpine, which is used in ophthalmology.
- *Ruta graveolens* L., formerly widely used as an emmenagogue and spasmolytic, shows strong phototoxic side effects. Many species (especially of the genus *Citrus*) are aromatic and used in foods, pharmacy and perfumery.

Solanaceae

Solanaceae with 2600 species includes some of the most important staples-the Potato (*Solanum tuberosum*) and several other medicinal and toxic plants, known for the highly active natural products. Typical for the family are alkaloids, especially of the tropane, nicotine and steroidal type. Many taxa are characterized by oxalic acid, which often forms typical structures (e.g. sand-like in *Atropa belladonna,* irregular crystals in *Datura stramonium*).

Important medicinal plants from the family are:

- *Atropa belladonna* L. (deadly nightshade, atropa), *Datura stramonium* L. (stramonium) and *Hyoscyamus niger* L. (henbane), which yield alkaloids with spasmolytic and anticholinergic properties; atropine is used in ophthalmology.

Zingiberaceae

This family are distributed in tropical regions and rich in essential oils with terpenes (borneol, camphene and cineole (all oxygenated monoterpenes), sesquiterpenes (zingiberene) and phenyl propanoid derivatives (cinnamic acid derivatives). Important medicinal plants from this family are as follows (Gurib-Fakim 2006):

- *Curcuma zanthorrhiza* Roxburgh (Temulawak, Javanese turmeric).
- *Curcuma longa* L. (syn. *C. domestica*, turmeric), a commonly used spice and popular remedy used, for example, for inflammatory, liver diseases, and in most Asian medical systems for a large variety of illnesses.
- *Elettaria cardamomum* (L.) Maton (cardamom), which is mostly used as a spice but also as a medicine.
- *Zingiber officinale* Roscoe (ginger), used for a large variety of illnesses, including travel sickness, respiratory and gastrointestinal disorders.

Medicinal, Pharmacological and Industrial Applications of Medicinal Plants

The fact that plant organisms produce chemical substances cause a positive or negative interfere with plant’s processes which can regulate human life based on what has been reported since ancient time. The value of medicinal plants and their molecules with therapeutic potential have historically proven, and nowadays still represent an important pool for the identification of novel drug leads.

Traditional Medicine

Traditional medicine refers to health practices, approaches, skills, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination of treat, diagnose and prevent illnesses or maintain well-being.

The first written records on medicinal applications of plants date back to 2600 BC which report the existence of a sophisticated medicinal system in Mesopotamia, comprising about 1000 plant-derived medicines. Egyptian medicine dates back to about 2900 BC, however, its most useful preserved record is the “Ebers Papyrus” from about 1550 BC, containing more than 700 drugs, mainly of plant origin (Borchardt 2002; Cragg and Newman 2013; Sneader 2005a, b). Traditional Chinese medicine has been extensively documented over thousands of years (Unschuld 1986), and the documentation of the Indian Ayurveda system dates back to the 1st millennium BC (Patwardhan 2005).

Up to know, millions of people around the world consume plant-based medicines as part of traditional medicine for a range of medical disorders. The use of traditional medicine in developing countries contributes directly to the socio-economic status and well-being of the rural communities (Tabuti et al. 2003; Chiranjibi et al. 2006). People especially herbalists and traditional healers generate income from medicinal plants.

Countries in Africa, Asia and Latin America use traditional medicine to help meet some of their primary health care needs. In Africa, up to 80% of the population uses traditional medicine for primary health care. In industrialized countries, adaptations of traditional medicine are termed "complementary" or "alternative".

To promote the proper use of traditional medicine/complementary or alternative, WHO has elaborated a Traditional Medicine Strategy (WHO 1998, 2002) that focuses on Policy, Safety/Quality/Efficacy, Access and Rational use of traditional medicine/complementary or alternative. The strategy aims to support Member States in developing proactive policies and implementing action plans that will strengthen the role traditional medicine plays in keeping populations healthy.

Traditional medicine has maintained its popularity in all regions of the developing world and its use is rapidly spreading in industrialized countries:

- In China, traditional herbal preparations account for 30–50% of the total medicinal consumption.
- In Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60% of children with high fever resulting from malaria is the use of herbal medicines at home.
- WHO estimates that in several African countries traditional birth attendants assist in the majority of births.
- In Europe, North America and other industrialized regions, over 50% of the population have used complementary or alternative medicine at least once.
- In San Francisco, London and South Africa, 75% of people living with HIV/AIDS use TM/CAM.
- 70% of the population in Canada has used complementary medicine at least once.
- In Germany, 90% of the population has used a natural remedy at some point in their life. Between 1995 and 2000, the number of doctors who had undergone special training in natural remedy medicine had almost doubled to 10,800.
- In the United States, 158 million of the adult population use complementary medicines and according to the USA Commission for Alternative and Complementary medicines, US$17 billion was spent on traditional remedies in 2000.
- In the United Kingdom, annual expenditure on alternative medicine is US$230 million.
- The global market for herbal medicines currently stands at over US$60 billion annually and is growing steadily (http://www.who.int/mediacentre/factsheets/2003/fs134/en/).

Phytotherapy

Herbalism (also herbal medicine or phytotherapy) is the study of botany and use of plants intended for medicinal purposes or for supplementing a diet. Plants have been the basis for medical treatments through much of human history, and such traditional medicine is still widely practiced today. Modern medicine recognizes herbalism as a form of alternative medicine, as the practice of herbalism is not strictly based on evidence gathered using the scientific method. Modern medicine makes use of many plant-derived compounds as the basis for evidence-based pharmaceutical drugs.

Closely related to herbalism, phytotherapy is the intended medical use of plants and plant extracts for therapeutic purposes (Heinrich 2016; Capasso et al. 2003). A possible differentiation with herbalism is that phytotherapy may require constituents in the plant extract be standardized by adhering to a minimum content of one or several active compounds in the therapeutic product (Heinrich 2016).

Modern phytotherapy may use conventional methods to assess herbal drug quality, although more typically relies on modern processes like high-performance liquid chromatography (HPLC), gas chromatography (GC), ultraviolet/visible spectrophotometry or atomic absorption spectroscopy to identify species, measure bacteriological contamination, assess potency, and create certificates of analysis for the material (Gad et al. 2013). Phytotherapy is distinct from homeopathy and anthroposophic medicine, and avoids mixing plant and synthetic bioactive substances.

Aromatherapy

The use of plants for therapeutic purposes has always been in human life, and is still valid despite the vast power and responsibility of the pharmaceutical chemistry, mainly based on the active principles of synthesis.

Nowadays, use of alternative and complementary therapies with mainstream medicine has gained the momentum. Aromatherapy is one of the complementary therapies which use essential oils as the major therapeutic agents to treat several diseases. The essential or volatile oils are extracted from the flowers, barks, stem, leaves, roots, fruits and other parts of the plant by various methods. This therapy is a natural way of healing a person's mind, body and soul (Worwood 2000). Literature survey reveals that this therapy has gained a lot of attention in the late 20th century and has become very popular in the 21st century due to its importance, popularity and widespread use, it is recognized as aroma science therapy (Esposito et al. 2014). These aroma molecules are very potent organic plant chemicals that make the surroundings free from disease, bacteria, virus and fungus (Liu et al. 2013). The penetration potential of these oils to reach the subcutaneous tissues is one of the important characters of this therapy.

Food Additives

Food safety is a global issue with significant implications for human health. The WHO annually reports that unsafe food results in the illnesses of at least 2 billion people worldwide which can be deadly.

Food additives are substances added to foods to keep them fresh or to enhance their colour, flavour or texture. Some additives have been used for centuries; for example, preserving food by pickling (with vinegar), salting, as with bacon, preserving sweets or using sulfur dioxide as with wines. With the advent of processed foods in the second half of the twentieth century, many more additives of plant-based origin have been introduced to food industry. The significance of medicinal aromatic and spice plants in food additives is raised by their antimicrobial (bactericidal and fungicidal) properties, owing to which they make highly valued preservatives for fresh vegetable or meat preparations as well as canned products (Davidson et al. 2005). Given the consumer demand for natural preservatives and/or consumer negative response to chemical compounds, it is imperative that more research is focused on the application of plant antimicrobials to food safety.

Cosmetic Products

The knowledge of herbal cosmetics is represented now-a-days by both orally transmitted folk information and newer information generated by modern scientific studies. Herbal products like extracts; oils and powders have been used in cosmetics as either active moieties or as excipients. Herbal extracts are primarily added to the cosmetic preparations due to several associated properties such as antioxidant (e.g. carotenoids, flavonoids and polyphenols) and anti-inflammatory characteristics (Glaser 2004; Draelos 2003).

The name itself suggests that herbal cosmetics are natural and free from all the harmful synthetic chemicals however it has proved to be toxic for skin. Instead of traditional synthetic products different plant parts and plant extracts are used in these products, e.g. aloe-vera gel and coconut oil. They also consist of natural nutrients like vitamin E that keeps skin healthy, glowing and beautiful (Akinyele and Odiyi 2007).

Medicinal Plants as a Future Source of New Drugs

The process of drug discovery is so long involves the identification of candidates, synthesis, characterization, screening, and assays for therapeutic efficacy. Despite competition from different drug discovery methods, natural products are still providing their fair share of new clinical candidates, new drugs and new drug leads.

These compounds were still a significant source of new drugs, especially in the anticancer, antihypertensive, anti-infectives, immuno-suppression, and neurological disease therapeutic areas, and some of them have since progressed further into clinical trials or onto the market (Butler 2004).

It is considered that because of the structural and biological diversity of their constituents, medicinal plants offer a unique and renewable resource for the discovering of potential new drugs and biological entities (Lahlou 2007). Therefore, plant-based metabolites can be predicted to remain an essential component in the search and development for new, safe and economical medicaments.

Unfortunately, as a result of ongoing climate changes and anthropogenic factors, a significant decrease in global vegetative species in future is predicted (Maclean and Wilson 2011; Thomas et al. 2004), endangering the sources of potential new drugs from nature and prompting urgent actions.

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Introduction to Environmental Challenges in All Over the World

Azadeh Kazemi and Mansour Ghorbanpour

Abstract The environment that we live in and make use of is being stripped off its precious components day by day. There are many angles from which the problem of environmental challenge can be studied. Similarly, many different views come into play if we need to find practical solutions to these challenges. There are innumerable factors associated with conservation of environment and also these challenges are interrelated. Different factors affected the environment of the Earth as it is in constant conflict with it. In this chapter we explain the most important challenges and the causes and effects of them on human, animal and plant life.

Keywords Environmental perturbations · Ecosystem · Living organisms · Pollution

Introduction

Definition of the environment is the complex of physical, chemical, and biotic factors (as climate, soil, and living things) that act upon an organism or an ecological community and ultimately determine its form and survival. Our environment in the world is constantly changing. There is no denying that. However, as our environment changes, so does the need to become increasingly aware of the problems that surround it. All across the world, people are facing a wealth of new and challenging environmental problems every day. Some of them are small and

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only affect a few ecosystems, but others are drastically changing the landscape of what we already know. The earth planet is poised at the brink of a severe environmental crisis. Current environmental problems make us vulnerable to disasters and tragedies, now and in the future. We are in a state of planetary emergency, with environmental problems piling up high around us. Unless we address the various issues prudently and seriously we are surely doomed for disaster. Current environmental problems require urgent attention.

The Major Current Environmental Problems

Environment Pollution

Environmental pollution has existed for centuries but only started to be significant following the industrial revolution in the 19th century. Pollution occurs when the natural environment cannot destroy an element without creating harm or damage to itself. The elements involved are not produced by nature, and the destroying process can vary from a few days to thousands of years (that is, for instance, the case for radioactive pollutants). In other words, pollution takes place when nature does not know how to decompose an element that has been brought to it in an unnatural way. Pollution must be taken seriously, as it has a negative effect on natural elements that are an absolute need for life to exist on earth, such as water and air. Indeed, without it, or if they were present on different quantities, animals—including humans—and plants could not survive. We can identify several types of pollution on Earth: air pollution, water pollution and soil pollution (Shafi 2005).

Causes of Environmental Pollution

1. Industries: Industries have been polluting our environment especially since the beginning of the industrial revolution, as mentioned above, notably due to the increasing use of fossil fuels. In the 19th century and for a significant part of the 20th century, coal has been use to make machines work faster, replacing human force. Though pollution by industries mainly causes air pollution, soil and water contamination can also occur. This is particularly the case for power-generating industries, such as plants producing electricity (May they be a dam, a nuclear reactor or some other type of plant). Also, the transportation of this energy can be harmful to the environment. We can take as an example the transportation of petrol through pipelines; if there is a leak in the pipeline, soil will automatically be polluted. At the same time, if the tanker transporting the petrol from its production plant to the place where it will be consumed leaks or sinks, the water will get contaminated.

2. Transportation: Ever since men abandoned animal power to travel, pollution of the environment has become higher and higher. Its levels have only been increasing until now. Similarly to industries, pollution caused by transport can mainly be attributed to fossil fuels. Indeed, humans went from horse carriages to cars, trains (which, before electricity, used to be propelled by coal), and airplanes. As the traffic is increasing every day, pollution follows that evolution.
3. Agricultural Activities: Agriculture is mainly responsible for the contamination of water and soil. This is caused by the increased use of pesticides, as well as by the intensive character of its production. Almost all pesticides are made from chemical substances and are meant to keep diseases and threatening animals away from the crops. However, by keeping these forms of life away, harm is almost always made to the surrounding environment as well. Furthermore, as agriculture gets more and more intensive to feed the increasing world population, more environments and ecosystems are destroyed to make space for the crops. Some of them like rapeseed-used to make oil-demand a lot of space for a relatively small output.
4. Trading Activities: Trading activities including the production and exchange of goods and services. Concerning goods, pollution can be caused by packaging (which often involves the use of plastic, which is made from fossil fuels) or transport, mainly.
5. Residences: Finally, residential areas provide their fair share of pollution as well. First, to be able to build homes, natural environment has to be destroyed in one way or another. Wildlife and plants are driven away and replaced by human constructions. As it requires the work of industries, construction itself is also a source of contamination of the environment. Then, when people settle in, they will produce waste every day, including a part that cannot be processed by the environment without harm yet (Harrison 2001).

Effects of Environmental Pollution

Now that we have identified the main causes of environmental pollution, let us study the negative effects it has:

1. Effects on Humans: The effects of environmental pollution on humans are mainly physical, but can also turn into neuron-affections in the long term. The best-known troubles to us are respiratory, in the form of allergies, asthma, irritation of the eyes and nasal passages, or other forms of respiratory infections. Notably, these well spread affections can be observed when air pollution is high in cities, when the weather gets hot, for instance. On top of that, environmental pollution has been proven to be a major factor in the development of cancer. This can happen for example when we eat reminiscences of pollutants used in the production of processed foods, or pesticides from the crops. Other, rarer, diseases include hepatitis, typhoid affections, diarrhea and hormonal disruptions.

2. Effects on Animals: Environmental pollution mainly affects animal by causing harm to their living environment, making it toxic for them to live in. Acid rains can change the composition of rivers and seas, making them toxic for fishes, an important quantity of ozone in the lower parts of the atmosphere can cause lung problems to all animals. Nitrogen and phosphates in water will cause overgrowth of toxic algae, preventing other forms of life to follow their normal course. Eventually, soil pollution will cause harm and sometimes even the destruction of microorganisms, which can have the dramatic effect of killing the first layers of the primary food chain.
3. Effects on Plants: As for animals, plants, and especially trees, can be destroyed by acid rains (and this will also have a negative effect on animals as well, as their natural environment will be modified), ozone in the lower atmosphere block the plant respiration, and harmful pollutants can be absorbed from the water or soil.
4. Effects on the Ecosystem: In short, environmental pollution, almost exclusively created by human activities, has a negative effect on the ecosystem, destroying crucial layers of it and causing an even more negative effect on the upper layers (Spellman 2009).

Types of Pollution

Air Pollution

The air that we breathe today is full of toxic and hazardous pollutants. Pollution in the environment could leave mother Earth sapped of its beauty and biodiversity. Each year millions of people die all around the world due to different pollution related problems. The key to live a healthy life is to identify the sources of air pollution and implement practical ways so that you can help stop air pollution.

Many people, when they think of air pollution, they think about smog and car emissions. This is what is called outdoor air pollution but it is more dangerous when it becomes indoor air pollution. Indoor air pollution occurs when certain air pollutants from particles and gases contaminate the air of indoor areas. These air pollutants can cause respiratory diseases or even cancer. Removing the air pollutants can improve the quality of your indoor air. Millions of people around the world prepare their meals using traditional methods (i.e. wood, charcoal, coal, dung, crop wastes) on open fires. Such inefficient practices can increase the amount of air pollutants inside the home and can also cause serious health problems. According to WHO, 4.3 million people a year die from the exposure to household air pollution. This type of pollution is significantly more dangerous due to how concentrated the air is in indoor environments. According to recent findings, over 2 million deaths occur every single year due to indoor air pollution. So what can we do about it? That is the question that many ask themselves every single day. Before you can fully comprehend the effects of indoor air pollution you must first be able to understand the causes of it as well as what we can do to improve our quality of air both indoors and outdoors (Agarwal 2005).

Water Pollution

Water is the greatest gift of nature. Humans have exploited this natural resource to a level where controlling water pollution is impossible. Here are some important reasons why water is considered as a precious natural resource. The misuse of this can cause damaging consequences and a threat to human life. Here is an interesting way to analyze how water pollution is gradually causing so many deaths worldwide. The use of hazardous chemicals in manufacturing industries and agriculture cause severe water pollution as waste from these industries goes directly into nearby rivers, lakes and ponds without getting treated to remove harmful toxins and compounds. This not only affects the quality of water but also pose danger to several endangered aquatic species. Increase in the water pollution can badly affect marine life and their habitats. Poor people who do not have access to clean drinking water are forced to drink untreated water which makes them prone to water related diseases which directly affects their health (Goel 2006). According to Wikipedia, “Water pollution is the contamination of water bodies (e.g. lakes, rivers, oceans, aquifers and groundwater). Water pollution occurs when pollutants are directly or indirectly discharged into water bodies without adequate treatment to remove harmful compounds. Water pollution affects plants and organisms living in these bodies of water. In almost all cases the effect is damaging not only to individual species and populations, but also to the natural biological communities.”

Soil Pollution

With the rise of concrete buildings and roads, one part of the Earth that we rarely see is the soil. It has many different names, such as dirt, mud and ground. However, it is definitely very important to us. The plants that feed us grow in soil and keeping it healthy is essential to maintaining a beautiful planet. However, like all other forms of nature, soil also suffers from pollution. The pollution of soil is a common thing these days, and it happens due to the presence of man-made elements. The main reason why the soil becomes contaminated is due to the presence of manmade waste. The waste produced from nature itself such as dead plants, carcasses of animals and rotten fruits and vegetables only adds to the fertility of the soil. However, our waste products are full of chemicals that are not originally found in nature and lead to soil pollution (Mani and Misra 2009).

Global Warming and Climate Change

Climate change is yet another environmental problem that has surfaced in last couple of decades. It occurs due to rise in global warming which occurs due to increase in temperature of atmosphere by burning of fossil fuels and release of harmful gases by industries. Climate change has various harmful effects but not limited to melting of polar ice, change in seasons, occurrence of new diseases, frequent occurrence of floods and change in overall weather scenario. For the purpose of current and future impacts, the term “climate change” has become synonymous with modern global warming (Demeritt 2001). The latter, in turn, refers to post–Industrial Revolution changes in global mean surface-air temperature that are hypothesized to have been caused by increased atmospheric concentrations of carbon dioxide (CO_2) Fan active greenhouse gas F during the eighteenth, nineteenth, and twentieth centuries. In 1765, the CO_2 atmospheric concentration was about 280 parts per million by volume (ppmv); in 2000, it is near 364 (ppmv). The atmospheric concentrations of other greenhouses gases (methane, nitrous oxide, chlorofluorocarbons) have also risen as a result of accelerated economic activity and energy use in the last two centuries. The mean concentration of water vapor F another key greenhouse gas F in the atmosphere has remained at a level of about 3000 (ppmv) throughout the Holocene period (the last 10,000 years).The rapid rise in fossil-fuel combustion as an energy source since the late eighteenth century has caused the observed increase in the atmospheric concentration of 20 CO_2. While the post–Industrial Revolution rise in CO_2 atmospheric concentration is beyond doubt, the question of whether or not the earth’s mean surface-air temperature has increased relative to the pre–Industrial Revolution level is the focus of intense research. Recent estimates indicate that it has increased between 0.31 and 0.61 °C during the last 150 years (Hansen et al. 2002). A cause-effect linkage between the rise in key greenhouse gases and the estimated increase in the global mean surface-air temperature remains shrouded in uncertainty. This is due to the complex variability of the earth’s climate and its interdependence with multiple terrestrial and extraterrestrial phenomena (Loáiciga 2003).

Overpopulation

The population of the planet is reaching unsustainable levels as it faces shortage of resources like water, fuel and food. Population explosion in less developed and developing countries is straining the already scarce resources. Intensive agriculture practiced to produce food damages the environment through use of chemical fertilizer, pesticides and insecticides. Overpopulation is one of the crucial current environmental problems.

Overpopulation is already affecting the 750 million people worldwide who have no access to potable water sources, and 2.5 billion are without proper sanitation. Many believe we've passed or are in the throes of a peak-water situation, meaning voracious demand has outpaced renewable supply, and ecological costs outweigh benefits of extraction. Besides industrial pollution, much of the water crisis is due to intensive agriculture designed to feed a growing number of humans: the latter half of the twentieth century saw irrigation systems nearly triple worldwide, from 100 million ha in 1950 to 280 million ha in 2000, to feed the burgeoning population. Consequently, many nations have dipped into non-renewable underground aquifers as global water supplies have been polluted or strained. The United Nations states that by 2025, 1.8 billion people may inhabit areas of "absolute water scarcity" (less than 1000 m^3 of water available per person per year), while two-thirds of the global population may be living in "water-stressed" regions (less than 1700 m^3 of water available per person per year). To put Western water consumption into perspective: sustaining the global population on average European or North American water habits would require water from 3.5 Earths.

Natural Resource Depletion

Natural resource depletion is another crucial current environmental problem. Fossil fuel consumption results in emission of Greenhouse gases, which is responsible for global warming and climate change. Globally, people are taking efforts to shift to renewable sources of energy like solar, wind, biogas and geothermal energy. The cost of installing the infrastructure and maintaining these sources has plummeted in the recent years.

What Causes Natural Resource Depletion?

One of the central causes to natural resource depletion is human activity, particularly as it relates to economic growth. Given the rise of the world population, Humans are becoming more and more reliant on food production for daily nutritional needs, which in turn is causing serious stress to the land and the environment. The main driving force leading to pressure on land resources has been increasing food production. Today food is needed for two billion more people than lived on the Earth three decades ago. Inefficient irrigation schemes can cause salinization and alkalization of soil, resulting in an estimated ten million hectares (38,610 square miles) of irrigated land abandoned annually. Humans also contribute to land degradation through poor soil management practices, deforestation, removal of natural vegetation, use of heavy machinery, overgrazing of livestock, and improper crop rotation" (Viotti and Kauppi 2013). Humans have been cutting trees for agriculture at very high rates. In addition, the increased uses of wood have continued to reduce the size of forests. As we are becoming more careless with our practices, as well as demanding more and more food, this in turn will continue to lead to natural resource depletion. As scholars point out, some of the underlying causes of natural resource depletion are the conditions facing humans. When humans need food, when they are in poverty (Viotti and Kauppi 2013), when they need employment, or ways to bring in income, they may look towards these practices such as deforestation, or agriculture without correct crop rotation, which in turn can and will continue to deplete the earth.

Forest Depletion

When looking at the world's forests, one finds a depletion of natural resources; "The net loss of the global forest area (deforestation plus reforestation) in the last decade of the 20th century was about 94 million ha (363,000 square miles), the equivalent of 2.4% of total world forests. Deforestation of tropical forests is almost 1% annually" (Viotti and Kauppi 2013). When looking at natural resource depletion in places such as Latin America, one will find that issues such as tropical deforestation is a very real and continued threat to natural resources and the environment as a whole (Rowntree et al. 2015).

In fact, tropical forests are among the most at risk areas of earth as it pertains to deforestation. Thus, not only are plant and animal life affected with this tropical deforestation, but these actions also lead to an increase of additional CO_2s. According to scholars, "Current estimates suggest that fully 20% of all human-causes GHG emissions result from cutting and burning tropical forests" (Rowntree et al. 2015). But, as people continue to harm the forests for wood, palm oil, or other resources, it is expected that the negative effects on natural resource depletion related to forests and tropical forests will continue. And because of the human causes of natural resource depletion, many have asked whether current behaviors towards natural resources can continue the way that they are going, and if so, what the effects of this behavior will be.

What Are the Effects of Natural Resource Depletion?

Current patterns of energy and natural resource use, agricultural practices, and urbanization appear to be largely unsustainable and require urgent remediation. Left unchecked, these patterns will lead to dangerous climate change and reduced economic growth, as a result of increased economic, social, and environmental costs and decreased productivity. Many of the world's natural resources have been greatly reduced do to human economic development in recent decades. In fact, the pace and scale of environmental damage has been well documented. More than one quarter of the world's land surface has been degraded as a result of soil erosion, salinization, nutrient depletion, and. Water withdrawals tripled in the past 50 years, leading to water scarcity and groundwater depletion. In developing countries, withdrawals are projected to increase by another 50% by 2030, by which time more than 5 billion people-two-thirds of the world's people-could be living in areas facing moderate to severe water stress (WRI forthcoming). Growth has also strained ecosystems. Roughly 60% of the world's ecosystem services are now of lower quality than they were 50 years ago, the current rate of species extinction is 100–1000 times higher than in prehuman days, and all of the planet's 13 hottest years on record have occurred since 1997 (Steer 2013).

There are also many economic effects as a result of current and historical natural resource depletion. For example, if one of the natural resources that are depleted is soil, then this will impact the agricultural output by those who are reliant on healthy soil for their income and economic livelihood. If individuals have depleted the soil, and the proper conditions no longer exist for efficient crop growth, then this will lead to lower output, and in turn will bring an individual, society, or state less income. In addition, from an international relations perspective, one also has to think about the effects of natural resource depletion on conflict. As it has been argued, "Conflict over natural resources may increase and significantly endanger development efforts in contexts where increased competition over natural resources spills over into the political sphere and leads to political violence" (Inforesources 2005).

Waste Disposal

Waste disposal is one of urgent current environmental problem. Waste management is the handling of discarded materials. Recycling and composting, which transform waste into useful products, are forms of waste management. The management of waste also includes disposal, such as landfilling. Waste can be almost anything, including food, leaves, newspapers, bottles, construction debris, and chemicals from a factory, candy wrappers, disposable diapers, old cars, or radioactive materials. People have always produced waste, but as industry and technology have evolved and the human population has grown, waste management has become increasingly complex. A primary objective of waste management today is to protect the public and the environment from potentially harmful effects of waste. Some waste materials are normally safe, but can become hazardous if not managed properly. For example, 1 gal (3.75 l) of used motor oil can potentially contaminate one million gal (3,790,000 l) of drinking water. Every individual, business, or organization must make decisions and take some responsibility regarding the management of his or her waste. On a larger scale, government agencies at the local, state, and federal levels enact and enforce regulations governing waste management. These agencies also educate the public about proper waste management. In addition, local government agencies may provide disposal or recycling services, or they may hire or authorize private companies to perform those functions (Guerrero et al. 2013).

Throughout history, there have been four basic methods of managing waste: dumping it, burning it, finding another use for it (reuse and recycling), and not creating the waste in the first place (waste prevention). How those four methods are utilized depends on the wastes being managed. Municipal solid waste is different from industrial, agricultural, or mining waste. Hazardous waste is a category that should be handled separately, although it sometimes is generated with the other types (Tchobanoglous and Kreith 2002).

The first humans did not worry much about waste management. They simply left their garbage where it dropped. However, as permanent communities developed, people began to dispose of their waste in designated dumping areas. The use of such “open dumps” for garbage is still common in many parts of the world. Open dumps have major disadvantages, however, especially in heavily populated areas. Toxic chemicals can filter down through a dump and contaminate groundwater. The liquid that filters through a dump or landfill is called leachate. Dumps may also generate methane, a flammable and explosive gas produced when organic wastes decompose under anaerobic (oxygen-poor) conditions (Tchobanoglous and Kreith 2002).

The landfill, also known as the “sanitary landfill,” was invented in England in the 1920s. At a landfill, the garbage is compacted and covered at the end of every day with several inches of soil. Landfilling became common in the United States in the 1940s. By the late 1950s, it was the dominant method for disposing municipal solid waste in the nation. Early landfills had significant problems with leachate and methane, but those have largely been resolved at facilities built since about the early 1970s. Well-engineered landfills are lined with several feet of clay and with thick plastic sheets. Leachate is collected at the bottom, drained through pipes, and processed. Methane gas is also safely piped out of many landfills. The dumping of waste does not just take place on land. Ocean dumping, in which barges carry garbage out to sea was once used as a disposal method by some United States coastal cities and is still practiced by some nations. Sewage sludge, or waste material from sewage treatment, was dumped at sea in huge quantities by New York City as recently as 1992, but this is now prohibited in the United States. Also called bio solids, sewage sludge is not generally considered solid waste, but it is sometimes composted with organic municipal solid waste (Christensen 1989).

Burning has a long history in municipal solid waste management. Some American cities began to burn their garbage in the late nineteenth century in devices called cremators. These were not very efficient, however, and cities went back to dumping and other methods. In the 1930s and 1940s, many cities built new types of more-efficient garbage burners known as incinerators. The early incinerators were rather dirty in terms of their emissions of air pollutants, and beginning in the 1950s they were gradually shut down. However, in the 1970s, waste burning enjoyed another revival. These newer incinerators, many of which are still in operation, are called “resource recovery” or “waste-to-energy” plants. In addition to burning garbage, they produce heat or electricity that can be used in nearby buildings or residences, or sold to a utility. Many local governments became interested in waste-to-energy plants following the energy crisis in 1973. However, since the mid-1980s, it became difficult to find locations to build these facilities, mainly because of public opposition focused on air-quality issues. Another problem with incineration is that it generates ash, which must be landfilled. Incinerators usually reduce the volume of garbage by 70–90%. The remainder of the incinerated waste comes out as ash that often contains high concentrations of toxic substances. Municipal solid waste will likely always be landfilled or burned to some extent. In the past 25 years, however, non-disposal methods such as waste prevention and recycling have become more common. Because of public concerns and the high costs of landfilling and burning (especially to build new facilities), local governments want to reduce the amount of waste that must be disposed in these ways. Municipal solid waste is a relatively small part of the overall waste generated in the United States. More than 95% of the total 4.5 billion tons of solid waste generated in the United States each year is agricultural, mining, or industrial waste. These wastes do not receive nearly as much attention as municipal solid waste, because most people do not have direct experience with them. Also, agricultural and mining wastes, which make up 88% of the overall total of solid waste, are largely handled at the places they are generated, that is, in the fields or at remote mining sites. Mining nearly always generates substantial waste, whether the material being mined is coal, clay, sand, gravel, building stone, or metallic ore. Early mining concentrated on the richest lodes of minerals. Because modern methods of mining are more efficient, they can extract the desired minerals from veins that are less rich. However, much more waste is produced in the process (Tchobanoglous and Kreith 2002).

Many of the plant and animal wastes generated by agriculture remain in the fields or rangelands. These wastes can be beneficial because they return organic matter and nutrients to the soil. However, modern techniques of raising large numbers of animals in small areas generate huge volumes of animal waste, or manure. Waste in such concentrated quantities must be managed carefully, or it can contaminate groundwater or surface water (Tchobanoglous and Kreith 2002).

Industrial wastes that are not hazardous have traditionally been sent to landfills or incinerators. The rising cost of disposal has prompted many companies to seek alternative methods for handling these wastes, such as waste prevention and recycling. Often a manufacturing plant can reclaim certain waste materials by feeding them back into the production process (Tchobanoglous and Kreith 2002).

Hazardous wastes are materials considered harmful or potentially harmful to human health or the environment. Wastes may be deemed hazardous because they are poisonous, flammable, or corrosive, or because they react with other substances in a dangerous way. Industrial operations have produced large quantities of hazardous waste for hundreds of years. Some hazardous wastes, such as mercury and dioxins, may be released as gases or vapors. Many hazardous industrial wastes are in liquid form. One of the greatest risks is that these wastes will contaminate water supplies. An estimated 60% of all hazardous industrial waste in the United States is disposed using a method called deep-well injection. With this technique, liquid wastes are injected through a well into an impervious rock formation that keeps the waste isolated from groundwater and surface water. Other methods of underground burial are also used to dispose hazardous industrial waste and other types of dangerous material. Pesticides used in farming may contaminate agricultural waste. Because of the enormous volumes of pesticides used in agriculture, the proper handling of unused pesticides is a daunting challenge for waste managers. Certain mining techniques also utilize toxic chemicals. Piles of mining and metal-processing waste, known as waste rock and tailings, may contain hazardous substances. Because of a reaction with the oxygen in the air, large amounts of toxic acids may form in waste rock and tailings and leach into surface waters (Tchobanoglous and Kreith 2002).

Public attitudes also play a pivotal role in decisions about waste management. Virtually every proposed new landfill or waste-to-energy plant is opposed by people who live near the site. Public officials and planners refer to this reaction as NIMBY, which stands for "Not in My Backyard." If an opposition group becomes vocal or powerful enough, a city or county council is not likely to approve a proposed waste-disposal project. The public also wields considerable influence with businesses. Recycling and waste prevention initiatives enjoy strong public support. About 19% of United States municipal solid waste was recycled or composted in 1994, 10% was incinerated, and 71% was landfilled. Preventing or reducing waste is typically the least expensive method for managing waste. Waste prevention may also reduce the amount of resources needed to manufacture or package a product. For example, most roll-on deodorants once came in a plastic bottle, which was inside a box. Beginning about 1992, deodorant manufacturers redesigned the bottle so that it would not tip-over easily on store shelves, which eliminated the need for the box as packaging. This is the type of waste prevention called source reduction. It can save businesses money, while also reducing waste. Waste prevention includes many different practices that result in using fewer materials or products, or using materials that are less toxic. For example, a chain of clothing stores can ship its products to its stores in reusable garment bags, instead of disposable plastic bags. Manufacturers of household batteries can reduce the amount of mercury in their batteries. In an office, employees can copy documents on both sides of a sheet of paper, instead of just one side. A family can use cloth instead of paper napkins (Tchobanoglous and Kreith 2002).

Composting grass clippings and tree leaves at home, rather than having them picked up for disposal or municipal composting is another form of waste prevention. A resident can leave grass clippings on the lawn after mowing (this is known as grass-cycling), or can compost leaves and grass in a backyard composting bin, or use them as mulch in the garden (Tchobanoglous and Kreith 2002).

When the current recycling boom began in the late 1980s, markets for the recyclables were not sufficiently considered. A result was that some recyclable materials were collected in large quantities but could not be sold, and some ended up going to landfills. Today, the development of recycling markets is a high priority. "Close the loop" is a catch-phrase in recycling education; it means that true recycling (i.e., the recycling loop) has not taken place until the new product is purchased and used. The high cost of government-created recycling programs is often criticized. Supporters of recycling argue it is still less expensive than landfilling or incineration, when all costs are considered. Another concern about recycling is that the recycling process itself may generate hazardous wastes that must be treated and disposed. Recycling of construction and demolition (C&D) debris is one of the growth areas for recycling. Although C&D debris is not normally considered a type of municipal solid waste, millions of tons of it have gone to municipal landfills over the years. If this material is separated at the construction or demolition site into separate piles of concrete, wood, and steel, it can usually be recycled. Composting is considered either a form of recycling, or a close relative. Composting occurs when organic waste—such as yard waste, food waste, and paper-is broken down by microbial processes. The resulting material, known as compost, can be used by landscapers and gardeners to improve the fertility of their soil. Yard waste, primarily grass clippings and tree leaves, makes up about one-fifth of the weight of municipal solid waste. Some states do not allow this waste to be disposed. These yard-waste bans have resulted in rapid growth for municipal composting programs. In these programs, yard waste is collected by trucks (separately from garbage and recyclables) and taken to a composting plant, where it is chopped up, heaped, and regularly turned until it becomes compost (Tchobanoglous and Kreith 2002).

Biological treatment, a technique for handling hazardous wastes, could be called a high-tech form of composting. Like composting, biological treatment employs microbes to break down wastes through a series of metabolic reactions. Many substances that are toxic, carcinogenic (cancer-causing), or undesirable in the environment for other reasons can be rendered harmless through this method. Extensive research on biological treatment is in progress. Genetic engineering, a controversial branch of biology dealing with the modification of genetic codes, is closely linked with biological treatment, and could produce significant advances in this field. Waste management became a particularly expensive proposition during the 1990s, especially for disposal. Consequently, waste managers constantly seek innovations that will improve efficiency and reduce costs. Several new ideas in land-filling involve the reclamation of useful resources from wastes.

For example, instead of just burning or releasing the methane gas that is generated within solid-waste landfills, some operators collect this gas, and then use it to produce power locally or sell it as fuel. At a few landfills, managers have experimented with a bold but relatively untested concept known as landfill mining. This involves digging up an existing landfill to recover recyclable materials, and sometimes to re-bury the garbage more efficiently. Landfill mining has been criticized as costly and impractical, but some operators believe it can save money under certain circumstances. In the high-tech world of incineration, new designs and concepts are constantly being tried. One waste-to-energy technology for solid waste being introduced to the United States is called fluidized-bed incineration. About 40% of incinerators in Japan use this technology, which is designed to have lower emissions of some air pollutants than conventional incinerators.

A 1994 United States Supreme Court ruling could increase the cost of incineration significantly. The Court ruled that some ash produced by municipal solid-waste incinerators must be treated as a hazardous waste, because of high levels of toxic substances such as lead and cadmium. This means that incinerator ash now has to be tested, and part or all of the material may have to go to a hazardous waste landfill rather than a standard landfill. A much smaller type of incinerator is used at many hospitals to burn medical wastes, such as blood, surgical waste, syringes, and laboratory waste. The safety of these medical waste incinerators has become a major issue in some communities. A study by the Environmental Protection Agency released in 1994 found that medical waste incinerators were leading sources of dioxin emissions into the air. The same study warned that dioxins, which can be formed by the burning of certain chemical compounds, pose a high risk of causing cancer and other health hazards in humans. As a hazardous waste, because of high levels of toxic substances such as lead and cadmium. This means that incinerator ash now has to be tested, and part or all of the material may have to go to a hazardous waste landfill rather than a standard landfill.

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Loss of Biodiversity

Biodiversity means the variety of life, including variation among genes, species, and functional traits.

- Richness: number of unique life forms
- Evenness: equitability among life forms
- Heterogeneity: dis-similarity among life forms.

Ecosystem functions are ecological processes that control the fluxes of energy, nutrients, and organic matter through an environment. Examples include …

- Primary production: the process by which plants use sunlight to convert inorganic matter into new biological tissue
- Nutrient cycling: the process by which biologically essential nutrients are captured, released, and then recaptured
- Decomposition: the process by which organic wastes like dead plants and animals are broken-down and recycled.

Ecosystem Services are the suite of benefits that ecosystems provide to humanity

- Provisioning services: Production of renewable resources (e.g., food, wood, freshwater)
- Regulating services: Processes that lessen environmental change (e.g., climate regulation, pest/disease control).

Loss of diversity across trophic levels has the potential to influence ecosystem functions even more Loss of diversity across trophic levels has the potential to influence ecosystem functions even more strongly than diversity loss within trophic levels Much work has shown that food web interactions are key mediators of ecosystem functioning, and that loss of higher consumers can cascade through a food web to influence plant biomass. Loss of one or a few top predator species can reduce plant biomass by at least as much as does the transformation of a diverse plant assemblage into a species monoculture. Loss of consumers can also alter vegetation structure, fire frequency, and even disease epidemics in a range of ecosystems 51. Strongly than diversity loss within trophic levels much work has shown that food web interactions are key mediators of ecosystem functioning, and that loss of higher consumers can cascade through a food web to influence plant biomass. Loss of one or a few top predator species can reduce plant biomass by at least as much as does the transformation of a diverse plant assemblage into a species monoculture. Loss of consumers can also alter vegetation structure, fire frequency, and even disease epidemics in a range of ecosystems. We can find the very complex relationship between ecosystem, Biodiversity and global change (Fig. 1).

Human activity is leading to the extinction of species and habitats and loss of bio-diversity. Eco systems, which took millions of years to perfect, are in danger when any species population is decimating. Balance of natural processes like

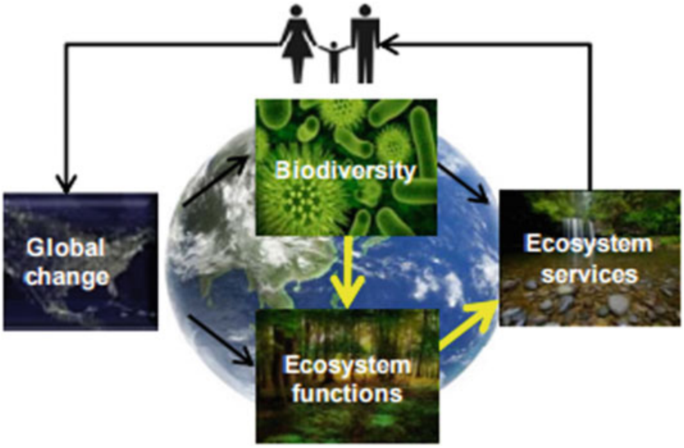


Fig. 1 Relationship between ecosystem, biodiversity and global change

pollination is crucial to the survival of the eco-system and human activity threatens the same. Another example is the destruction of coral reefs in the various oceans, which support the rich marine life (Cardinale 2012).

Ozone Layer Depletion

The ozone layer is not really a layer at all, but has become known as such because most ozone particles are scattered between 19 and 30 km (12–30 miles) up in the Earth's atmosphere, in a region called the stratosphere. The concentration of ozone in the ozone layer is usually under 10 parts ozone per million. Without the ozone layer, a lot of ultraviolet (UV) radiation from the Sun would not be stopped reaching the Earth's surface, causing untold damage to most living species. In the 1970s, scientists discovered that chlorofluorocarbons (CFCs) could destroy ozone in the stratosphere. Ozone is created in the stratosphere when UV radiation from the Sun strikes molecules of oxygen (O_2) and causes the two oxygen atoms to split apart. If a freed atom bumps into another O_2, it joins up, forming ozone (O_3). This process is known as photolysis. Ozone is also naturally broken down in the stratosphere by sunlight and by a chemical reaction with various compounds containing nitrogen, hydrogen and chlorine. These chemicals all occur naturally in the atmosphere in very small amounts. In an unpolluted atmosphere there is a balance between the amount of ozone being produced and the amount of ozone being destroyed. As a result, the total concentration of ozone in the stratosphere remains relatively constant. At different temperatures and pressures (i.e. varying altitudes within the stratosphere), there are different formation and destruction rates. Thus, the amount of ozone within the stratosphere varies according to altitude. Ozone concentrations are highest between 19 and 23 km. Most of the ozone in the stratosphere is formed over the equator where the level of sunshine striking the Earth is greatest. It is transported by winds towards higher latitudes. Consequently, the amount of stratospheric ozone above a location on the Earth varies naturally with latitude, season, and from day-to-day. Under normal circumstances highest ozone values are found over the Canadian Arctic and Siberia, whilst the lowest values are found around the equator. The ozone layer over Canada is normally thicker in winter and early spring, varying naturally by about 25% between January and July. Weather conditions can also cause considerable daily variations.

Ultraviolet radiations (UVR), are high energy electromagnetic waves emitted from the Sun. UV radiation includes UV-A, the least dangerous form of UV radiation, UV-B, and UV-C, which is the most dangerous. UV-C is unable to reach the Earth's surface due to stratospheric ozone's ability to absorb it. The real threat comes from UV-B, which can enter the Earth's atmosphere, and has adverse effects (Fig. 2).

Ozone layer depletion first captured the attention of the whole world in the latter half of 1970, and since then, a lot of research has been done to find its possible effects and causes. Various studies have been undertaken to find out possible solutions. Let us take a look at some of the causes and effects of ozone layer depletion.

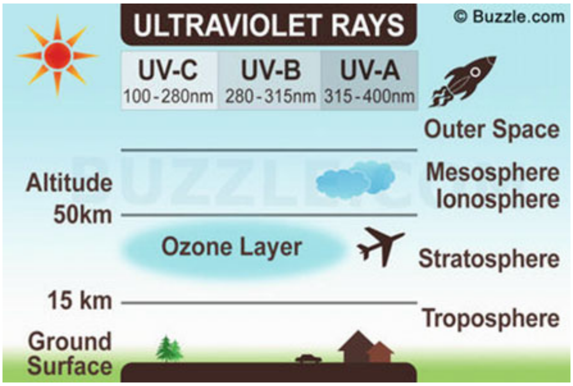


Fig. 2 Different type of Ultraviolet radiations

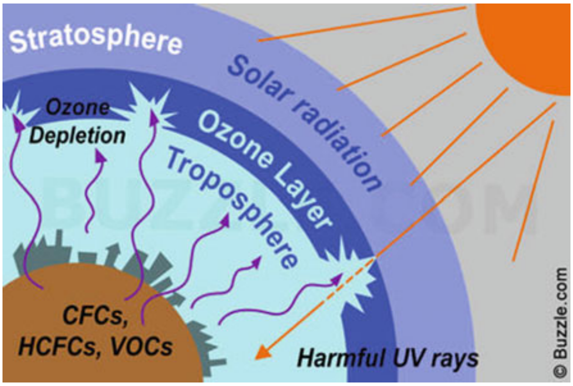
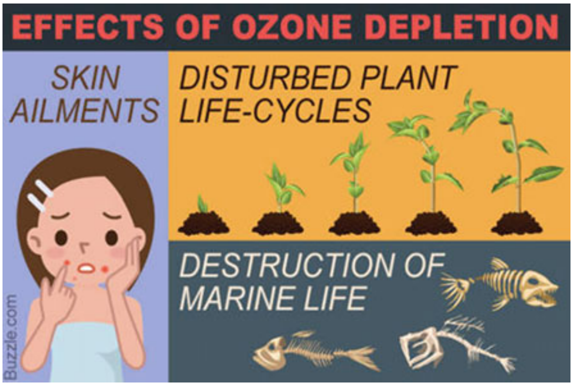


Fig. 3 Ozone Depletion Ozone by CFCs, VOCs, HCFCs

Causes of Ozone Depletion Ozone is a triatomic form of oxygen (O_3), found in the Earth's atmosphere. A combination of low temperatures, elevated chlorine, and bromine concentrations in the upper stratosphere are responsible for the destruction of ozone. The production and emission of chlorofluorocarbons (CFCs), is the leading cause of ozone layer depletion. CFC's account for almost 80% of the total depletion of ozone. Other ozone-depleting substances (ODS), include hydro chlorofluorocarbons (HCFCs), and volatile organic compounds (VOCs). These are often found in vehicle emissions, byproducts of industrial processes, refrigerants, and aerosols. ODS are relatively stable in the lower atmosphere of the Earth, but in the stratosphere, they are exposed to ultraviolet radiation and thus, they break down to release a free chlorine atom. This free chlorine atom reacts with an ozone molecule (O_3), and forms chlorine monoxide (ClO), and a molecule of oxygen. Now, ClO reacts with an ozone molecule to form a chlorine atom, and two molecules of oxygen. The free chlorine molecule again reacts with ozone to form chlorine monoxide. The process continues, and this results in the depletion of the ozone layer (Fig. 3).

Fig. 4 Effects of ozone depletion on Humans, Amphibians, Marine Ecosystemsand Plants



As ozone depletes in the stratosphere, it forms a ‘hole’ in the layer. This hole enables harmful ultraviolet rays to enter the Earth’s atmosphere. Ultraviolet rays of the Sun are associated with a number of health-related and environmental issues. Let us take a look at how ozone depletion affects different life forms (Fig. 4).

Impact on Humans

- Skin cancer: Exposure to ultraviolet rays poses an increased risk of developing several types of skin cancers, including malignant melanoma, and basal and squamous cell carcinoma.
- Eye damage: Direct exposure to UV radiations can result in photo keratitis (snow blindness), and cataracts.
- Immune system damage: Effects of UV rays include impairment of the immune system. Increased exposure to UV rays weakens the response of the immune system.
- Accelerated aging of skin: Constant exposure to UV radiation can cause photo allergy, which results in the outbreak of rashes in fair-skinned people.
- Other effects: Ozone chemicals can cause difficulty in breathing, chest pain, throat irritation, and hamper lung functioning.

Effects on Amphibians

Ozone depletion is listed as one of the causes for the declining numbers of amphibian species. Ozone depletion affects many species at every stage of their life cycle. Some of the effects are mentioned below. Hampers growth and development in larvae

- Changes behavior and habits
- Causes deformities in some species
- Decreases immunity. Some species have become more vulnerable to diseases and death
- Retinal damage and blindness in some species.

Effects on Marine Ecosystems

Plankton (phytoplankton and bacteriaplankton) is threatened by increased UV radiation. Marine phytoplankton plays a fundamental role in both the food chain as well as the oceanic carbon cycle. It plays an important role in converting atmospheric carbon dioxide into oxygen. Ultraviolet rays can influence the survival rates of these microscopic organisms, by affecting their orientation and mobility. This eventually disturbs and affects the entire ecosystem.

Impact on Plants

- In some species of plants, UV radiation can alter the time of flowering, as well as the number of flowers produced by a plant.
- Plant growth can be directly affected by UV-B radiation. Despite mechanisms to reduce or repair these effects, physiological and developmental processes of plants are affected.

Other Effects

Another observation is an increase in the ozone present in the lower atmosphere due to the decrease in the ozone in the stratosphere. Ozone present in the lower atmosphere is regarded as a pollutant and a greenhouse gas that can contribute to global warming and climate change. However, studies have pointed out that the lifespan of lower atmospheric ozone is quite less, compared to stratospheric ozone. At the same time, increase in the level of ozone in the lower atmosphere can enhance the ability of sunlight to synthesize vitamin D, which can be regarded as an important beneficial effect of ozone layer depletion.

Growing concern for ozone depletion led to the adoption of the Montreal Protocol in 1987, in order to reduce and control industrial emission of chlorofluorocarbons (CFCs). Such international agreements have succeeded to a great extent in reducing the emission of these compounds. However, more cooperation and understanding among all the countries is required to solve the problem completely. You too can do your bit to save the ozone. Use/buy more recycled products, save energy, use public transport, and most importantly, spread awareness. Our individual efforts can go a long way in saving the Earth's blanket.

This is one of the most important current environmental problems (Sivasakthivel and Reddy 2001).

Acid Rain

Acid rain, or acid deposition, is a broad term that includes any form of precipitation with acidic components, such as sulfuric or nitric acid that fall to the ground from the atmosphere in wet or dry forms. This can include rain, snow, fog, hail or even dust that is acidic.

Causes of Acid Rain

Acid rain results when sulfur dioxide (SO_2) and nitrogen oxides (NO_X) are emitted into the atmosphere and transported by wind and air currents. The SO_2 and NO_X react with water, oxygen and other chemicals to form sulfuric and nitric acids. These then mix with water and other materials before falling to the ground.

While a small portion of the SO_2 and NO_X that cause acid rain is from natural sources such as volcanoes, most of it comes from the burning of fossil fuels. The major sources of SO_2 and NO_X in the atmosphere are:

- Burning of fossil fuels to generate electricity. Two thirds of SO_2 and one fourth of NO_X in the atmosphere come from electric power generators.
- Vehicles and heavy equipment.
- Manufacturing, oil refineries and other industries.

Winds can blow SO_2 and NO_X over long distances and across borders making acid rain a problem for everyone and not just those who live close to these sources.

Forms of Acid Deposition

Wet Deposition

Wet deposition is what we most commonly think of as acid rain. The sulfuric and nitric acids formed in the atmosphere fall to the ground mixed with rain, snow, fog, or hail.

Dry Deposition

Acidic particles and gases can also deposit from the atmosphere in the absence of moisture as dry deposition. The acidic particles and gases may deposit to surfaces (water bodies, vegetation, buildings) quickly or may react during atmospheric transport to form larger particles that can be harmful to human health. When the accumulated acids are washed off a surface by the next rain, this acidic water flows over and through the ground, and can harm plants and wildlife, such as insects and fish.

The amount of acidity in the atmosphere that deposits to earth through dry deposition depends on the amount of rainfall an area receives. For example, in desert areas the ratio of dry to wet deposition is higher than an area that receives several inches of rain each year.

Measuring Acid Rain

Acidity and alkalinity are measured using a pH scale for which 7.0 is neutral. The lower a substance's pH (less than 7), the more acidic it is; the higher a substance's pH (greater than 7), the more alkaline it is. Normal rain has a pH of about 5.6; it is slightly acidic because carbon dioxide (CO_2) dissolves into it forming weak carbonic acid. Acid rain usually has a pH between 4.2 and 4.4 (Fig. 5).

Policymakers, research scientists, ecologists, and modelers rely on the National Atmospheric Deposition Program's (NADP) National Trends Network (NTN) for measurements of wet deposition. The NADP/NTN collects acid rain at more than 250 monitoring sites throughout the US, Canada, Alaska, Hawaii and the US Virgin Islands. Unlike wet deposition, dry deposition is difficult and expensive to measure. Dry deposition estimates for nitrogen and sulfur pollutants are provided by the Clean Air Status and Trends Network (CASTNET). Air concentrations are measured by CASTNET at more than 90 locations.

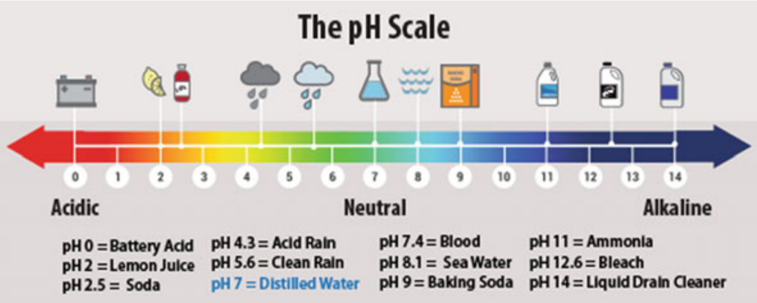


Fig. 5 The pH scale

When acid deposition is washed into lakes and streams, it can cause some to turn acidic. The Long-Term Monitoring (LTM) Network measures and monitors surface water chemistry at over 280 sites to provide valuable information on aquatic ecosystem health and how water bodies respond to changes in acid-causing emissions and acid deposition (EPA 2008).

Genetic Engineering

Genetic modification of food using biotechnology is called genetic engineering. Genetic modification of food results in increased toxins and diseases as genes from an allergic plant can transfer to target plant. Genetically modified crops can cause serious environmental problems as an engineered gene may prove toxic to wildlife. Another drawback is that increased use of toxins to make insect resistant plant can cause resultant organisms to become resistant to antibiotics.

Genetic Engineering Risks and Impacts

Any technology that offers benefits will usually come with risks as well. In order to make wise decisions about using a technology, we must understand its potential impacts well enough to decide whether the risks are acceptably low.

What are the risks posed by the use of genetic engineering (GE) in agriculture? The answers fall mostly into two categories: risks to human health, and environmental impacts.

GE Health Risks

Health risks of genetic engineering have sometimes been described in exaggerated, alarmist terms, implying that foods made from GE crops are inherently unsafe. There is no evidence, for instance, that refined products derived from GE crops, such as starch, sugar and oils, are different than those derived from conventionally bred crops. It is also an exaggeration, however, to state that there are no health risks associated with GE. For one thing, not enough is known: research on the effects of specific genes has been limited-and tightly controlled by the industry. But we do know of ways in which genetically engineered crops could cause health problems. For instance, genes from an allergenic plant could transfer this unwanted trait to the target plant. This phenomenon was documented in 1996, as soybeans with a Brazil nut gene-added to improve their value as animal feed-produced an allergic response in test subjects with Brazil nut allergies. Unintended consequences like these underscore the need for effective regulation of GE products. In the absence of a rigorous approval process, there is nothing to ensure that GE crops that cause health problems will always be identified and kept off the market.

GE Environmental Impacts

Genetically engineered crops can potentially cause environmental problems that result directly from the engineered traits. For instance, an engineered gene may cause a GE crop (or a wild relative of that crop) to become invasive or toxic to wildlife. But the most damaging impact of GE in agriculture so far is the phenomenon of pesticide resistance. Millions of acres of U.S. farmland are now infested by weeds that have become resistant to the herbicide glyphosate. Overuse of Monsanto's "Roundup Ready" trait, which is engineered to tolerate the herbicide, has promoted the accelerated development of resistance in several weed species. Looking for ways to fight back against these "super weeds," farmers are now turning to older, more toxic herbicides such as 2,4-D and dicamba. As if on cue, agribusiness companies have begun to develop new GE crops engineered to tolerate these older herbicides-with no guarantee that the Roundup Ready story will not repeat itself, producing a new wave of resistant weeds. And this issue is not confined to herbicides: recent reports suggest a growing problem of corn rootworms resistant to the insecticide Bt, which some corn varieties have been engineered to produce.

GE and Industrial Agriculture

As the super weed crisis illustrates, current applications of genetic engineering have become a key component of an unsustainable approach to food production: industrial agriculture, with its dependence on monoculture-supported by costly chemical inputs-at the expense of the long-term health and productivity of the farm.

A different approach to farming is available-what UCS calls "healthy farms." This approach is not only more sustainable than industrial agriculture, but often more cost-effective. Yet as long as the marketplace of agricultural products and policies is dominated by the industrial model, prioritizing expensive products over knowledge-based agro ecological approaches, healthy farm solutions face an uphill battle.

In the case of GE, better solutions include crop breeding (often assisted by molecular biology techniques) and agro ecological practices such as crop rotation, cover crops, and integrated crop/livestock management.

Such healthy farm practices are the future of U.S. agriculture-and policymakers can help speed the transition by supporting research and education on them. In the meantime, stronger regulation of the biotechnology industry is needed to minimize health and environmental risks from GE products (Nicholl 2008).

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Rarity of Endemic Medicinal Plants and Role of Herbaria for Their Conservation Against Environmental Challenges

Rizwana Khanum

Abstract Endemic plants are restricted to particular place, topography and climate. There are about 34 hotspot range from Tropical Andes with 15,000 endemic plants and covering 5.0% of total endemic flora Atlantic forest with 8000 endemic plants and covering 2.7% of total endemic World flora etc. The small population size and distribution range of endemic species, their unique biological features, especially the loss of dispersal ability, tough ecological specialization, and predicted low genetic diversity, render them extremely vulnerable to environmental changes. Because population size and population identity can influence growth and survival differently across environmental stress gradients. But endemic species grows naturally in a single geographical area, the size of which could be either narrow or relatively large and not all endemic species are rare, just as not all rare species must necessarily be endemic. Serious consequences for biodiversity/deforestation and forest degradation are the habitat loss mainly due to climatic changes for vegetation clearing, mostly for agricultural expansion and thus considered to be the most important driver of population decline and species extinctions worldwide. The impact of deforestation is more severe in hotspots containing high concentrations of globally endemic species. Other than climate change herbarium collections used for investigations include documentation of shifts in plant biodiversity with increasing urbanization, population extinction of environmentally sensitive plants, conservation priorities for taxa in endangered habitats, and human-induced reductions in individual plant size. The Pakistan Museum of Natural History (PMNH) being unique of its kind and paying major role for combating most of the issues for endemic plants.

Keywords Endemic plants · Environmental changes · Pakistan · PMNH · Conservation

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Introduction

More than 270,000 vascular plant species reported in whole World (Walter and Gillet 1998). Endemic taxa are those which occur in restricted geographical region (Stace 1989).

This sort of changeability is the consequence of complex interactions of different features of life history of local environmental conditions, populations and ecological and physiological requirements of particular species (Barnett and Kohn 1991). Mostly rare and endangered species confined to small populations in geographically isolated places. Rare or endemic plants may be locally common but occur in only few habitats that are geographically restricted (Kruckeberg and Rabinowitz 1985). We can say that they may also be both limited and geographically restricted, indicating specific adaptation to habitats that are rare (Rabinowitz 1981). All locally rare plants don't exhibit the same patterns due to differences of ecological and evolutionary processes that give rise to rarity (Kruckeberg and Rabinowitz 1985). The limitation of genetic diversity within and among populations of locally rare plants depends on whether a species is rare naturally or it has recently become rare as a consequence of stochastic events. Stochastic events may be anthropogenic or environmental. Naturally small plant populations may show genetic systems adjusted to close inbreeding and adaptations that counteract the disadvantage of rarity, contrary to species that have experienced severe reductions in population through anthropogenic activities like habitat destruction, fragmentation, or degradation (Barnett and Kohn 1991). Cain (1944) describes that plant endemics are usually considered to have one of two distinct historical origins.

> In the first treatment of the subject, Cain classified endemics as being either "new" or "old." New endemics are recently formed species confined to a region by geographic barriers; old endemics are formerly widespread species capable of spreading again (Cain 1944).

The major factors determining the stability between new and old endemics are the age and isolation of an area. This definition would be later refined for the California flora by Stebbins and Major (1965), who classified

> Species as neo- or paleoendemics. Neoendemics originate after a single founder event and have had inadequate time to spread; such taxa are also referred to as "insular endemics." Paleoendemics form via a pathway of biotype depletion, where environmental changes may lead to altered competitive regimes and habitat-specific population extirpation. As a result, paleoendemics now occupy a small subset of their former range.

Distribution and Significance of Endemic Plants

According to Kier et al. (2009) estimated endemism richness of vascular plants for 90 biogeographic regions covering the whole terrestrial area (except for Antarctica). The data shows that range equivalents of vascular plants for all 90 regions yield a

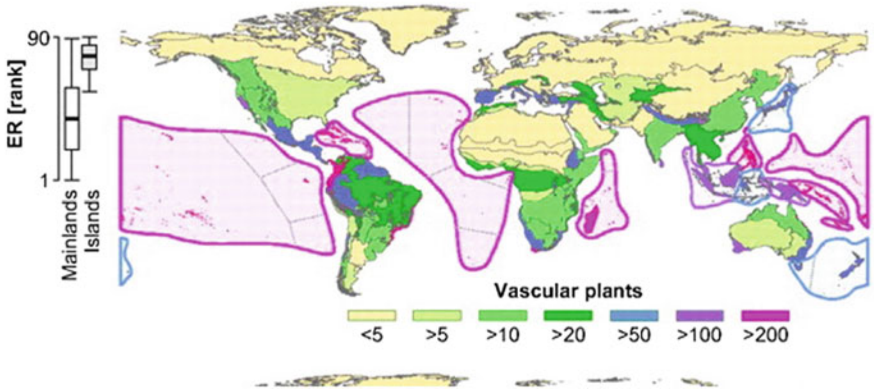

Fig. 1 Distribution of vascular plants in World. *Source* Kier et al. (2009)

total of 315,903. The island regions spanned a smaller latitudinal extent and were closer to the equator; possibly deform the overall representation as species richness increases and average range size decreases toward the equator. Thus that's why half of the top 20 regions in terms of endemism richness per standard area were island regions (Kier et al. 2009, Fig. 1).

The hotspots of World hold at least 150,000 plants as endemics, representing a remarkable 50% of the world's vascular plant species. This is an absolute not an accurate figure because it does not include species that are endemic to the combined area of two or more hotspots. The hotspot is an area must have at least 1500 species of endemic plants. There are about 34 hotspot range from Tropical Andes with 15,000 endemic plants and covering 5.0% of total endemic flora Atlantic forest with 8000 endemic plants and covering 2.7% of total endemic World flora etc. (CPEF Initiative).

Endemic plants are restricted to particular place, topography and climate (Kruckeberg and Rabinowitz 1985) (Fig. 2). Such native unique plants and animals that have evolved together depend upon each other for survival (Peirson 2010). These plants and animals form a complex network of relationships, an intricate web of life with each species' life cycle highly dependent on the others, also known as an ecosystem (Bascompte and Jordano 2006). For example, native plants do a better job of providing food and shelter for native wild animals than do introduced plants. Native plants are the foundation of our natural ecosystems and protect biodiversity. Biodiversity, short for biological diversity, describes the variety of life found in an ecosystem. These endemic plants are used in the development of new foods, medicines and industrial products. These plants have developed their own natural defenses against many pests and diseases, requiring less pesticide use. Reducing or eliminating the use of pesticides keeps these toxins out of our watersheds (Mahomoodally et al. 2005; Balick et al. 1996; Barik et al. 2007; Bibi et al. 2015).

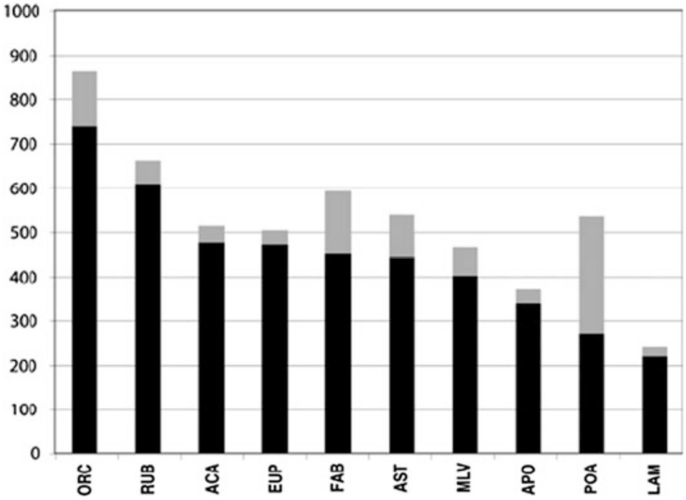

Fig. 2 The-ten-families-with-the-largest-number-of-endemic-species-in-the-Malagasy [ORC: Orchidaceae (862 Species), RUB: Rubiaceae (660 species), ACA: Acanthaceae (512 species), EUP: Euphorbiaceae (504 species), FAB: Fabaceae (592 species), AST: Asteraceae (540 species), MLV: Malvaceae (466 species), APO: Apocynaceae (371 species), POA: Poaceae (536 species), LAM: Lamiaceae (240 species)]. *Source* Callmander et al. (2011)

Major Threats

The endemic plants affected by various natural and anthropogenic factors. These will be discussed below.

Small Population Size

The small population size and distribution range of endemic species, their unique biological features, especially the loss of dispersal ability, tough ecological specialization, and predicted low genetic diversity, render them extremely vulnerable to environmental changes (Whittaker and Fernández-Palacios 2007; Patiño et al. 2013; Sonne et al. 2016). Because population size and population identity can influence growth and survival differently across environmental stress gradients. Small populations are more exposed than large ones to extinction because of environmental stochasticity, genetic drift and inbreeding. (Frankham 2005; Lande 1993; Kaljund and Jaaska 2010). But endemic species, grows naturally in a single geographical area, the size of which could be either narrow or relatively large and not all endemic species are rare, just as not all rare species must necessarily be endemic (Isik 2011; Oakley and Winn 2012). Further the studies showed that the pollination limitation is likely to be the main limiting factor in size of population of these endemic (Machaka-Houri et al. 2012; Ashman et al. 2004; Franzén et al. 2009; Knight et al. 2006). The small population because of a reduction of species richness and abundance of pollinators, a change in the pattern of foraging behaviour of flower-visiting insects, a reduction in pollinator visits per flower or average pollen loads per visit (Lamont et al. 1993; Jennersten 1988; Didham et al. 1996; Aizen and Feinsinger 1994a, b).

Habitat Fragmentation

The habitat fragmentation will disturb interactions between plant–pollinator resulting declines in plant reproduction. Additionally loss of one mutualistic partner in fragmented parts of the habitat has been predicted to causing extinctions within the fragment (Rathcke and Jules 1993; Kearns et al. 1998).

We predict high levels of pollination failure in rare plants with combinations of these characteristics as, for example, in dioecious species with fragmented populations pollinated by insects with a small foraging range. Spira (2001) considers that insurance traits such as wind pollination, self pollination and asexual reproduction are unlikely to compensate rare plants in the long term for a continuous decline in pollination and argues that the key to slowing the rate of species loss is habitat preservation. Of more immediate extinction threat are those rare plants without compensation for pollination failure (importantly those requiring regular or high levels of seed recruitment for survival), especially if reductions in pollination levels have been brought about by recent environmental change. Pollination and seed levels among plants in a community must reach a high enough threshold to account for reductions as a result of, for example, herbivory and loss of seed viability. Observations of successful pollination among threatened or rare plants alone, although providing evidence of viability, need to be accompanied by evidence of recruitment from seed, because pollination levels recorded might not be sufficient to achieve population replacement (Wilcock 2002; Spira 2001; Weekley and Race 2001; Warburton et al. 2000; Bakker and Berendse 1999; Wilcock and Neiland 1998).

Thus the understanding of the interactions between the levels of self-compatibility and patterns of pollinator activity are mandatory to predict the impacts of habitat fragmentation. The reproductive success is affected by fragmentation depends upon interaction between the quantity and quality of pollen a plant receives and the level of self-compatibility (Aizen et al. 1994a, 2002). Repeated fires may affect population sizes and reduced plant densities, increased population fragmentation and even localized extinctions, especially for obligate seeders (Bradstock et al. 1996).

Thus we can say that habitat fragmentation includes three components (1) pure loss of habitat, (2) reduced fragment size, and (3) increased spatial isolation of remnant fragments. In addition, small populations may be more prone to extinction due to the loss of genetic variation. A decreasing population size may result in erosion of genetic variation through the loss of alleles by random genetic drift. In addition, increased selfing (in plants) and mating among closely related individuals in small populations may result in inbreeding and a reduction of the number of heterozygotes (Andren 1994; Berge et al. 1998; Schaal and Leverich 1996; Booy et al. 2000; Buza et al. 2000; Cruzan 2001; Culley and Grubb 2003).

Clearance of Native Forest

The loss of forest between 2000 and 2005 is about 7.3 million ha annually (FAO 2006, 2009) Serious consequences for biodiversity/deforestation and forest degradation are the habitat loss for vegetation clearing, mostly for agricultural expansion and thus considered to be the most important driver of population decline and species extinctions worldwide (Gibbs et al. 2010). The impact of deforestation is more severe in hotspots containing high concentrations of globally endemic species. With the current level of deforestation, most of the land area of forest supporting endemic have wiped out and much of the remaining native vegetation is highly fragmented (Pandit et al. 2007; Laurance 1991; Myers 1998; Ross et al. 2002; Allison and Hobbs 2006; Debuse et al. 2007; Ford 2011; Gibbs et al. 2010). The land-use change and their consequences for deforestation are important aspects for forest degradation (Singh et al. 2001; Sodhi et al. 2009; Echeverria et al. 2006).

Spread of Invasive Species

Invasive species as any species that successfully invades a forest type (ecosystem) where it was previously unknown, causing biological change or economic harm in that ecosystem. Those invasive species that originate from outside the ecosystem are a major cause of species extinction (Levine et al. 2002; Norton 2009). Most introductions of invasive alien species come due to human actions (Mack et al. 2000). Further, the success of invasive species depends upon series of factors like habitat alteration and degradation, community structure alteration, and over-exploitation. The systems by which invasive species effect local ecosystem change include: competition with native or endemic species, alteration of ecosystem functioning, and even genetic contamination. As a consequence, it altered community structure, altered biodiversity (including extinctions), homogenization of flora and/or fauna, and, ultimately, reduced ecosystem services (Diamond 1989; Chapin et al. 2000; Shea and Chesson 2002; Hooper et al. 2005; Thompson et al. 2009) The examples of the loss of native flora and fauna due to invasive species are numerous (Aarssen and Turkington 1985; Batten et al. 2006, 2008) and comparative studies like *Lolium* *perenne* and *Trifolium repens* (Aarssen and Turkington 1985), *Taraxacum ceratophorum* and its invasive congener *T. officinale* (Brock et al. 2005), native perennial and exotic annual grasses, invasive tropical shrub Clidemia hirta (DeWalt et al. 2004), *Centaurea solstitialis* invasion success is influenced by *Nassella pulchra* (Morghan and Rice 2005), *Lythrum salicaria* (Lythraceae) with co-occurring native species (Nagel and Griffin 2001) etc.

Exploitation

Over-exploitation occurs when the number of individuals that are removed annually from a population exceeds the natural annual increment of that population so that it can no longer sustain itself without intervention, which leads to decline and threatens its existence. While over-exploitation affects many renewable natural resources (e.g., over-harvesting of timber, fuelwood, grazing areas, hunting, fishing), it has been severe in tropical grasslands and savannahs, and in marine ecosystems, while it is increasingly having a negative impact in tropical forests and coastal areas (MEA 2005). Over-exploitation of species may result in degradation of the ecosystems followed by the loss of genetic diversity and the extinction of species.

Environmental Challenges

Climate change considered to be as one of the utmost challenges to humankind and all other life. Medicinal plants and more precisely endemic medicinal plants are highly valuable to human livelihood (Anderson et al. 2005; Bajracharya et al. 2007; Calinger et al. 2013; Salick et al. 2014). According to the assessment of WHO about 60% of the world population and 80% of the population of developing countries depends on traditional medicine mainly derived from plants (WHO 2002). Medicinal plants are already affected by anthropogenic climate changes around the world. According to reports of Intergovernmental panel on climate change (IPCC 2007), there will be further increase in temperatures from 1.4 to 5.8 °C by the year 2100 and by 2033 there will be extreme and unpredictable weather incidents. For example more frequent and heavier monsoon rainfall, frequent and hotter summer days, less frequent and lower dry season rainfall, Stronger and more frequent storms with high winds etc. Key factors affecting endemic populations are climate change, habitat destruction, overharvesting and decline in natural pollinators resulting from the use of insecticides (Koopowitz 2001; Vaasa and Rosenberg 2004; Byg and Salick 2009).

The endemic plant species are considered more prone to extinction as more vulnerable to climate change and thus face high risk. Therefore, an improved knowledge of the factors responsible for such change requires intensive and continuous field survey. Further research on threatened endemic medicinal plant under climate change scenario is fundamental for developing conservation strategies as well as practicing of cultivating medicinal plants through the involvement of local communities with the traditional indigenous knowledge can prevent the impact of climate change (Parmesan 2006; Benning et al. 2002; Keutgen et al. 1997; Mooney et al. 1991). Thus may be presumed that endemic plants face extraordinarily high risk from climate change because their narrow edaphic niches limit their possibilities to adapt through migration (Harrison et al. 2009).

Thus species endemic to regions or ecosystems that are especially susceptible to climate change, for example *Rhodiola rosea* of the Canadian Arctic an snow lotus (*Saussurea laniceps*, right—in the wild, for sale and drying) (Susan 2009) of the Tibetan mountains are specifically identified as medicinal species that could face significant threats from climate change, *Tylophora hirsuta* in Northern parts of Pakistan (Sauve 2004; Brown et al. 2002; Lutz 2009; Eklabya et al. 2010; Khanum et al. 2013).

There are various indirect factors like people's livelihoods, population growth, governance limitations, lack of transparency in decision-making, deficient normative frameworks, insufficient resources for implementation and enforcement of laws and regulations, growing international demand for agricultural products, increasing interest in biofuels, confused sector policies, local cultural and demographic factors, climate change, and perverse subsidies.. These complex interactions of factors have made it difficult to change the course of deforestation, particularly in developing countries apart from direct driver of agriculture for deforestation (MEA 2005; FAO 2006, 2009; Kanninen et al. 2007; Flynn et al. 2009; Laliberté et al. 2010).

Conservational Implications (Policy Initiative)

The Sixth Conference of the Parties (COP6) to the Convention on Biological Diversity (CBD) held in April, 2002 adopted the Global Strategy for Plant Conservation (GSPC), which includes 16 outcome-oriented targets for 2010. The long-term objective of the GSPC is to halt the continuing loss of plant diversity. At COP7 held last year in Kuala Lumpur, the CBD moved to integrate the targets of the GSPC into all its relevant thematic and cross-cutting programs. A move to integrate the GTI and the GSPC is reflected in a growing trend to include conservation assessments in taxonomic works. The conservation community urgently needs input from taxonomists; P. Raven has pointed out that "The GSPC is the greatest opportunity so far for plant conservation" (Bramwell et al. 2002). Mainly IUCN develop criteria in 1994 and revised in 2001 and based on risk of extinction can classify (IUCN 1994, 2001). However Red Lists for tropical countries are incomplete and it is nearly impossible to assess how many species are truly threatened at a global scale or at a regional/hot spot level. All species must be assessed (or reassessed) using the most recent Red List criteria (IUCN 2001) to determine those that are "threatened", whether critically Endangered (CR), Endangered (EN), or Vulnerable (VU) (i.e., those facing an “extremely high risk” of extinction, a “very high risk”, or a “high risk”) (IUCN 2004; IUCN & SSC 2004).

In addition to this, strengthening of a protectionist policy is clearly the most practical means to enhance the value of remaining native forests. Focusing on the most damaged ecosystems, is likely where we will observe our greatest achievement. Similarly increasing popularity of private agreements between landholders and governments for the gazetting of privately owned native vegetation remnants, private acquisition of large tracts of land by non-governmental organizations, replanting schemes funded, biodiversity corridor plans etc. (Sodhi et al. 2009; Ford 2011).

Thus policies to achieve a forest transition include measures to develop the supply of wood and to manage the demand for it. So investments in the area and productivity of plantations, as well as in the adoption of sustainable forest management practices in natural forests require for rising wood supply without declining forest area requires. Interventions from demand-side include institutions that promote ecoconsumerism, like through certification schemes, substitution of wood by other products and recycling and reuse efforts etc. A forest transition can be enhanced by policies that increase the value of intact forests through a combination of payments for ecosystem services and land-use zoning schemes, including protected areas. These policies are reviewed elsewhere (Auld et al. 2008; Dauvergne and Lister 2010; Lambin and Meyfroidt 2011). The most important implications of the degree to which Worlds native forests have disappeared or been degraded are that management must emphasize the maintenance of existing primary forest patches, as well as focus on the regeneration of matrix areas between fragments to increase native habitat area, connectivity and ecosystem functions (Newton and Oldfield 2008).

Ramsar Convention, CITES, Convention on Biological Diversity, The United Nations Framework Convention on Climate Change (UNFCCC or FCCC), The United Nations Convention to Combat Desertification (UNCCD), Kyoto Protocol are also develop for various inquiry based issues for terrestrial to wetland plants including endemic medicinal ones (Matthews 1993; Yadab 2008; http://unfccc.int/press/fact_sheets/items/4977.php; http://www.cbd.int/history/; http://www.unccd.int/convention/menu.php).

Role of Herbaria for Conservation of Medicinal Endemic and Pakistan’ Overview

> A voucher can be broadly defined as a representative sample of an expertly identified organism that is deposited and stored at a facility from which researchers may later obtain the specimen for examination and further study (Culley 2013).

The distribution of a plant species is typically plotted from locality data on herbarium specimens in floristic and taxonomic studies, which typically provide the best existing information regarding distribution. Herbarium specimens are also frequently used to assess changes usually expansions, in the range of a species over time (e.g., Stuckey 1980; Pyšek 1991; Weber 1998; Delisle et al. 2003), study the occurrence and distribution of plant pathogens (e.g., Ristaino 1988; Koponen et al. 2000; Antonovics et al. 2003; Applequist et al. 2007), and sometimes to identify temporal changes in phenotype, genotype or phenology within a specified range (e.g., Parkhurst 1978; Saltonstall 2002; Primack et al. 2004). In addition to these uses, herbarium records may play a significant role in evaluation of conservation status, conservation priorities or changes over time in species frequency (e.g., Burgman et al. 1995; MacDougall et al. 1998; Funk et al. 1999; Ter Steege et al. 2000; Golding 2001; Hedenas et al. 2002; Schatz 2002; Willis et al. 2003; Ungricht et al. 2005). But inadequate geographic coverage or various collection biases resulted in limited accuracy in predicting distribution (e.g., Rich and Woodruff 1992; Heyligers 1998; MacDougall et al. 1998; Ungricht et al. 2005). An additional source of error may be that previously documented populations are no longer present; Kropf and Bernhardt (2004) found that of 13 populations of *Anthyllis montana* subsp. *jacquinii*, documented by 305 herbarium collections, seven were apparently extinct. As the assumption may be made by potential end users that a dot map based on herbarium specimens represents current presence at the indicated localities, especially for conservation purposes it would be valuable to have some estimate of the validity of historical data in predicting current distributions. Furthermore, even if a previously existing species does not occur in later collections from the same locality, this may specify not actual loss of the population but chance failure to relocate it, collection at the wrong time of the year, etc. (Markos et al. 2016; Ames and Spooner 2008).

Example The herbarium data collections indicate various trends including species population status. For example, Nilsson and Nilsson (1983) determined that the noticeable rate of species turnover on six carefully surveyed small islands was up to three times the actual rate. The basic purpose of the study was to analyze the predictive value of herbarium collections by attempting to relocate historical populations of Echinacea that were documented in herbarium collections from the state of Missouri. Echinacea is endemic to North America; the most recent revision recognized four species (Binns et al. 2002), most including multiple varieties that correspond to the nine species in the treatment by McGregor (1968). Thus, the four taxa native to Missouri are *E. atrorubens* (Nutt.) Nutt. var.paradoxa (J.B. Norton) Cronq., *E. pallida* (Nutt.) Nutt. var. *pallida*, *E. pallida* var. *simulata* (McGregor) Binns, and *E. purpurea* (L.) Moench. According to Binns et al.'s (2002) classification, but *E. paradoxa* (J.B. Norton) Britton, *E. pallida*, *E. simulata* McGregor, and *E. purpurea* according to McGregor's (1968) classification, which remains the more commonly used at present. Among them two species like *Echinacea pallida* var. *pallida* and *E. purpurea* are in commercial demand for their medicinal value.

Similarly, impacts of climate change on plants using herbarium specimens to show how temperature variation causes changes in geographic distributions (Feeley 2012) and flowering times (Primack et al. 2004; Lavoie and Lachance 2006; Miller-Rushing et al. 2006; Robbirt et al. 2011; Panchen et al. 2012; Calinger et al. 2013). Furthermore, herbarium data have been used to locate alpine areas for monitoring of especially sensitive native plants (Gallagher et al. 2009), and evaluate diversity gradients in different areas of the world in relation to climate change (Droissart et al. 2012). Historical specimens also been used to observe the relation of plants with their changing air quality and alterations in nutrient cycling. For example, Lewis and Clark were analyzed using carbon isotope ratios to generate baseline data on the amount of carbon present in the atmosphere over 200 year ago in herbarium specimens (Teece et al. 2002). In addition, it was proved that reduction of stimatatl density of leaves is related to rising CO_2 levels over the past 200 year (Woodward 1987; Beerling and Chaloner 1993), and human activities within the past 100 year resulted in increasing levels of nitrogen and other nutrients in bryophytes (Peñuelas and Filella 2001).

Other than climate change herbarium collections used for investigations include documentation of shifts in plant biodiversity with increasing urbanization (Dolan et al. 2011), population extinction of environmentally sensitive plants (Lienert et al. 2002), conservation priorities for taxa in endangered habitats (MacDougall et al. 1998), and human-induced reductions in individual plant size (Law and Salick 2005). The specimens have also been used to examine the invasive species across a region, as in the cases of the ornamental Callery pear tree (*Pyrus calleryana* Decne.) following its commercial introduction in the United States in 1962 (Vincent 2005), and *Typha* species, which have been spreading across North America for well over 100 year (Shih and Finkelstein 2008).

Although the prospective for degradation due to age and storage conditions, DNA can sometimes be obtained from historical and modern herbarium specimens thus allowing genetic comparisons across time to examine population bottlenecks and other processes (e.g., Wandeler et al. 2007; Lister et al. 2010; Zuntini et al. 2013; Khanum et al. 2016).

Role of Pakistan Museum of Natural History

Endemic plants have special importance and give uniqueness to a region. Pakistan has also rich in endemic plants and 7.8% flora containing 400 species belonging to c. 169 genera and 45 families are endemic in Pakistan (Ali 2008). Regarding the

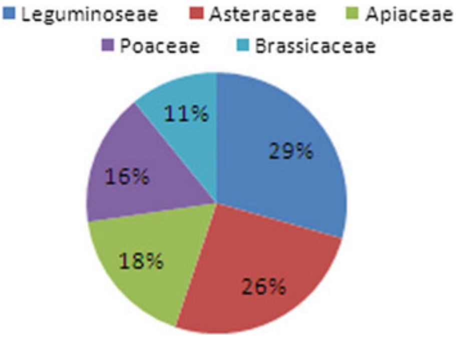

Fig. 3 Distributional trend of endemic plants from Pakistan (Main families). *Source* Ali (2008)

regional distribution most of endemic plants restricted to north of the country including Kashmir mountains. According to Ali and Qaiser (1986) Leguminoseae, Asteraceae, Apiaceae, Poaceae and Brassicaceae are among the families having more of endemic plants (Fig. 3). Among these the genus "*Astragalus*" contains 37 the largest recorded endemic species. North of country include most of flora from Hindukush and Himalayan region like Chitral and Kararkorum range like Gilgit & Baltistan.

Pakistan Museum of Natural History (PMNH) is unique one representing most of floral specimens from various geographic regions and established in 1976 (www.pmh.gov.pk). Apart from PMNH, several universities are also collecting plants like KAR in University of Karachi, IBD in Qiad I Azam University etc. But the uniqueness of PMNH makes it prominent with all facilities that could be provided to researcher, scholar and other students.

Now the collection/section for endemic plants is underway through the funding of Rufford, through which each of the unique plant could be assessable and explored for various studies range from conservation (In situ or ex situ), phonological changes, population size etc.

Endemic medicinal plants of Pakistan and climate change.

According to assessment based on herbarium (PMNH, NARC, KAR) as well as field data medicinal plants like *Pentatropis spiralis*, *Tylophora hirsuta*, *and Vincetoxicumarnottianum* under the future climate change scenario, the Maxent model predicted habitat gains for *P. spiralis* in southern Punjab and Balochistan, **BUT** loss of habitat in south-eastern Sindh. *Vincetoxicum arnottianum* as well as *T. hirsuta* would gain new habitat in upper Peaks of northern parts of Pakistan. *T. hirsuta* is predicted to lose most of the habitats in northern Punjab and in parches from lower peaks of Galliat, Zhob, Qalat etc. (Khanum et al. 2013, Fig. 4).

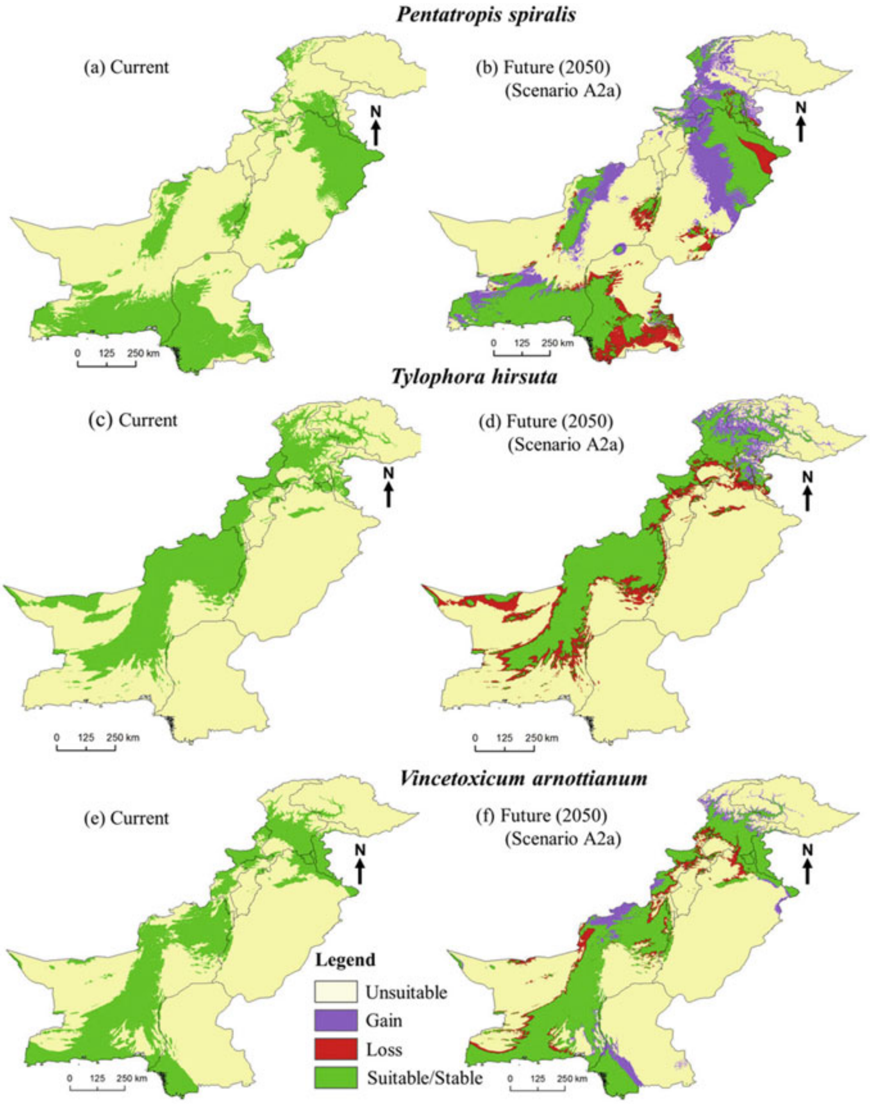

Fig. 4 Effect of climate change on medicinal endemic plants of Pakistan (Khanum et al. 2013)

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Metabolic Responses of Medicinal Plants to Global Warming, Temperature and Heat Stress

Shabir H. Wani, Nisha Kapoor and Ritu Mahajan

Abstract Global warming has resulted in strong heat waves which has severely affected the growth and development of the plants. The changes in distinct metabolic pathways have hampered the adaptive responses of plants to different environmental stresses. Extreme variations in temperature during summers have a significant impact on agricultural production worldwide because the heat waves cause yield losses that risks the future global food security. However, the plants have made certain adjustments to cope up with the adverse environmental conditions which includes the production of compatible solutes that could maintain the cell turgor by stabilizing the osmotic regulation. Even at the molecular level, various modifications in the expression of genes protect the plants from heat stress. Further, the collaboration of molecular biologists and plant breeders, to develop new genotypes by identifying and introgressing the tolerance genes that can result in plants with acceptance to wide range of environmental stresses.

Keywords Heat shock · Osmolytes · Phytohormones · Secondary metabolites · Stress tolerance

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Introduction

Abiotic stress such as extreme temperatures, drought, flooding, salinity etc., are one of the main outcomes of global climate change (Wani et al. 2008, 2016; Gosal et al. 2009; Wani and Sah 2014). Temperature is an important factor that determines the rate of plant growth and development and each species has a specific temperature requirement. In recent years recurrence of heat waves or tremendous temperature events are predictable (Bhatla and Tripathi 2014). These extreme temperature events are of short-term durations where temperature increases over 5 °C above the normal temperatures. As a result the plants are at high risk due to these climatic changes and strong heat waves whose intensity is very high as observed in recent years. As a result the growth and development of plants is severely affected (Cleland et al. 2012). The effects of increased temperature exhibit a larger impact on vegetative growth and metabolites present in the plants. Higher temperatures associated with climate change have affected the yield responses as different species are based on the plant's cardinal temperature requirements (Hatfield and Prueger 2015). Unfavorable environmental conditions adversely affect the plant growth and different metabolic pathways involved in signaling, physiological regulation and defense responses. The involvement of primary metabolites such as sugars, amino acids and various intermediates of Krebs cycle directly involved in photosynthesis are also affected. Also, the induction of secondary metabolites due to heat stress could act as an effective mechanism of cross-protection against biotic threats, providing a link between abiotic and biotic stress responses (Arbona et al. 2013). The genotypic changes that show more response to elevated climatic changes are adapted better (Springate and Kover 2014). Continuous evolutionary processes have resulted in vast metabolic diversity in plants with infinite reservoir of miscellaneous functions. Metabolic adjustments in response to heat stress involve fine adjustments in amino acid, carbohydrates and amine metabolic pathways. If there is proper activation of early metabolic responses in plants imposed due to stress, then it can help the cells to restore chemical imbalances which are vital for its survival. There are array of mechanisms along with changes in the genetic composition that depends upon the speed with which the temperature changes. Plants that induce changes in morphology or physiology with respect to changing environmental conditions can tolerate extreme stressful environmental conditions.

Change in Flowering Time

As the temperature increases crop flowering and maturing also changes. Wheeler et al. (1996) observed the decline in the yield of wheat by $\sim$5–8% per 1 °C rise in mean seasonal temperature. Also along with elevation in temperature some other factors, such as the enhanced rate of net photosynthesis at elevated CO_2 also decreases the yield.

In the life cycle of most plants, the timing of flowering is a critical stage of development when seed number is determined. It was observed that the temperature (>32–36 °C) greatly reduces the seed set and finally the crop yield, if they coincide with a brief critical period of only 1–3 d around the time of flowering (Jagadish et al. 2008).

Effect of Elevated CO_2 on Plant Development and Morphology

The effect of CO_2 is directly on plants due to photosynthetic gas exchange while the indirect effect of CO_2 it is a potent greenhouse gas that contributes to global warming. Increased CO_2 stimulates photosynthetic carbon assimilation rates by an average of 31% across 40 species (Ainsworth and Long 2005). He also observed that elevated CO_2 in C_3 species results is increase in above ground biomass by an average of 20%. In soybean elevated CO_2 results in an increased number of leaf nodes and increased leaf size (Dermody et al. 2006), increased root length, altered root depth distribution and nodulation (Gray et al. 2016), and increased pod number and seed yield (Bishop et al. 2014). Heat stress hampers photosynthesis due to reduction of soluble proteins and Rubisco binding proteins (Hasanuzzaman et al. 2013). Heat imposes negative impacts on leaf of plant like reduced leaf water potential, reduced leaf area and pre-mature leaf senescence which have negative impacts on total photosynthesis performance of plant. Even the reduced activity of sucrose phosphate synthase, ADP-glucose pyrophosphorylase, and invertase affects starch and sucrose synthesis (Rodríguez et al. 2005). In photosynthesis related processes, where Ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (Rubisco) catalyzes the first step in net photosynthetic CO_2 assimilation and photorespiratory carbon oxidation. The enzyme due to competitive inhibition by O_2 is inefficient as a catalyst for the carboxylation of RuBP. At low temperatures, all the metabolic reactions, including those of sucrose synthesis, are slower (Sage and Kubien 2007). Rubisco helps in protein synthesis which were measured in the leaves of cotton plants under control (28 °C) and also those at heat-stress (41 °C) conditions. In response to high temperature, de novo protein synthesis quickly shifted from mostly expression of Rubisco large and small subunits to the major heat-shock proteins, while de novo synthesis of the constitutively expressed 47- and 43-kDa activase polypeptides was not appreciably altered (Law et al. 2001).

Heat Stress and Secondary Metabolite Production

Plant metabolism contributes towards heat stress tolerance by providing the energy and production of secondary metabolites essential for cellular homeostasis. Temperature has great influence on secondary metabolite production in the plants

(Ramakrishna and Ravishankar 2011). Jochum et al. (2007) reported the enhancement of ginsenoside and reduction in photosynthesis in *P. quinquefolius* when there was 5 °C increase in temperature.

Carotenoids are necessary for photoprotection of photosynthesis and play an important role as a precursor in signaling during the plant development under stress conditions. They enhance the nutritional quality and plant yield. Various reports indicate that the plants exposed to high-temperature stress show reduced chlorophyll biosynthesis (Reda and Mandoura 2011) as it is the first process occurring in plastids which is affected or degraded by the high temperature. Mohanty et al. (2006) observed decrease in 5-aminolevulinate dehydratase, the first enzyme of pyrrole biosynthetic pathway in cucumber and wheat under high-temperature. Davison et al. (2002) studied overexpression of the chyB gene that encodes β-carotene hydroxylase in *Arabidopsis thaliana*. He observed twofold increase in the size of the xanthophyll cycle pool and tolerance of the plants to conditions of high light and high temperature. Similarly, Velikova et al. (2012) studied the increased stability in permeability of the thylakoid membrane in potato exposed to at mild heat treatment of 35 °C for 2 h. This study indicated that thylakoids and thylakoid membranes were maintained by de-epoxidized xanthophylls during heat stress. A remarkable reduction in productivity of anthocycnins was observed in suspension cultures of *Perilla frutescens*, when the high temperature was increased to 28 °C, while the productivity of the pigment was optimal at 25 °C (Zhong and Yoshida 1993). Thimmaraju et al. (2003) observed the influence of different temperatures while studying the kinetics of release of the anthocyanin pigment from hairy root cultures of *Beta vulgaris*.

High Temperature and Osmolyte Accumulation

The accumulation of low molecular weight water-soluble compounds called "osmolytes" is generally adopted by many plants to fight the environmental stresses. Production and accumulation of compatible solutes in response to high temperature is the most common defensive mechanism adapted by the plants. The chemicals include amino acids (asparagine, proline, serine), amines (polyamines and glycinebetaine), and γ-amino-N-butyric acid (GABA). Even the total sugars, including fructose, sucrose, trehalose, raffinose and proline (Wani and Gosal 2010; Wani et al. 2013; Harsh et al. 2016) as well as pools of anti-oxidants such as glutathione (GSH) and ascorbate (de Leonardis et al. 2015; Ding et al. 2016) accumulate in response to heat stress. This results in overall some of the protective functions of compatible osmoprotectants i.e. preservation of cellular turgor, stabilization of proteins, cellular structures and osmotic balance during environmental stress. The accumulation of aromatic amino acids viz. tryptophan, tyrosine and phenylalanine in shikimic acid in metabolism pathway under stress conditions causes a change in metabolism that results in the secondary metabolite production, thereby providing stress tolerance to the plants (Suguiyama et al. 2014).

Sakamoto and Murata (2002) reported an important role of Glycinebetaine (GB), an amphoteric quaternary amine, in plants under various stresses, such as salinity or high temperature though the capacity to synthesize GB under stress conditions differs from species to species (Ashraf and Foolad 2007). Certain medicinal plants like tobacco (*Nicotiana tabacum*) naturally do not produce GB under stress conditions. However, genetic engineering has allowed the introduction of GB-biosynthetic pathways into such GB-deficit species (Quan et al. 2004). Further, the osmolyte production under heat stress is thought to increase protein stability and stabilize the structure of the membrane bilayer (Mirzaei et al. 2012).

Phytohormones and Thermotolerance

Plants already have sophisticated mechanism that can even sense (even 1 °C) increase or decrease in temperature (Kumar and Wigge 2010; Sanghera et al. 2011; Ahammed et al. 2013). There are several multiple pathways, regulatory networks and cellular compartments involved in the plants against heat stress (Mittler et al. 2012). Many phytohormones like ABA, salicylic acid and ethylene increase their levels under heat stress as they act as strong tools for overcoming the adverse effects of abiotic stresses in plants (Bita and Gerats 2013; Kim et al. 2016). Salicylic acid is involved in the regulation of important plant physiological processes such as photosynthesis, nitrogen metabolism, proline metabolism, antioxidant defense system and plant-water relations under stress conditions (Khan et al. 2015; Gorni and Pacheco 2016) and thereby provides protection in plants against abiotic stresses. Further, requirement for certain hormone and its response to signaling depends and varies upon the kinds of thermotolerance. Salicylic acid improves plant tolerance to heat stress as reported by Clarke et al. (2004) in *Ocimum basilicum*. Xu et al. (2016) reported that in *Aquilaria sinensis* heat shock of 50 °C for 30 min resulted in upregulation in the expression of Jasmonic Acid (JA) pathway genes and induced production of JA is mandatory for the enhanced production of agarwood sesquiterpene using suspension cultures. In *Capsicum annuum* WRKY proteins (CaWRKY6) plays a significant role in response to high temperature response (Cai et al. 2015). Expression of these proteins can be induced by exogenous application of Jasmonic acid, Absicic acid and ethylene. Suppression of WRKY gene by virus-induced gene silencing increases its susceptibility to heat stress which is associated with the downregulation of JA, ethylene, and ABA-induced marker gene expression. However, overexpression of CaWRKY6 improves tolerance to heat stress.

Heat Stress and Reproductive Development

Heat stress has great impact on sexual reproduction and flowering as high temperature is most lethal at the stage of flower bud initiation and hence finally results in reduced crop plant productivity (Thakur et al. 2010). This could be due to reduced water and nutrient transport during reproductive development. Heat stress causes the down-regulation of sucrose synthase, a number of cell wall and vacuolar invertases in the developing pollen grains. This results in the disruption of sucrose and starch turnover and thus accumulatation of soluble carbohydrates is in reduced levels (Sato et al. 2006). In tomato the accumulation of soluble phenolics, increased phenylalanine ammonia-lyase activity and decreased peroxidase and polyphenol oxidase activity was observed in response to heat stress (Rivero et al. 2001).

Physiological Aspects of Heat Stress

Plants respond to heat by a progressive adjustment at physiological status and metabolic level with constant and temporary metabolic alterations. Jin et al. (2015) observed changes in physiological and metabolic levels in *Portulaca oleracea* L. after treatments with drought, heat and combined stresses. These treatments given individually or combined with other stress treatments resulted in increased level of malondialdehyde, electrolyte leakage and activities of superoxide dismutase, peroxidase, while decline in chlorophyll content was observed. Further, the content of an amino acid asparagine was increased under heat condition. This is due to the association of amino acids with the substrates for protein synthesis and thus could help in quick recovery of the plant metabolism after stress (Fig. 1).

In plants exposed to high temperatures, Glycine betaine plays an important role (Sakamoto and Murata 2002). Glycine betaine is produced in chloroplasts and maintains the activation of Rubisco by sequestering Rubisco activase near thylakoids thereby preventing its thermal inactivation (Allakhverdiev et al. 2008). Sucrose being the principal end product of photosynthesis, translocates from source leaves to sink organs through the phloem. Sucrose and its cleavage products responds to stresses through carbon allocation and sugar signaling thereby regulating the plant development and Roitsch and González (2004). Li et al. (2012) observed high cell wall and vacuolar invertases and increased sucrose content that contributes to heat tolerance in young tomato fruits. It was observed in tomato that at low concentrations sucrose acts as signaling molecule while in high concentrations it becomes a ROS scavenger (Sugio et al. 2009).

Enhanced synthesis of secondary metabolites under heat stress conditions also protects against oxidative damage (Bita and Gerats 2013). It was also suggested that accumulation of anthocyanin under heat stress provide to decrease leaf osmotic potential, which finally results resulting in an increased uptake and reduced transpirational loss of water. Meiri et al. (2010) reported that in *Arabidopsis* plants

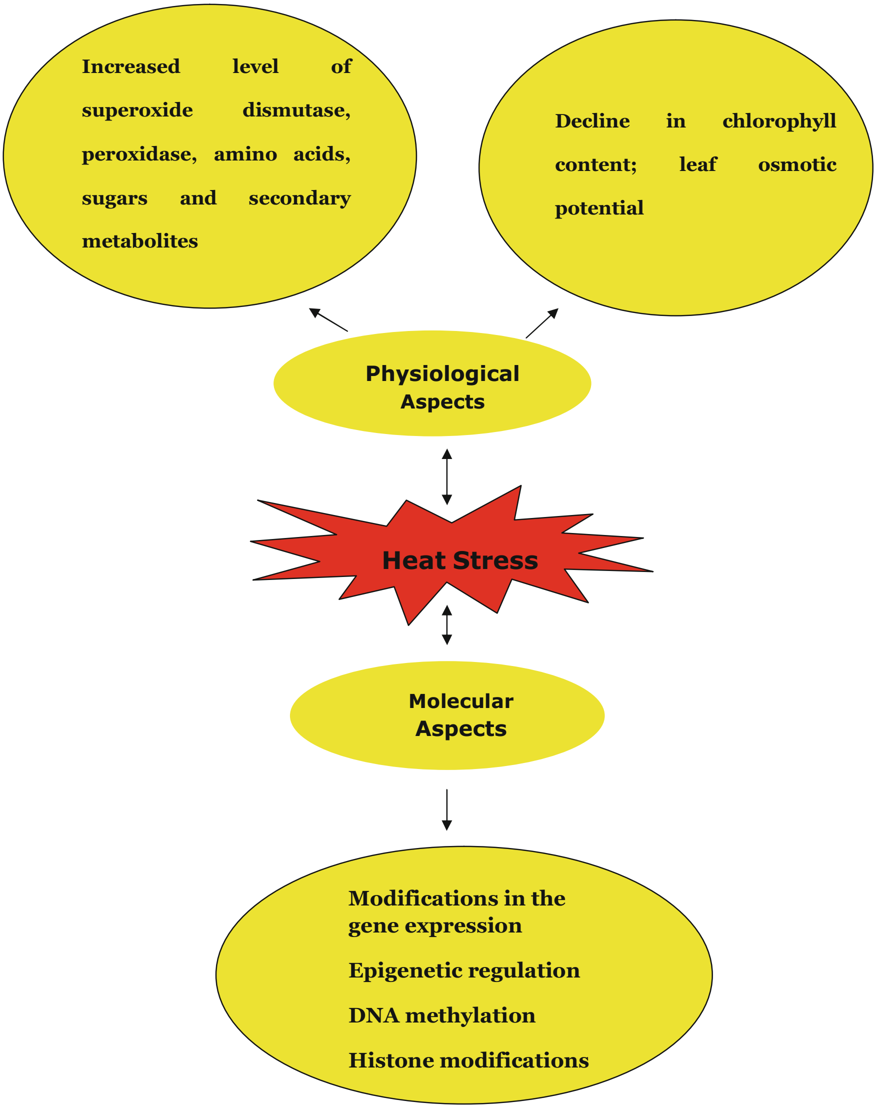

Fig. 1 Physiological and molecular aspects of heat stress in the medicinal plants

overexpressing the chyB gene that codes for b-carotene hydroxylase, an enzyme involved in the zeaxanthin biosynthetic pathway, show more tolerance to increased temperatures. It was also suggested that the prevention from oxidative damage to membranes is due to the action of zeaxanthin.

Molecular Aspects of Heat Stress

At the molecular level, heat stress causes modifications in the expression of genes involved in protecting the plants from heat stress (Zhu et al. 2013). These are mostly the genes responsible for the expression of osmoprotectants, detoxifying enzymes, transporter, and regulatory proteins. In such conditions of heat stress, modification of physiological and biochemical processes by gene expression changes slowly leads to the development of heat tolerance in the form of acclimation or adaptation of a plant to high temperature (Hasanuzzaman et al. 2010). Studied have revealed that the exogenous applications of protectants in the form of osmoprotectants phytohormones, signaling molecules, polyamines and trace elements are also effective in mitigating heat stress-induced damage in plants (Asthir 2015).

Studies on epigenetic regulation of heat responses, including DNA methylation, histone modifications, histone variants, ATP-dependent chromatin remodeling, histone chaperones, small RNAs, long non-coding RNAs and other undefined epigenetic mechanisms also play a significant role to prevent heat-related damages (Liu et al. 2015). In cotton anthers, one histone methyltransferase, one histone monoubiquitination gene and two jumonji C (jmjC) domain-containing genes are down-regulated upon high temperature (Min et al. 2014).

The expression profiles of AsA-related genes in response to temperature stress conditions have been reported earlier in apples and tomatoes (Ma et al. 2008; Ioannidi et al. 2009). Heat stress also results in increase or decrease of expression level of certain genes. Li et al. (2016) studied that the expression levels of genes CsMDHAR, CsDHAR1, and CsDHAR2 increased sharply after 1 h, in response to heat stress in 'Yingshuang' variety of *Camellia sinensis.*

Conclusions and Future Prospects

High temperature stress has become the foremost concern for crop production worldwide because it seriously affects the overall growth, development and productivity of the plants. Though in recent years many studies have focused on the response of plants to heat stress but till date a total understanding of thermo tolerance mechanisms remains subtle. Plants exposed to extreme temperatures often show a common response in the form of oxidative stress that cause damage to lipids, proteins, and nucleic acids in plants. Various phytohormones are involved in adapting the plant against heat stress. The high rate of emission of greenhouse gases from different sources is responsible for a gradual increase in the world's ambient temperature, further resulting in global warming. Therefore, the response of plant and the mechanisms underlying for its adaptation to elevated temperatures needs to be better understood for important medicinal plants. Also, the metabolic adjustments to stress are vital for acquiring stress tolerance. Still there is need to study numerous genes that are involved in the synthesis of stress-associated metabolites which get altered as per the requirement of plants under stress conditions.

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Effects of Toxic Gases, Ozone, Carbon Dioxide, and Wastes on Plant Secondary Metabolism

Vinay Kumar, Tushar Khare, Sagar Arya, Varsha Shriram and Shabir H. Wani

Abstract Various kinds of human activities along with environmental interactions or changes are occasioning the addition and accumulation of hazardous entities in the environment. The subsequent result of this is negative effects of these factors on living systems including plants. Factors such as heavy metals, toxic gases, ozone, and carbon dioxide have a major impact on plant growth and secondary metabolism of the plants. Secondary metabolites are the key players in plant adaptation to these environmental stresses and play a role in mitigating the negative effects of these stresses. Both primary and secondary metabolisms are altered under these stress environments, however, plants have evolved to endure these conditions through inducing several regulating mechanisms such as evapotranspiration of available water, controlled openings and closings of stomata as per the availability of water, over accumulation of various osmoprotectants and osmoregulators, induction of antioxidant machinery and fine tuning of transcriptional and post-transcriptional regulations of gene expressions. In most of the plants, the ultimate result of these

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defensive adaptations is regulated production of the secondary metabolites. In this chapter, we have discussed the effects of toxic gases, ozone, carbon dioxide as well as other wastes including the nanoparticles-wastes on plant secondary metabolites.

Keywords Toxic gases · Secondary metabolism · Secondary metabolites · Ozone · Carbon dioxide · Heavy metals · Nanoparticles · Wastes

Abbreviations

PSM	Plant Secondary Metabolites
CO_2	Carbon Dioxide
O_3	Ozone
SO_2	Sulfur Dioxide
H_2S	Hydrogen Sulfide
Cd	Cadmium
Cr	Chromium
Ni	Nickel
As	Arsenic
Ag	Silver
Au	Gold
NAA	Naphthalene acetic acid
NSC	Non-structural Carbohydrates

Introduction

Environmental stresses include drought, salinity, extreme temperatures, toxic gases, ozone, carbon dioxide, and other wastes released into environment because of climatic aberrations (Gosal et al. 2009; Wani et al. 2010; Wani and Gosal 2011; Wani and Hossain 2015). Plants being sessile organisms face several environmental perturbations during their life cycles (Kumar and Khare 2016). These environmental signals induce several changes in plants at physiological, biochemical and molecular levels (Sanghera et al. 2011; Wani et al. 2013, 2017; Khare et al. 2015). Both primary and secondary metabolisms get affected under change in environment or climate, however, plants have evolved to sustain under these conditions via inducing several counter-balancing mechanisms such as regulated use and evapotranspiration of available water, controlled openings and closings of stomata as per the availability of water, overaccumulation of various osmoprotectants and osmoregulators, induction of antioxidant machinery and fine tuning of transcriptional and post-transcriptional regulations of gene expressions (Kumar et al. 2010; Wani and Gosal 2010; Khare et al. 2015; Kumar and Khare 2015; Wani et al. 2016a, b; Wani and Kumar 2015; Shriram et al. 2016). In the past two centuries, air pollution problems have been aggravated due to population burst, rapid industrialization and other related anthropogenic activities to meet the global food and feed demands. There is a threatening increase in the atmospheric concentrations of various greenhouse gases namely carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) and toxic gas pollutants like sulfur dioxide (SO_2) nitrogen oxides (NO_x), besides secondary pollutants like ozone (O_3). There is a remarkable increase in the concentrations of greenhouse gases owing to various anthropogenic activities including industrialization in recent past.

Though there is considerable literature indicating the effects of these pollutants on plant growth, development and primary metabolism and metabolites. Though there is enough evidence indicating that plant primary metabolism and secondary metabolism are closely knitted (Fig. 1). Plant secondary metabolites are highly specialized products usually biosynthesized by the plants using their primary

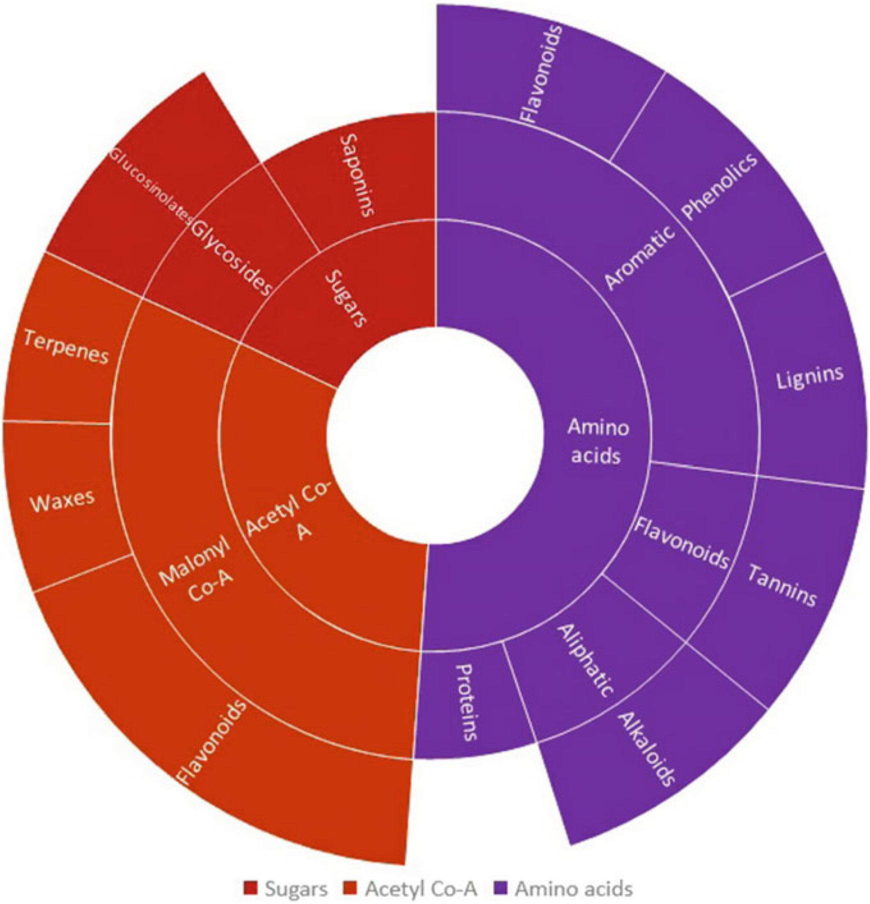

Fig. 1 Generalized biosynthetic relation in plant primary and secondary metabolites

metabolites as substrates. However, there is lack of substantial data and in-depth understanding about the impacts of toxic gases, greenhouse gases and ozone on plant secondary metabolism as a whole and on the production of plant secondary metabolites (PSMs). Nevertheless, there is a recent upsurge on the significance of altered plant secondary metabolism and enhanced production of PSMs under the influence of these gases as well as other wastes including the nanoparticles-wastes released into the environment. We are presenting herein the review of these issues through this chapter.

Effects of CO_2 on Plant Secondary Metabolites

Greenhouse gases together constitute as a major environmental challenge for medicinal plants. Among those, CO_2 is one of the major causes with a tremendous rise since industrialization took place. About half of the anthropogenic CO_2 emission between 1750 and 2011 has occurred in the last 40 years (IPCC 2014) and yet we are not certain about the potential effects of this abrupt change on medicinal plants. Since medicinal plants are potent sources for PSMs, they possess significant plasticity to adapt with the changing environments. Though, this metabolic plasticity conferred due to PSMs may affect other secondary metabolites which are usually the basis for their medicinal activity (Mishra 2016). For example; when *Digitalis lanata* known for its use in heart failures (Rahimtoola 2004) was treated with elevated CO_2, there was a 3.5-fold increase in digoxin, a cardenolide glycoside. In the same experiment where digoxin showed enhancement, other three glycosides viz. digitoxin, digitoxigenin and digoxin-mono-digitoxoside showed a decline in their concentration (Stuhlfauth et al. 1987; Stuhlfauth and Fock 1990). In addition, time also plays a crucial role in deciding the metabolic flux in relation to PSMs. For instance, in a typical time related experiment on *Hymenocallis littoralis* whose bulbs are known for their antineoplastic and antiviral properties, the elevated CO_2 resulted in increase in 3 types of alkaloids (pancratistatin, 7-deoxynarciclasine and 7-deoxy-trans dihydronarciclasin) in the first year and decrease in their concentration for the subsequent year (Idso et al. 2000). Similarly, in *Ginkgo biloba* a traditional Chinese medicinal plant used in Alzheimer's disease, vascular or mixed dementia (Weinmann et al. 2010) elevated CO_2 and O_3 together resulted in altered terpenoid content, 15% increase in quercetin aglycon and 10% decrease in keampferol aglycon, 15% in isorhamnetin and bilobalide to some extent (Huang et al. 2010). In a typical study on *Papaver setigerum*, elevated CO_2 levels from 300–600 μmol mol^{-1} corresponding roughly to the concentrations that prevailed during middle of the twentieth century, the present concentration, and near and long-term projections for the current century (Ziska et al. 2008) resulted in enhancement of four alkaloids viz. morphine, codeine, papaverine and noscapine. Further, it is also predicted that increase in CO_2 may result in high plant carbon: nutrient ratios producing excess of non-structural carbohydrates (NSCs). These

NSCs may be then are accessible for incorporation in C-based secondary metabolites (Heyworth et al. 1998). Pertaining to this prediction, a test carried out on *Hypericum perforatum* showed that elevated CO_2 resulted in enhancement of hypericin, pseudohypericin and hyperforin belonging to the class of phenolics (Zobayed and Saxena 2004). After observing the potential effects of secondary metabolites from Broccoli on cancer and cardiovascular diseases (Mahn and Reyes 2012), it was essential to evaluate effect of CO_2 on this plant. For this, widely exploited Broccoli (*Brassica oleracea*) var. italica Plenck was subjected to elevated CO_2, and this experiment showed increase in methylsulfinylalkyl glucosinolates glucoraphanin and glucoiberin derived from Glucosinolates (Schonhof et al. 2007). In a similar experiment on *Catharanthus roseus*, widely known for its anti-cancerous, anti-viral and diuretic properties (Ezuruike and Prieto 2014) when treated with elevated CO_2 showed increase in almost all of the PSMs viz. alkaloids, flavonoids, phenolic and tannins (Saravanan and Karthi 2014). In *Zingiber officinale*, elevated CO_2 resulted in increase in Flavonoid and Phenolic content (Ghasemzadeh et al. 2010). It was observed that with elevated CO_2, *Quercus ilicifolia* showed increase in tannins and phenolic content (Stiling and Cornelissen 2007). Ibrahim and Jaafar (2012) subjected *Eleais guineensis* (Oil Palm) to elevated CO_2 levels (400–1200 μmol mol^{-1}). In this study, the authors observed increase in flavonoids and phenolic contents attributed it to increase in primary metabolite phenylalanine a metabolic precursor for most of the secondary metabolites. Further on identical lines, Ibrahim et al. (2014) working on *Labisia pumila* showed that there was an increase in flavonoids and phenolics in response to increased artificial atmospheric CO_2 levels. These findings were more inclined towards increase in levels of secondary metabolites as a response to elevated CO_2 as compared to present atmospheric CO_2 concentration. But in a study carried out on *Pseudotsuga manziesii*, it was seen that the level of terpenes specifically monoterpenes decreased significantly (Snow et al. 2003).

Similar studies performed in vitro will play a crucial role in assessing the effect of CO_2 in in vivo conditions. A typical in vitro study has focused on *Panax ginseng* suspension culture of roots, a plant that frequently featured in prescriptions of traditional Chinese, Japanese and Korean medicine for cancer, immunomodulation and other stress related ailments (Wang et al. 2007; Chang et al. 2003). Elevated CO_2 levels in this suspension culture showed increase in phenolic and flavonoid contents (Ali et al. 2005). Thus, such findings are very essential to correlate the effects of CO_2 on in vivo studies with that of in vitro.

By reviewing the overall trend in such findings, it is very essential to focus on entire secondary metabolome of medicinal plants, beside evaluating the effects with respect to time duration, seasonal variation, temperature, nutrient availability etc. since other parameters either singly or in combination are going to play a significant role in altering the metabolic plasticity of medicinal plants. Looking at the paramount productive threshold of such metabolic alterations, appropriate conservatory practices are needed before these plants lose their bioactive components in the long run.

Effects of Ozone on Plant Secondary Metabolites

Ozone is widely known for its bioprotective activity against ultra violet radiations. However, its surface concentration i.e., ground level O_3 is increasing due to the rise in O_3 precursor emission in many pollution prone areas. Ground-level ozone pollution is already decreasing global crop yields (from 2.2–5.5% for maize to 3.9–15% and 8.5–14% for wheat and soybean, respectively), to differing extents depending on genotype and environmental conditions. These ill effects are also seen on medicinal plants. However, due to very limited focus on O_3 related effects on medicinal plants and their PSMs content it has become mandatory to evaluate its potential consequences.

In a study done on *Melissa officinalis*, a traditional medicinal plant used for treatment in dementia, anxiety and central nervous system (CNS) related disorders with elevated ozone concentrations showed that the total anthocyanins increased to a substantial extent along with phenolics and tannins (Pellegrini et al. 2011; Shakeri et al. 2016). A group of scientists from Brazil conducted an experiment to check effect of chronic ozone exposure on *Capsicum baccatum*. It was found that pericarp of ozone exposed plants showed 50% decrease in capsaicin and dihydrocapsaicin also the seeds showed significant reduction in capsaicin but no change in dihydrocapsaicin as compared to the control plants. Additionally, total carotenoid and phenolic content in the pericarp increased by 52.8 and 17% respectively (Bortolin et al. 2016). A similar study on Ecophysiological and antioxidant traits of *Salvia officinalis* under ozone stress (120 ± 13 ppb for 90 consecutive days) showed an increase in phenolic content; notably in Gallic acid (2-fold increase), Catechinic acid (increase was observed once in the total fumigation period of 90 days), Caffeic acid (8-fold increase) and Rosmarinic acid (122% increase on 60th day of treatment) (Pellegrini et al. 2015). Another experiment on *Betula pendula* with elevated O_3 displayed an increase in hyperoside a flavonoid, with decreased papyriferic acid a triterpenoid and dehydrosalidroside hyperoside, betuloside belonging to phenolics (Lavola et al. 1994). In a similar O_3 elevation experiment on *Pinus taeda* L, unveiled an increasing in condensed tannins without any rise in total concentration of phenols indicating that the O_3 related increase in foliar tannins was due to change in allocation within the phenolic group rather than to increase in total phenolics (Jordan et al. 1991). Albeit O_3 related effects are known on edible crops, similar effects are yet to be diagnosed on medicinal plants on a wide scale and plan proper conservatory policies/practices.

Effect of ozone as an indicator of secondary metabolites alterations in *in vitro* conditions has also studied in recent past. A study on *Pueraria thomsnii* suspension cultures showed an increase in puerarin production by cells treated with ozone, the increase was prominent 20 h after the treatment (Sun et al. 2012). The highest puerarin production was seen about 35 h after ozone treatment, which was 2.6-fold of the control. This outcome indicates that exposure to ozone might be a potential tool to improve puerarin production of *P. thomsnii* cells. Along with puerarin, O_3 exposure also indicated an increase in levels of ABA which was much higher than that of the control cells. The highest ABA production was observed at about 15 h after ozone treatment, which was about 11 times that of the control (Sun et al. 2012).

On identical lines, a study was carried out about ozone exposure on *Hypericum perforatum* cell suspension culture by Xu et al. (2011). In this experiment 6-day old cell culture were exposed to 30–180 nL L^{-1} ozone for 0–6 h. It was observed that cell suspension (5-day old) treated with 90 nL L^{-1} ozone dose showed maximum hypericin production (harvested on 21st day). Also, hypericin produced was maximum when cells were exposed 15th day of the suspension culture and harvested on 21st day. The ozone exposure time was optimized to be 3 h for highest hypericin production keeping the above parameters unchanged (Xu et al. 2011). Various secondary metabolites with altered production under the influence of higher CO_2 and O_3 levels are given in Fig. 2.

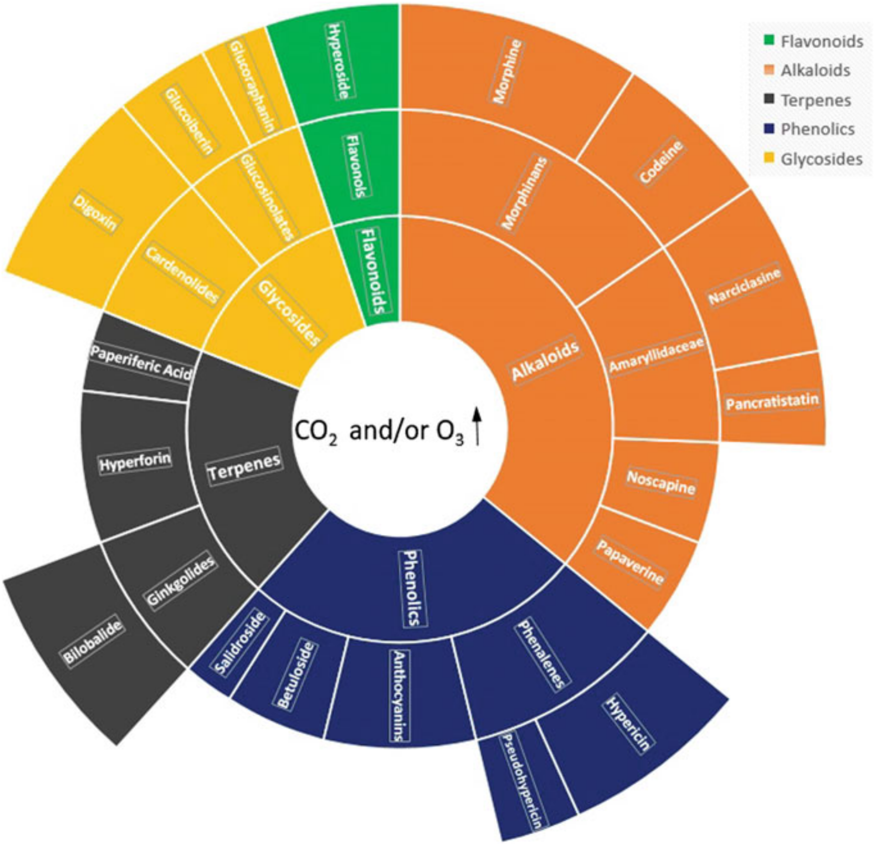

Fig. 2 Secondary metabolites altered in selected medicinal plants (covered in the text portion) in response to elevated Carbon dioxide (CO_2) and Ozone (O_3) levels

Effects of Toxic Gases on Plant Secondary Metabolites

Sulfur dioxide is one of the major air pollutants having ability to get enter in the plant system via roots as well as via stomatal opening by means of photosynthesis and respiration. Depending on the type of the plant and different environmental factors, differential responses of the plants against SO_2 exposure have been observed. Some responses include damage to the photosystem (Swanepoel et al. 2007), changes in the stomatal density and perturbations in efficiency of carbon fixation (Chung et al. 2011; Haworth et al. 2012; Silva et al. 2015). The atmospheric SO_2 along with H_2S also acts as sulfur source which can be up-taken through stomata of the plants apart from the sulfate uptake from the roots. Owing to the importance of the sulfur in many important pathways, this stomatal uptake influences the metabolic profile of the plant. Glucosinolates are sulfur containing secondary metabolites which plays significant role in sulfur storage which helps in re-distribution of sulfur during sulfur deprived condition (Falk et al. 2007). Two members from genus *Brassica* have been exposed to the 0.25 $\mu l\ l^{-1}$ of SO_2 for seven days to investigate the deviations in the glucosinolate content (Aghajanzadeh et al. 2015). The glucosinolate content showed negligible change in the shoot under sulfur deprived as well as sufficient conditions. But under sulfur deprivation environment, when foliarly absorbed sulfur was the only source of sulfur, glucosinolate content in root; notably some fraction of indolic glucosinolates showed reduction. Sulfur plays crucial role in the grapes and wine industry which is applied in several chemical forms. So, the overall plant profile including secondary metabolism alters notably. The SO_2 induced re-programming is observed in grape berry transcriptome allied with biotic defense responses as well as oxidative signaling. The SO_2 induced fumigation showed altered anthocyanin synthesis although the minor abundance of flavon-3-ol transcript after fumigation with SO_2 indicates no rapid degradation of anthocyanin (Giraud et al. 2012).

With respect to the various approaches to supply sulfur to the plants, there is keen interest in understanding the effect of one more sulfur containing gas; hydrogen sulfide (H_2S). The high dosage of H_2S is proved to be responsible for defoliation, leaf lesions, decreased growth rate, and tissue death in some plants (Montesinos-Pereira et al. 2016). But contrastingly, H_2S have also been reported to act as fundamental molecule produced by plants which works to control plant functioning (Zhang et al. 2010). This is also a signaling molecule which is proved to promote the antioxidant activities in many plants against abiotic stresses. The application of H_2S alleviated the antioxidant potential and quality in some plants as well (Montesinos-Pereira et al. 2016). The Bronco cabbage (*Brassica oleracea*) was applied with the incrementing levels of sodium hydrosulfide (as H_2S donor) to check the physiological and antioxidative changes. It was reported that the lower levels of treatment showed increased contents of carotenoids, anthocyanins, flavonols, total phenolics and sinigrin (Montesinos-Pereira et al. 2016). Hydrogen sulfide has been also reported to mediate nicotine biosynthesis in *Nicotiana tabacum* when the growth of plants is induced under high temperature (Chen et al. 2016).

Effects of Heavy Metal Wastes on Plant Secondary Metabolites

Toxic heavy metals such as cadmium (Cd), chromium (Cr), Nickel (Ni), Arsenic (Ar) etc. have been severely incorporated in the environment via variable sources including industrial effluent, fertilizers, pesticides and metal smelters. In soil they are present as free metal ions, metal complexes in soluble form, exchangeable metal ions, and insoluble or precipitated oxides, carbonates, hydroxides or they may also form a part of structural silicates (Rai et al. 2004). Plants exposed to heavy metal contaminated environment tend to change the secondary metabolite profile. This interaction may lead to either suppression or stimulation of the secondary bioactive compounds. The heavy metal exposure is a cause of induction of oxidative stress triggering formation of highly active signaling molecules which further helps in production of secondary metabolites which affects the medicinal potency of the plant (Nasim and Dhir 2010).

Chromium (Cr) is a carcinogenic heavy metal which is released in the environment via carpet, textile, leather tanning or electroplating industry. *Ocimum tenuiflorum* L. from the family Lamiaceae was cultivated in Hoagland solution (5%) containing variable concentrations of Cr(IV) (0, 10, 20, 50, 100 μM) to analyze the Euginol content, a major component of *Ocimum* oil. Significant increment in eugenol content up to 100 μM in comparison to control was observed. Approximately 25% increase was observed in eugenol content when plants were exposed to 20 μM chromium for 72 h. Effect of chromium on two therapeutically important secondary metabolites phyllanthin and hypophyllanthin from *Phyllanthus amarus* was studied by Rai and Mehrotra (2008). Increment in production of both the secondary metabolites was observed under increasing concentrations (20, 50, 100 μM) without much increase in the accumulation of chromium in leaves. Cadmium (Cd) is another non-essential, toxic heavy metal which is widespread in the environment. The constant increase in the cadmium levels is observed in the soil throughout the world. The phyllanthin and hypophyllanthin levels were analyzed in *Phyllanthus amarus* under various concentrations of cadmium. The quantity of both the secondary metabolites showed increment up to 50 ppm of cadmium treatment which reduced further at 100 ppm of cadmium (Rai et al. 2005). In another experiment, cadmium treatment was proved to improve the biosynthesis of artemisinic acid, arteannuin B and artemisinin in medicinal plant *Artemisia annua* L. (Zhou et al. 2016). Nickel (Ni) is a heavy metal which is present in industrially contaminated as well as pristine soils. A medicinal plant, St. John's wort (*Hypericum perforatum* L.) was screened for the effect of nickel on its secondary metabolite profile by Murch et al. (2002). The plants showed 15–20 fold reduction in amount of pseudohypericin and hypericin, whereas ability of plants to synthesize or amass hyperforin was completely vanished. Another heavy metal Arsenic (As) which enters the environment via weathering, biological activities as well as volcanic eruptions is also a component of pesticides/chemical fertilizers and

Table 1 Effects of ozone and carbon dioxide on plant secondary metabolite production

Component	Treatment	Medicinal Plant	Affected secondary metabolites	References
O_3	Elevated O_3	*Capsicum baccatum*	Capsaicin↓, Dihydrocapsaicin↓, Carotenids↑, Phenolics↑	Bortolin et al. (2016)
O_3	Elevated O_3	*Salvia officinalis*	Gallic acid↑, Catechinic acid↑, Caffeic acid↑, Rosmarinic acid↑	Pellegrini et al. (2015)
CO_2	Elevated CO_2 and Light intensity	*Labisia pumila*	Flavonoids↑, Phenolics↑	Ibrahim et al. (2014)
CO_2	Elevated CO_2	*Catharanthus roseus*	Phenolics↑, Flavonoids↑, Tannins↑, Alkaloids↑	Saravanan and Karthi (2014), Singh and Agarwal (2015)
O_3	Elevated O_3	*Melissa officinalis*	Phenolics↑, Anthocyanins↑, Tannins↑	Tonelli et al. (2015), Pellegrini et al. (2011)
O_3	Elevated O_3	*Pueraria thomsnii*	Puerarin↑, ABA↑	Sun et al. (2011)
O_3	Elevated O_3	*Hypericum perforatum*	Hypericin↑	Xu et al. (2011)
CO_2	Elevated CO_2	*Zingiber officinale*	Flavonoids↑, Phenolics↑	Ghasemzadeh et al. (2010, 2011)
O_3 and CO_2	Elevated O_3 and CO_2	*Ginkgo biloba*	Tannins↓, Quercetinaglycon↑, Keampferolaglycon↓, Isorhamnetin↓, Bilobalide↓	Huang et al. (2010), He et al. (2009)
CO_2	Elevated CO_2	*Papaver setigerum*	Morphine↑, Codeine↑, Papaverine↑ and Noscapine↑	Ziska et al. (2008)
CO_2	Elevated CO_2	*Quercus ilicifolia*	Tannins↑, Phenolics↑	Stiling and Cornelissen (2007)
CO_2	Elevated CO_2	*Brassica oleracea* var. italica Plenck	Methylsulfinylalkyl glucosinolates glucoraphanin↑, Glucoiberin↑	Schonhof et al. (2007)
CO_2	Elevated CO_2	*Hypericum perforatum L.*	Hypericin↑, Pseudohypericin↑, Hyperforin↑	Mosaleeyanon et al. (2005), Zobayed and Saxena (2004)
CO_2	Elevated CO_2	*Panax ginseng* C. A. Mayer	Phenolics↑, Flavonoids↑	Ali et al. (2005)

(continued)

Table 1 (continued)

Component	Treatment	Medicinal Plant	Affected secondary metabolites	References
CO_2	Elevated CO_2	*Pseudotsuga manziesii*	Monoterpenes↓	Snow et al. (2003)
CO_2	Elevated CO_2	*Hymenocallis littoralis* (Bulbs)	Pancratistatin↑, 7-deoxynarciclasine↑, 7-deoxy-trans dihydronarciclasin↑	Idso et al. (2000)
O_3	Elevated O_3	*Betula pendula*	Dehydrosalidroside hyperoside↓, Betuloside↓, Platyfylloside↓, Salidroside↓, papyriferic acid↑, hyperoside↑	Saleem et al. (2001), Lavola et al. (1994)
O_3	Elevated O_3	*Loblolly pine*	Tannins↑	Jordan et al. (1991)
CO_2	Elevated CO_2	*Digitalis lanata*	Digoxin↑, Cardenolide↑	Stuhlfauth and Fock (1990), Stuhlfauth et al. (1987)

residues from mining (Cao et al. 2009). A traditional Chinese medicinal plant *Sculellaria baicalensis* Georgi was screened accumulation and uptake of arsenic. The experiment concluded that the concentration of five flavone compounds were nor expressively by lower arsenic concentration. But the higher concentration of arsenic showed inhibition of baicalin and wogoninside formation whereas generation of baicalein, wogonin and oroxylin A was accelerated (Cao et al. 2009). Chamomile plant (*Matricaria chamomilla*) was grown in nutrient solution containing copper (Cu) (3, 60, 120 μM) for ten days (Kováčik et al. 2008). In methanolic extracts total eleven secondary active compounds were examined (protocatechuic, *p*-hydroxybenzoic, vanillic, chlorogenic, salicylic acid, gentisic, syringic, caffeic, sinapic and *o*-/*p*-coumaric acid). The detected compounds showed increment at 60 μM copper treatment whereas concentrations of the same were either lower or showed no change compared to the control at 120 μM (Kováčik et al. 2008) (Table 1).

Nanoparticle Wastes and Plant Secondary Metabolites

The synthesis of numerous types of nanoparticles has gained unprecedented attention in recent years, due to their vast array of applications (Mapara et al. 2015). Nanoparticles are the tiny entities ranging from the size 1 to 100 nm, formed with metal or metal oxides as a base. The waste materials out from industries, medical products and agriculture are emerging as sources for increasing the nano-waste amount in the environment. As plants are immobile with two foremost sinks of the environment, water and soil; they cannot escape the severe effects and successive metabolism changes due to nano-pollution (Marslin et al. 2017). The induction of reactive oxygen species in plants due to the interaction with nanoparticles alters the secondary metabolism. Increment in important secondary metabolite artemisinin was observed in the hairy root cultures of *Artemisia annua* treated with 900 mg L^{-1} of silver (Ag) nanoparticles for 20 days. The increase in amount (~3.9 folds) can be correlated with signalling molecule production (hydrogen peroxide), lipid peroxidation levels and catalase activity (Zhang et al. 2013). Silver nanoparticles also showed positive growth in anthocyanin and flavonoid synthesis in *Arabidopsis* as the expression level for the genes responsible for their synthesis showed up-regulation (Garcia-Sanchez et al. 2015). Improvement in content of a steroidal saponin, diosgenin in fenugreek (*Trigonella foenum-graecum*) was observed under the influence of silver nanoparticles (2 μg kg^{-1}) (Jasim et al. 2017). In barley plant, treatment with cadmium oxide nanoparticles was proved to be responsible for increment in ferulic acid and isovitexin. The concentration of cadmium oxide nanoparticles in air was approximately 2×10^5 particles cm^{-3}. On the similar line, callus tissue of *Prunella vulgaris*, a plant with important antiviral properties was cultivated in medium fortified with naphthalene acetic acid (NAA) along with gold (Au) and/or silver (Ag) nanoparticles (Fazal et al. 2016). Authors recorded maximum accumulation of phenolics and flavonoids along with the enhanced callus induction (Fazal et al. 2016). A generalized scheme is presented in Fig. 3 to

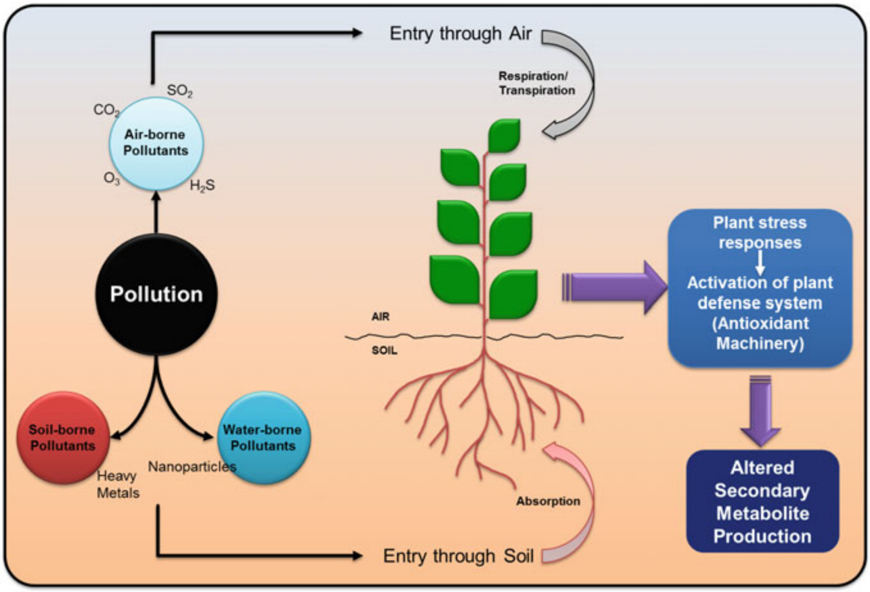

Fig. 3 Toxic gases (SO_2, H_2S), Ozone (O_3), Carbon dioxide (CO_2) and waste (Heavy metal waste, Nano-waste) mediated alteration in plant secondary metabolism

summarize the effects of toxic gases, O_3, CO_2 and wastes (heavy metal wastes and nano-wastes) on plant secondary metabolism.

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Plant Secondary Metabolites and Some Plant Growth Regulators Elicited by UV Irradiation, Light And/Or Shade

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Abstract Classification of plant secondary metabolites and characterization of major plant growth regulators are shortly described. A short account is also given to light, shade and ultraviolet radiation and their impact on plants. Recent investigations regarding secondary metabolite production and alterations in endogenous level of plant growth regulators in medicinal plants grown under light, shade or UV radiation are reviewed and discussed. Some conclusions and future perspectives to enlarge the investigations in this direction are also given.

Keywords Light · Plant growth regulators · Plant secondary metabolites · Shade · UV-irradiation

Abbreviations

ABA	Abscisic acid
CK	Cytokinin
FR	Far red light
GA	Gibberellic acid
IAA	Indole-3-yl acetic acid
PAR	Photosynthetically active radiation
R	Red light
ROS	Reactive oxygen species
SAR	Shade avoidance response
UV	Ultraviolet radiation

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Light and Shade—Impact on Plants

Photosynthetically active radiation (PAR, 400–700 nm) is part of the visible light (380–760 nm). The predominant wavelengths sensed by plant photoreceptors and pigments corresponds to blue (400–500 nm) and red (R, 600–700 nm) and, to a lesser extent, green (500–600 nm) light. Due to phytochromes, plants perceive a small fraction of near-infrared radiation or far-red (FR) light, with a sensitivity peak at 730 nm, which is important for plant development (reviewed by Huché-Thélier et al. 2016).

Daylight includes nearly equal proportions of R and FR light but their ratio is lowered under the canopy as photosynthetic pigments absorb red light (Ruberti et al. 2012). Plants shaded within a canopy will have reduced R/FR and blue/green ratios, sensed by phytochromes and cryptochromes, respectively. FR photons are not effective to induce photosynthesis, so low R/FR ratio may quickly provoke changes in gene expression and physiological responses, controlling phenotypic plasticity and allowing plants to compete better with surrounding plants (Keuskamp et al. 2010). Foliar shade or reduced PAR can be perceived by cryptochrome and phytochrome photoreceptors due to reduced intensity of blue and R/FR light, respectively. In addition, shade affects environmental conditions as ground and air temperature, humidity, and soil chemistry (Song et al. 2012).

In general, plants developed two opposite strategies in response to competition for light: *shade avoidance* and *shade tolerance* (Ruberti et al. 2012).

Flowering plants sense the reduction in R/FR ratio due to phytochrome photoreceptors which is an early signal of neighbor proximity and activate complex of developmental responses like elongated stems, petioles, hypocotyls and internodes, smaller leaves/reduction in leaf development/, apical dominance, suppression of branching and early flowering (Keuskamp et al. 2010; Ruberti et al. 2012; Gommers et al. 2013; Park and Runkle 2017). Stimulation of elongation growth is the most impressive of shade avoidance responses (SAR). The response is rapid and reversible when returning plants to light with high R/FR ratio.

In contrast to shade avoidance, *shade tolerant plants* (shade-tolerance response) inhibit shade avoidance characteristics and increase leaf expansion causing interception of more radiation and simultaneous chlorophyll reorganization in order to improve photosynthetic efficiency (Gommers et al. 2013; Park and Runkle 2017).

UV Radiation—Classification and Impact on Plants

According to the International Commission on Illumination, Ultraviolet (UV) wavelength (400–200 nm) is a small part of the solar radiation reaching the Earth's surface, which is divided into UV-A (315–400 nm), UV-B (280–315 nm) and UV-C (200–280 nm), and it affects negatively all living organisms. The harmful effect of UV radiation increases towards the shorter wavelengths. UV-C quickly provokes high levels of injuries because of its highest energy, but due to its absorption by the atmosphere UV-C radiation does not reach the Earth's surface (Häder et al. 2007). UV-B radiation is absorbed by the stratospheric ozone and only small part of it reaches the Earth's surface. Plants sense UV-B radiation with UVR8 photoreceptor but UV-A could be sensed by phototropins and cryptochromes (reviewed by Verdaguer et al. 2017). In addition, UV penetration via plant tissues decreases as wavelength decreases, so UV-B radiation cannot reach such deeper target sites in the leaves as UV-A can. Due to the measures taken through the Montreal Protocol regulating atmosphere pollution with ozone depleting substances, ozone layer started to build up again and the levels of UV-B irradiation on the Earth's surface tend to decrease (Björn 2015). Along with the stratospheric ozone amount, the spectral irradiance of the environmental UV depends on a number of other factors like the angle at which the solar radiation reaches the Earth's atmosphere, i.e., the "solar zenith angle" (including time of day, season and latitude), altitude, clouds, surface reflection, etc. (Barnes et al. 2015). Since there is no selective absorber for the long-wave UV-A radiation, its intensity is much higher than UV-B but it is not so biologically relevant (Vass et al. 2005).

High doses of UV-B and UV-C radiation affect negatively growth, development, photosynthesis, and other important processes in plants; cause overproduction of reactive oxygen species (ROS) and development of oxidative stress; can decrease cell viability and lead to cell death (Barnes et al. 2015). However, low UV-B or UV-C doses may trigger acclimation responses in plants, including induced biosynthesis of secondary metabolites (Kreft et al. 2002; Jansen et al. 2008; Rai et al. 2011; Nadeau et al. 2012; Barnes et al. 2015).

Secondary Metabolites

Secondary metabolites are organic compounds naturally occurring in plants, which derive through methylation, hydroxylation, glycosylation or other biochemical reactions from primary metabolites (carbohydrates, proteins, amino acids, lipids) (Korkina 2007). Secondary metabolites could be classified into several categories according to various features—for example based on their chemical structure, biosynthetic pathway or their solubility in different solvents, etc. Major popular classification is related to the presence or absence of nitrogen in their chemical structure (Gershenzon 2002). Thus secondary metabolites form two major groups: (1) nitrogen containing—alkaloids, non-protein amino acids, amines, cyanogenic glycosides, and glucosinolates; and (2) nitrogen not containing—terpenes (mono-, sesqui-, di-, tri-, tetraterpenes, steroids, saponins), phenolics (phenolic acids and phenylpropanoids), polyketides and polyacetylenes (Fig. 1).

Secondary metabolites are involved in variety of plant physiological and developmental processes like: formation of root nodules; germination of pollens, pigmentation of leaves and petals; contribution in stress responses as signaling molecules or scavengers of free radicals, which are formed after UV irradiation and

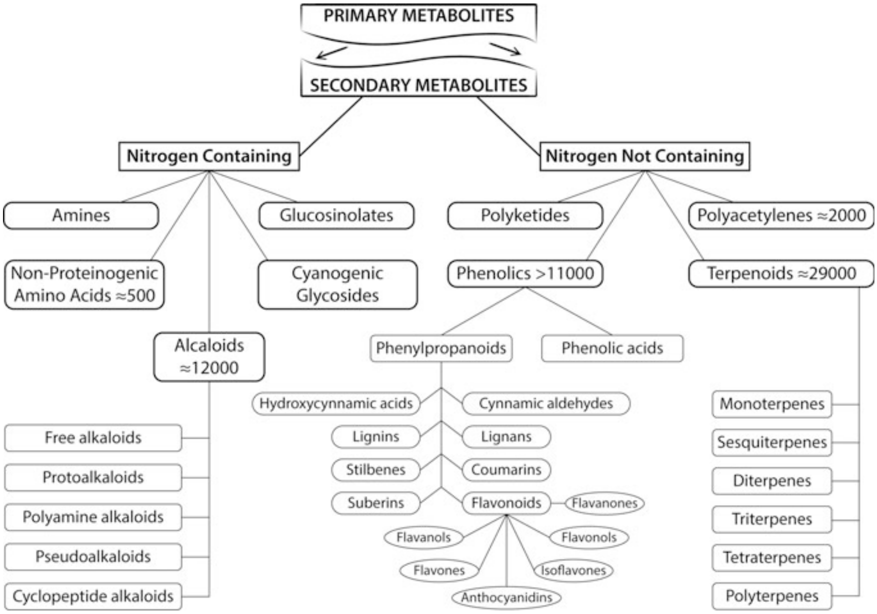

Fig. 1 Major secondary metabolites subgroups

invasion of pathogens (Gershenzon 2002; Edreva 2005; Korkina 2007; Edreva et al. 2007, 2008; Buer et al. 2010; Samanta et al. 2011). Plant secondary metabolites possess biological activities, which are important for human life and health and therefore are used in traditional and modern medicine, food industry, perfumery and cosmetics (Havsteen 2002; Korkina 2007; Dinkova-Kostova 2008; Jansen et al. 2008; Zhang and Björn 2009; Caputi and Aprea 2011; Wijesinghe and Jeon 2011). Many alkaloids are neurotransmitters, which are used in medicine as antiarrhythmic, antihypertensive, anesthetic, analgesic, antipyretic compounds. Terpenes, terpenoids, plant-derived phenylpropanoids (especially flavonoids) and their derivatives are among the most common biologically active components in food, wines, beer, spices, aromas, fragrances, and essential oils. Taking in account their protective properties, secondary metabolites are of great medicinal interest, especially as free radical scavengers and antioxidants, anticancer, anti-virus, antibacterial, antiprotozoal, anti-inflammatory agents and UV screeners.

Plant Growth Regulators and Phytohormones

Endogenous plant growth regulators are diverse group of organic compounds, which are synthesized within plant cells. Plant growth regulators function as endogenous stimuli modulating plant responses at molecular and physiological level. Phytohormones as part of the natural plant growth regulators are found in small quantities in plant cells, playing signaling role and mediating plant responses to normal or stressful conditions. Plant hormones act at their site of synthesis or in other plant organs via long distance transport. Some major plant growth regulators are shown on Fig. 2.

Auxins

Auxins are phytohormones consisting of indole or phenyl ring and a carboxylic group. The naturally occurring auxins in plants include indole-3-yl acetic acid, 4-chloroindole-3-acetic acid, phenylacetic acid, indole-3-butyric acid, and indole-3-propionic acid. They positively influence cell division and enlargement, bud formation, root initiation, growth of stems, roots, and fruits, participate in other physiological processes as phototropism, geotropism and hydrotropism. Auxins also contribute in plant adaptive stress responses to different stresses (Kazan 2013), including UV irradiation (Vanhaelewyn et al. 2016).

Cytokinins

Cytokinins are plant hormones that promote cell division, growth and differentiation, control apical dominance, axillary bud growth, retard leaf senescence, and along with auxins they control almost all aspects of plant growth. Naturally occurring cytokinins are structural analogues of adenine, bearing an isoprenoid side chain at N^6 position. Cytokinins are found in plants as free bases, ribosides and ribotides, and the most active among them are *trans*-Zeatin and N^6-Isopentenyladenine. There are also a number of highly active synthetic compounds such as Kinetin and N^6-Benzyladenine. O'Brien and Benkova (2013) have reviewed cytokinin involvement in plant stress responses.

Gibberellins

Gibberellins are tetracyclic diterpenoid carboxylic acids. They can be divided in two classes based on the presence of either 19 or 20 carbons. More than 125 gibberellins are known but only a few of 19-carbon gibberellins like GA_1, GA_3, GA_4 and GA_7 have important biological activity and act as phytohormones in higher plants. Gibberellins render positive effects on a number of developmental processes such as seed germination, leaf expansion, stem elongation, flower initiation, flower and fruit development, leaf and fruit senescence. Although limited information exists in relation to UV-induced alteration of gibberellins (Hectors et al.

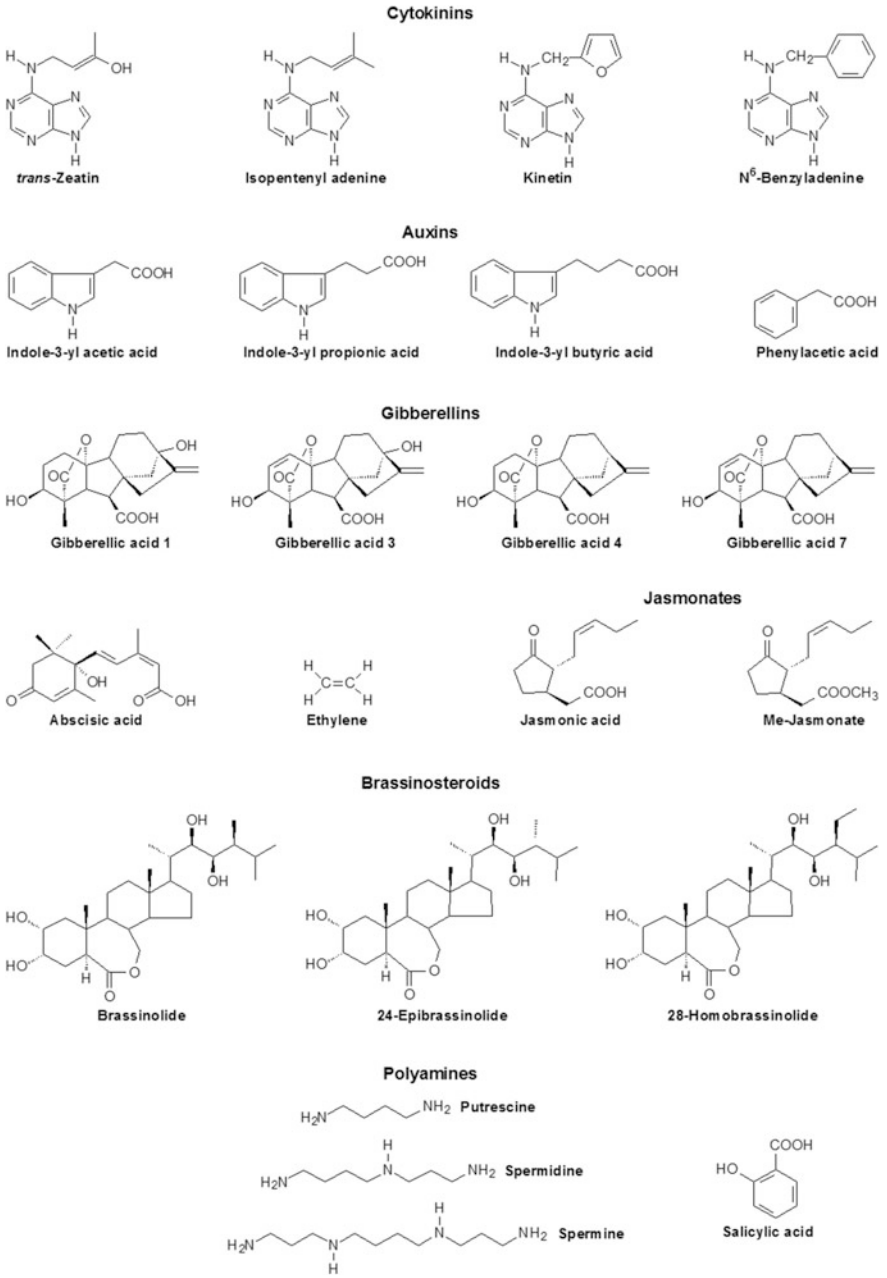

Fig. 2 Phytohormones and plant growth regulators

2007), there is evidence for their roles in plant abiotic stress response and adaptation (Wani et al. 2016).

Abscisic Acid

Abscisic acid is an isoprenoid plant hormone, which controls important physiological processes like seed dormancy, stomata movements, embryo morphogenesis, and synthesis of storage proteins and lipids. ABA contributes to a number of stress adaptive responses such as drought (Wani et al. 2016) and UV radiation (Tossi et al. 2012).

Ethylene

Ethylene is gaseous phytohormone that is induced during certain stages of plant growth and development such as seed germination, root hair and adventitious root growth induction, ripening of fruits, senescence of leaves and flowers, abscission of leaves, etc. Ethylene can also be induced by a variety of external stimuli such as mechanical wounding, diverse environmental stresses (Wani et al. 2016). Ethylene and/or its biosynthetic precursor 1-aminocyclopropane-1carboxylic acid accumulate after UV irradiance (Katerova et al. 2009; Pan et al. 2014).

Brassinosteroids

Brassinosteroids are a new class of plant hormones that regulate multiple physiological and developmental processes such as cell division, elongation and expansion, vascular differentiation, pollen tube growth, seed germination, reproductive development, and confer resistance of plants against various abiotic stresses (Wani et al. 2016). The most bioactive brassinosteroinds are brassinolide, 28-homobrassinolide, and 24-epibrassinolide.

Jasmonates

Jasmonates are involved in physiological processes such as growth inhibition, flower development, leaf senescence and abscission. Jasmonates possess also signaling functions in direct or indirect plant responses to both abiotic and biotic stresses (Wani et al. 2016) including UV stress (reviewed by Vanhaelewyn et al. 2016).

Polyamines

The tetraamine spermine, triamine spermidine, and diamine putrescine are the major polyamines found among all plant species. They are aliphatic nitrogen-containing compounds. Polyamines contribute to a number of growth and developmental processes like cell division, growth, and differentiation; dormancy breaking of tubers and germination of seeds; morphogenesis; development of flower buds; fruit growth; cell, organ, and tissue senescence. Because of their polycationic nature, polyamines possess antioxidant and free radical scavenging properties and contribute to plant tolerance against different abiotic stresses, including UV-irradiation (Groppa and Benavides 2008; Gill and Tuteja 2010; Todorova et al. 2014).

Salicylic Acid

Salicylic acid is an endogenous growth regulator of phenolic nature, which participates in physiological processes as seed germination, vegetative growth and development, flowering, senescence, photosynthesis, transpiration, ion uptake and transport (Rivas-San Vicente and Plasencia 2011). Salicylic acid is involved mainly in induction of plant reactions against biotic stresses as a part of the signal transduction pathway leading to systemic acquired resistance. Evidence exists that salicylic acid plays an important role in UV stress responses (reviewed by Vanhaelewyn et al. 2016).

Impact of UV Radiation on the Synthesis of Secondary Metabolites in Medicinal Plants

Compared to plants grown under white light, an enhanced production of antioxidants like phenolics (geraniin and ellagic acid) and carotenoids were found in in vitro cultures of *Phyllanthus tenellus* grown under white light plus UV-A radiation (Victório et al. 2011). Additional UV-A radiation had negative effect on branching and number of shoots, which reduced proliferation rate, but the thickness of abaxial epidermis and palisade parenhima were increased as a protective reaction against UV-A. Bernal et al. (2015) reported a reduction in leaf concentration of specific quercitin and kaempferol derivatives in seedlings of *Laurus nobilis* grown under low levels of supplemental UV-A, as compared with plants grown under ambient UV light. The content of total phenolics and total flavonoid in sowthistle (*Ixeris dentata* Nakai) were significantly enhanced after 3d and 5d of continuous exposure to UV-A, compared with control, but plant growth was not inhibited (Lee et al. 2013). On the contrary, the rise in total phenolics and total flavonoid contents found after either continuous or gradual UV-B exposure of sowthistle was accompanied with growth inhibition. The expression of the majority genes involved in peppermint (*Mentha x piperita* L.) terpenoid biosynthesis were modulated by exposure to UV-B (7.1 kJm^{-2} day^{-1} UV_{BE}) radiation of plants grown in field and in a growth chamber, but it did not correlate with the amount of most essential oil compounds (Dolzhenko et al. 2010). Authors documented enhanced phenolic compounds like flavonoids eriocitrin, hesperidin and kaempferol 7-*O*-rutinoside in UV-B treated plants. The interaction between terpenoid and flavonoid production in response to UV-B was proven by the higher essential oils amount in growth chamber plants associated with lower total phenolic contents; and the decreased terpenoid concentrations in field grown peppermint connected with increased content of phenolic compounds. It was concluded that field grown plants were better adapted to increasing UV-B irradiation than peppermints in growth chamber due to enhanced flavonoid concentration. UV-B-induced accumulation of monoterpene *trans*-ocimene was shown in leaves (higher in mature than in developing leaves) of linalool-rich commercial variety of sweet basilicum lacking phenylpropanoids in its essential oil (Ioannidis et al. 2002). Wang et al. (2016) reported that UV-B exposure (40 min at 40 $\mu W\ cm^{-2}$) induced tanshinone accumulation in *Salvia miltiorrhiza* without inhibition of root growth. Tanshinones are known as major bioactive diterpenoids of *S. miltiorrhiza* roots used for the treatment of cardiocerebral diseases and authors recommended UV-B irradiation as an effective elicitor of tanshinone production. Other authors revealed that short term high intensity (3d, 1.13 Wm^{-2}) and long term low intensity (15d, 0.43 Wm^{-2}) UV-B irradiation induced nearly 1.5 fold higher glycyrrhizin (an oleanane-type triterpenoid saponin, natural sweetener possessing anti-tumor and anti-viral activities) production in roots of only 3 month old *Glycyrrhiza uralensis* than in control plants (Afreen et al. 2005). Using similar model system, Afreen et al. (2006), compared concentration of melatonin (N-acetyl-5-methoxytryptamine, an indole amine) in different tissues (seed, root, leaf and stem) of *G. uralensis* and noted the highest amount in root tissues, which increased with plant development. The highest melatonin concentration in roots was found after exposure to UV-B radiation, as compared with exposure to red, blue or white light, but it decreased under longer exposure period. The authors assumed that melatonin protects *G. uralensis* plant against UV-B triggered oxidative damage. In addition, Solhaug et al. (2003) showed that induction of melanin and parietin (an anthraquinone, possessing antifungal activity) pigments synthesis in *Lobaria pulmonaria* and *Xanthoria parietina* lichens required presence of UV-B light. Spitaler et al. (2006) evaluated the altitudinal variation of phytochemical diversity in flowering heads (mostly unaffected by seasonal variations in regard to secondary metabolite contents) from *Arnica montana*, grown between 590 and 2230 m (at 9 sites near Innsbruck, Austria). Increased ratio of flavonoids with vicinal free hydroxy groups in ring B of their chemical structure to flavonoids without this trait, and enhanced contents of caffeic acid derivatives (most notably 1-methoxyoxaloyl-3,5-dicaffeoylquinic acid) with elevation was reported. The authors demonstrated for the first time the induction of phenolics as major factor in ROS scavenging system in genetically homogenous populations grown along an altitudinal gradient and most probably linked with augmented UV-B light. In opposite, sesquiterpene lactones were not observed to correlate with altitude, which was explained with the fact they did not absorb UV-B radiation nor possess radical scavenging activity (Spitaler et al. 2006). It was reported that total hypericins content of the plants (defined as the sum of quinones protohypericin, hypericin, protopseudohypericin and pseudohypericin) also has the tendency to increase with altitude in four spontaneously growing *Hypericum* species collected during the flowering stage on the island of Crete (Xenophontos et al. 2008). The authors had proposed that the reason for the increasing levels of total hypericins with increasing altitude might be a complex of enhanced UV-B radiation accompanied by higher light intensities and lower air temperature. In another study concentration of hyperforin, pseudohypericin and hypericin was reported to alter after exposure to UV-B (single/daily/increasing daily dose) in *Hypericum perforatum* (St. John's wort) during the vegetative stage of plants (Brechner et al. 2011). Single UV-B exposure led to significant transient production of hyperforin (a terpenoid) which was enhanced to a concentration comparable to the beginning stages of flowering (when the active ingredient concentrations in control plants are highest). The authors stated that the information obtained might be valuable to optimize total product harvest for continuous production in controlled environments (Brechner et al. 2011). Hyperforin and hypericin are the main active ingredients which contribute to the antidepressant action of St. John's wort and hypericin and pseudohypericin possess anti-viral and anti-retroviral activity. Germ et al. (2010) reported UV-B induction of flavonoids and tannins production in leaves of St. John's wort. In opposite to Xenophontos et al. (2008), Germ et al. (2010) noted that hypericin concentration in leaves decreased in plants exposed to enhanced UV-B light and assumed that plants compete for the energy needed for synthesis of flavonoids, hypericin and tannins as the synthesis of UV-B absorbing compound is an energy-consuming process. Similarly, a higher yield of biomass, curcumin and curcuminoid content of rhizome was reported for turmeric grown under UV exclusion conditions compared with full sun (Ferreira et al. 2016). Other authors found that in tarbush (*Flourensia cernua* DC) UV restriction had positive effect on total volatile concentrations but did not affected total phenolics (Estell et al. 2016). In particular, concentrations of camphene, sabinene, β-pinene, borneol, bornyl acetate, Z-jasmone, α-terpinene, *cis*-chrysanthenol + pinocarvone, β-acoradienol, drima-7,9(11)-diene, ledol, flourensadiol were higher for plants grown under UV restricted than under control conditions. Sun et al. (2010) examined the effect of different radiation time applied by device with UV-B intensity of 82.90 $\mu W\ cm^{-2}$ on flavonoids concentration in freshly collected *Ginkgo biloba* leaves. It was reported that younger leaves and moderate irradiation time of 120 min UV-B significantly enhanced content of quercetin, kaempferol and isorhamnetin as well as total flavonoids concentration. The authors wished to present an innovation method enriching health-related compounds for food and pharmaceutical technology. Similarly, Ning et al. (2012) studied the effect of UV-B or UV-A radiation on secondary metabolites in freshly collected flower buds of medicinal plant *Lonicera japonica* Thunb. Four kinds of secoiridoid glycoside (secologanic acid, secoxyloganin, secologanin and (E)-

aldosecologanin) and three kinds of isochlorogenic acid (3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid and 4,5-di-*O*-caffeoylquinic acid) were reported to rise after UV application. Iridoids or iridoid-rich plants have been noted to possess wide range of biological activities like anti-arthritic, anti-inflammatory, antibacterial, antifungal, anticancer, anticoagulant, antioxidant, antivirus, antispasmodic, immunomodulatory, wound-healing and neuroprotective activities. In addition, antioxidant power measured by DPPH assay showed that methanol extracts of flower buds was higher in UV-B treated flower buds than in UV-A treated.

Study on *C. roseus* showed that UV-B exposure inhibited growth and caused oxidative damages but induced alkaloids vinblastine, vindoline, and catharanthine, considered to act as UV-B protectants (Guo et al. 2014). Interestingly, simultaneous nitrate nutrition alleviates negative effect of UV-B exposure and increased substantially amounts of three alkaloids studied. Peebles et al. (2009) examined the role of the endogenous production of jasmonic acid via octadecanoid pathway in the production of terpenoid indole alkaloids in *C. roseus* hairy roots using octadecanoid pathway inhibitors. The results obtained led the authors to assume that the octadecanoid pathway does not actively control generation of terpenoid indole alkaloids under normal or UV-B stress conditions in *C. roseus*. Leaf concentration of another monoterpene indole alkaloid brachycerine, possessing antioxidant and antimutagenic activities was also noted to enhance significantly in UV-B-treated cuttings of *Psychotria brachyceras* Müll. Arg. (do Nascimento et al. 2012). The results obtained led the authors to suppose that brachycerine probably participate in acute UV-B responses and at least partially its accumulation might be regulated at transcriptional level. In other study (Kreft et al. 2002), rutin and tannin concentrations were reported to reduce in the following order: under ambient > UV-B enhanced (simulating 17% O_3 depletion) > UV-B depleted (by Mylar foil) conditions in field grown *Fagopyrum esculentum* Moench (buckwheat). The highest amounts of these compounds were determined in flowers, followed by leaves and stems. Concentration of flavonol conjugates and betacyanins in halophyte *Mesembryanthemum crystallinum* was also reported to increase even when the plant was exposed to very low wavelength UV-B (like 280 or 295 nm) and the amount of feruloylglucose (the precursor of flavonol conjugates and acylated betacyanins) was much higher in leaves than in leaf tips (Ibdah et al. 2002). The authors reported that accumulation of flavonols and betacyanins could be illustrated by a weakly sigmoid dose function along with an exponential reduction of the response function of the plant with increasing wavelength. Other authors observed that three closely related species of the sub-Arctic dwarf shrubs (*Vaccinium myrtillus* L., *Vaccinium vitis-idaea* L., and *Vaccinium uliginosum* L.) grown outdoors showed different strategies in UV-B response concerning the content and distribution of UV-absorbing phenolic compounds in leaves (Semerdjieva et al. 2003). Methanol-extractable UV-B absorbing compound amounts were highest in *V. myrtillus*, increased with UV-B irradiation, distributed all over the leaf but concentrated in cells containing chlorophyll. The majority of phenolic compounds in *V. vitis-idaea* were cell-wall bound, concentrated in the walls of epidermis and their pool was enhanced with

UV-B dose. The authors assumed that the difference in strategies for UV screening found in those two plants could be connected with leaf longevity. The response of *V. uliginosum* was found to be flexible. This plasticity in response between the other two plants was explained with the fact that plant is deciduous as *V. myrtillus*, but possesses leaves with structural similarity to *V. vitis-idaea*. Kumari and Agrawal (2010) also applied supplemental UV-B radiation (1.8 and 3.6 kJ m^{-2} d^{-1} above ambient) on the aromatic perennial herb *Cymbopogon citratus* (D.C.) Staph in field conditions and reported that only higher dose inhibited biomass production. A reduction of chlorophyll content without significant alteration in photosynthesis, and induction of carotenoids and phenolic compounds was noted in UV-B treated plants. Authors demonstrated the positive outcome of the supplemental low dose of UV-B radiation on volatile oils production. In addition, dense waxy deposition was observed on the adaxial surface of leaves irradiated with lower UV-B dose. UV-B was reported to be necessary for the normal development of oil glands, in particular for the filling of glandular trichomes of sweet basil (Ioannidis et al. 2002). Lack of significant difference in wax content was shown for UV-B treated (5 and 10 kJm^{-2} d^{-1}) and control Stellaria plants, pertaining to "sun" and "shade" ecotypes (Sangtarash et al. 2009). In both ecotypes UV-B reduced growth and dry weight but increased carotenoids and flavonoids. Other authors irradiated white asparagus (*Asparagus officinalis* L. cv. Gijnlim) spears (apical and basal parts) with UV-B (0.54 or 1.08 kJ m^{-2}) and showed that concentration of flavonol quercetin-4′-*O*-monoglucoside increased with UV-B dose, which was accompanied with rise in activity of polyphenol-related enzymes (phenylalanine ammonia-lyase and peroxidase) (Eichholz et al. 2012).

Reifenrath and Müller (2007) reported increased relative contents of quercetin flavonols at the cost of kaempferols in *Sinapsis alba* L. leaves in UV-treated (short term UV-B plus UV-A) plants. Total flavonoid amounts in young leaves of *Nasturtium officinale* L. exposed to UV were also enhanced but revealed to be much lower when compared to *S. alba*, which was associated with the shady habitat. In both species hydroxycinnamic acid contents were unaffected, however, as important partners of the *Brassicaceae* defence system glucosinolates and myrosinases responded in species-specific manner to UV exposure. The authors concluded that compared to old leaves, young leaves (rich in nitrogen and soluble protein) were efficiently protected to UV light due to high flavonoid and glucosinolate amounts in *S. alba*, or enhanced flavonoid levels and myrosinase activities in *N. officinale* (Reifenrath and Müller 2007). Other authors explored UV-B elicitor mediated changes on secondary plant metabolites in *Vaccinium corymbosum* L. (highbush blueberries) after harvest by application of different doses (0.075 and 0.15 Wh m^{-2}) and adaptation times (2 and 24 h) (Eichholz et al. 2011). Both UV-B treatments enhanced the relative peak area of volatile secondary metabolites (C6-aldehydes, terpenes and ketones), responsible for the valuable flavor, stress response, environmental interaction (herbivore attack), antimicrobial and anticarcinogenic assets. The rise in volatile secondary metabolites was detected only after 2 h adaptation time but an opposite tendency was reported 22 h later. Expectedly, the degradation products of aldehydes, viz. alcoholic compounds exhibited opposite results. Total phenolic compounds, responsible for the antioxidative properties of bluberries, increased rapidly with UV-B intensity. Similarly, an increment of phenolic compounds (flavonols, anthocyanins, hydroxycinnamic and hydroxybenzoic acids) was shown in UV-B treated *Ribes nigrum* L. (black currant) irrespective of the adaptation time (Huyskens-Keil et al. 2007). Based on the obtained results the authors suggested that flavonols and phenolic acids have antioxidant protection activity on UV-B mediated tissue damage and concluded that anthocyanins absorbed UV radiation within a short time. Exposure of fresh mulberry leaves in vitro to UV-B light also induced production of two 2-aryl-benzofuran secondary metabolite compounds—chalcomoracin, possessing antibacterial activity, as well as accumulation of its precursor moracin N (Gu et al. 2010). Global metabolite profiling of UV-induced changes showed that phenylpropanoid-related metabolites shikimic acid, quinic acid and phenylalanine were markedly increased in lemon balm (*Melissa officinalis*) exposed for 2 h to UV-B irradiation as compared with control plants (Kim et al. 2012). *Kalanchoe pinnata* extracts had higher diversity of phenolic compounds and increased quantity of quercitrin after UV-B irradiation (Nascimento et al. 2015). Interestingly, Hoffmann et al. (2015) reported that pre-acclimation of UV-B stressed pepper (*Capsicum annuum*) plants with lower PAR or blue (30%) light intensity had poorer photosynthetic performance and lower amount of epidermal flavonols than those pre-acclimated under high PAR (300 umol m^{-2} s^{-1}) or blue (62%) light. It was concluded that high amount of blue light resulted in better acclimation and recovery of *C. annuum* plants to UV-B irradiation and induced accumulation of more epidermal flavonols. Low dose UV-B exposure (3 h, 2.8 Wm^{-2}) induced artemisinin (endoperoxide sesquiterpene lactone, an antimalarial drug) and flavonoid yield as well as concentrations of UV-B absorbing anthocyanins and phenolics for in vitro propagated *Artemisia annua* plantlets (Pandey and Pandey-Rai 2014). Higher artemisinin accumulation was shown to be a result of significant up-regulation of *HMGR*, *DXR*, *IPPi*, *FPS*, *ADS*, *CYP71AV1* and *RED1* gene transcripts. Authors proved that 3 h irradiation period is optimal for artemisinin yield and better plant tolerance to UV-B stress. It was suggested that short-term UV-B irradiation could be a safe way for in vitro grown *A. annua* to propagate high artemisinin producing plantlets. Low dose of UV-B radiation (1.44 $kJm^{-2}d^{-1}$) also induced artemisinin biosynthesis in *A. annua* seedlings (Pan et al. 2014). Compared to control, prolonged (14d) and short-term UV-B (4.2 kJ $m^{-2}d^{-1}$) and UV-C (5.7 kJ $m^{-2}d^{-1}$) pre-treatments on *A. annua* seedlings (before transplantation to field) augmented artemisinin and flavonoid contents at all developmental stages (Rai et al. 2011). Overexpression of genes associated with artemisinin/isoprenoid biosynthesis (*HMGR, CPR, ADC*) was noted as well. UV-B and UV-C pre-treatments increased carotenoid but decreased chlorophyll content. Despite the positive alteration in artemisinin and flavonoid contents, the morphological and physiological changes under UV-C pre-treatment revealed significant plant damage. Therefore, authors recommend short-term pre-treatment with UV-B but not UV-C in greenhouse before transplantation to field as a potential elicitor for sustained production of artemisinin by *A. annua.* Twenty-four hours UV-C irradiation was also effective in the reddening of yellow saffron thistle florets to yield carthamin which is applied as a colour additive for processed foods, in cosmetic and medicinal industry (Saito 2001). Concentrations of calycosin 7-*O*-β-glucoside, pseudobaptigenin and hesperidin were significantly increased by UV-C radiation (for 20d) as compared with white light in *Cyclopia subternata* (honeybush) callus but growth was not affected (Kokotkiewicz et al. 2014). Nadeau et al. (2012) studied the effect of hormetic UV-C dose on glucosinolates (secondary metabolites derived from amino acids, the precursors of bioactive compounds with anti-cancer properties like sulforaphane and indole-3-carbinol) in broccoli florets. It was reported that UV-C tended to enhance 4-methoxyglucobrassicin, 4-hydroxyglucobrassicin and glucoraphanin in broccoli florets. So they suggested that hormetic dose of UV-C had biochemical significance to enlarge potential health effect of broccoli in cancer prevention by increasing bioactive compounds.

Impact of UV Radiation on the Metabolism of Plant Growth Regulators in Medicinal Plants

UV-B treated (5, and 10 $kJm^{-2}\ d^{-1}$) and control Stellaria plants, pertaining to “sun” and “shade” ecotypes, showed lack of significant difference in ethylene production (Sangtarash et al. 2009). Seedlings of *Artemisia annua* L, irradiated with low dose of UV-B radiation (1.44 $kJ\ m^{-2}\ d^{-1}$) had elevated endogenous content of ABA, ROS and up-regulation of genes responding to stress, ROS generation, hormone (ethylene, ABA) stimulus and cell cycle control (Pan et al. 2014). In comparison with controls, prolonged UV-B exposure (35d, $8hd^{-1}$, 0.029 $Jm^{-2}\ s^{-1}$) of *Trichosanthes kirilowii* Maxim seedlings resulted in accumulation of ABA but reduction of IAA and gibberellic acid ($GA_{1/3}$) with time (Liu and Zhong 2009). Leaf zeatin ribozide content gradually increased as the treatment time continued but there was no clear difference with control plants. Compared to full sun, UV exclusion does not alter the content of endogenous spermidine concentration but increased concentration of the diamine putrescine and decreased spermine content in rhizome of turmeric (Ferreira et al. 2016). In addition, turmeric grown under full sun had some degree of UV morphogenic responses, compared with plants grown under UV exclusion which led the authors to the assumption that it was caused by inactivation or blockage of auxin transport. The content of free diamine putrescine increased in the leaves and especially in the roots of *Salvia officinalis* L. after 10 min irradiation with UV-B (Radyukina et al. 2010). UV-B irradiation induced also the accumulation of soluble conjugates of polyamines spermine and spermidine, which are probably involved in plant defense mechanism against UV-B stress.

Light and Shade—Impact on the Metabolism of Secondary Metabolites and Some Plant Growth Regulators

Using elaborate GC-MS analysis, Carvalho et al. (2016) tested the hypothesis that spectral quality may control vital sensory qualities of basil (*Ocimum basilicum* L.) herb. LED-based treatment of basil with blue/red/yellow or blue/red/green wavelengths induced production of monoterpenoids and reduced phenylpropanoids but blue/red/far red treatments increased the levels of most sesquiterpenoid volatile molecules. Compared to greenhouse conditions, blue/red/green wavelengths promoted higher amounts of eugenol, linalool and 1,8-cineole. This study shows that specific light irradiation with narrow-bandwidth light could influence important herbal traits like aroma in sweet basil. It was noted that due to the quick adjustment of plants to the new light environment, volatile profiles were maintained for about one week after treatment. Authors speculated that specific light programs might be scheduled to obtain healthier food products decreasing the toxic compounds as methyleugenol in basil. The effect of light quality (white, yellow, blue, red) on camptothecin (quinoline alkaloid) production was studied in *Camptotheca acuminata* seedlings (Liu et al. 2015). It was reported that compared with white light, red light provoked highest leaf biomass and camptothecin yield, whereas blue light led to highest camptothecin content, higher activities of camptothecin biosynthesis-related enzymes (TSB, b-subunit of tryptophan synthase and TDC, tryptophan decarboxylase) and corresponding transcript levels of *TSB, TDC1*, and *TDC2* genes, but lowest leaf biomass. Authors concluded that red light had best effect for increasing production of camptothecin in leaves of *C. acuminata* seedlings and suggested that light quality manipulation could be an effective approach to get high camptothecin yield. Hoffmann et al. (2015) reported that *Capsicum annuum* plants grown under high PAR (300 µmol m^{-2} s^{-1}) intensity or 62% blue light had increased epidermal flavonols as compared with plants grown under lower PAR light intensity (100 µmol m^{-2} s^{-1}) or lower levels of blue light (30%). Concentrations of calycosin 7-*O*-β-glucoside, pseudobaptigenin and hesperidin were significantly increased by blue (on 14d of experiment) as compared with white light but growth was not affected by different wavebands (white, red, blue, yellow, complete darkness) treatment studied in *Cyclopia subternata* (honeybush) callus (Kokotkiewicz et al. 2014). The highest statistically different result for formononetin accumulation was obtained after 14d irradiation with red and blue light. Ahmad et al. (2016) also demonstrated that blue light augmented total phenolic and total flavonoid contents for callus cultures of *Stevia rebaudiana*. It was shown that treatment with blue LED spectrum or cool fluorescent lamp with 27 µmol m^{-2} s^{-1} was the best for an optimal in vitro growth of *Achillea millefolium* but there was variation in number, amount and profile of volatile compounds under the influence of quality and light intensity (Alvarenga et al. 2015). Among the major volatile constituents identified (sabinene, 1,8 cineole, borneol, β-caryophyllene and β-cubebene) in *A. millefolium*, the highest quantities were identified after blue (1,8-cineole and β-caryophyllene), fluorescent (borneol), red (sabinene) and white (β-cubebene) light treatment. The authors suggested that it might be possible to yield the compounds of interest by adjustment of the ambient light quality and/or intensity (Alvarenga et al. 2015).

The amount of scutellarin (flavone glycoside) was shown to be higher in sun-developed leaves than in shade-developed leaves of *Erigeron breviscapus* (Zhou et al. 2016). Scutellarin is the main active substance of this herbal heliophyte. Its content decreased during the first 10d after transfer from shade to high light but 40d after transfer it had higher amounts than before the transfer. Compared to low light intensity (35 μmol $s^{-1}m^{-2}$, simulating deep shade), high light treatment (175 μmol $s^{-1}m^{-2}$, simulating moderate shade) induced accumulation of foliar verbascoside and iridoid glycosides (aucubin and catalpol) in both above and belowground plant tissue of *Plantago lanceolata* (Miehe-Steier et al. 2015). The highest flavone content of the typical heliophyte *Lithocarpus litseifolius* (sweet tea) was obtained under 40% shading (one shading net) and the lowest was found under 80% (two shading nets) shading (Li et al. 2016). In order to increase flavone content in the leaves, it was suggested that *L. litseifolius* should be sown in lower locations of a sunny slope or the upper location of a shady slope. Partial shade was noted to cause reduction in total phenolics but had no effect on total volatile concentrations of tarbush as compared with control plants (Estell et al. 2016). However, concentrations of camphene, sabinene, β-pinene, borneol, bornyl acetate, and Z-jasmone were higher for plants grown under partial shade than controls. Grbic et al. (2016) explored the effect of natural light and colored plastic films (changing R/FR and blue/R ratios) on secondary metabolite concentrations and some morphometric parameters of *Perilla frutescens*, which is well known for its beneficial medicinal properties. Compared with natural light, colored plastic films used caused significant reduction in polyphenol and anthocyanin concentrations but do not alter flavonoid content in *P. frutescens*. A 2-year field study exploring the effects of light using cladding materials (polyethylene films named Clarixblue and Patilite, which cut off more than 90% of UV-B waveband, and shade net that reduces solar radiation by 70%) on yarrow (*Achilea collina* cv. SPAK) grown in the Aplps did not observe any SAR effects and the authors hypothesized that it could be a shade-tolerant species (Giorgi et al. 2014). However, phenylpropanoid levels (caffeic acid derivatives as 5-*O*-caffeoylquinic, 3,5-di-*O*-caffeoylquinic and 4,5-di-*O*-caffeoylquinic acids) in inflorescences were reduced significantly in shaded plants compared with full sun. Authors identified switch in carbon allocation between leaves and inflorescences after PAR reduction by shade net. It was reported that UV-B radiation has no significant effect on the biosynthesis of phenylpropanoids in yarrow (leaves and inflorescence) because their concentrations under Patilite and Clarixblue films were similar to those measured under full sun. Study of Podyma et al. (2010) on southern sweet-grass (*Poaceae*) indicated an opportunity to obtain high yield and quality of leaves in the system of organic cultivation. Shading (50 and 70% by shading nets) led to accumulation of coumarin and 3,4-dihydrocoumarin but reduction of bergapten in comparison with non-shaded plants. The highest content of phenolic compounds (astragalin, rutin and chlorogenic, elagic, ferulic, *o*-coumaric, rosmarinic acids) and cumulative yield of leaves were found in plants grown under 50% shading. Recent study on *Glechoma longituba* explored photo-acclimation mechanisms under different light intensities and suggested that it is extremely shade-tolerant plant (Zhang et al. 2015). Ursolic acid and oleanolic acid are the major bioactive compounds in *G. longituba* which possess various pharmacological activities and are used as markers to estimate medicinal quality of the plant. It was reported that ursolic acid and oleanolic acid yield in *G. longituba* as well as the yield and qualities of the medicinal plant were maximal at 16 and 33% of sunlight levels. The study confirmed that appropriate light control might be used to improve the yield and medicinal properties of *G. longituba*. The effects of shade were studied in herbaceous peony (*Paeonia lactiflora* Pall.), which is high-light demanding plant containing many bioactive compounds with pharmaceutical importance as paeoniflorin, oleanolic acid and ursolic acid (Zhao et al. 2012). Study showed that various morphology parameters were higher in sun-exposed compared to shade grown peony. Shade reduced soluble sugar, soluble protein and malondyaldehyde contents, which caused delayed initial flowering date, lengthened flowering time, reduced flower fresh weight, faded flower color and others. It was found that reduced anthocyanin content was the reason of fading flower color under shade. During the period with highest anthocyanin content (flower-bud stage S1 and initiating bloom stage S2) it was found to be higher in sun exposed plants due to expression of upstream anthocyanin biosynthesis genes (*PlPAL* and *PlCHS*) and of downstream (*PlF3H*) genes. Matos et al. (2009) explored phenotypic plasticity to a wide natural light gradient in leaves from four canopy positions (the intercepted PAR differed by a factor of 25 between the sun-exposed leaves and those in shade) in coffee trees, which are considered to be shade-requiring species. Compared with shaded leaves, sun-exposed leaves had lower content of neoxanthin, violaxanthin and α-carotene but higher concentrations of lutein, antheraxanthin, zeaxanthin and β-carotene. Compared with shaded leaves, sun-exposed leaves had better developed photoprotection mechanisms, including augmentation of zeaxanthin pools and de-epoxidation state of the xanthophyll cycle.

An extensive study of light effects (different R/FR ratio and PAR levels) on plant growth regulators was elaborated for *Stellaria longipes* plants occupying different habitats. One with prairie-origin i.e., ‘shade’ ecotype (tall phenotype, highly responsive to low R/FR ratio) and another with alpine-origin i.e., ‘sun’ ecotype (dwarf phenotype, not responsive to low R/FR ratio). Kurepin et al. (2015) quantified ABA, IAA and GA levels for shoots harvested from plants during active stem elongation growth using normal or low R/FR ratio light. Both ecotypes synthesized high amounts of GA_1 under low R/FR light but differ in their catabolism of GA_1. The ‘sun’ ecotype had very high amounts of GA_8 under both light conditions and lack of increased growth response. IAA concentration in ‘shade’ ecotype showed stable rise for plants grown under low R/FR compared to sunlight but reduction or no change was observed in ‘sun’ ecotype. ABA concentration was decreased significantly for both ecotypes grown under low R/FR as compared to normal R/FR light. The result was not surprising as auxin is known to play important role in stem elongation increase observed for SAR. Reduced levels of ABA found for shade-adapted ‘shade’ ecotype Stellaria plants grown under shade is in line with ABA to IAA and/or ABA to GA antagonism associated with stem elongation. Authors concluded that the low R/FR ratio light-mediated increase in stem elongation, observed for the low R/FR light-adapted ‘shade’ ecotype Stellaria plants is regulated by enhanced IAA and GA_1 biosynthesis. In addition, rise in endogenous GAs (GA_{19}, GA_{20}, GA_1 and GA_8) concentrations was reported for both ecotypes grown under low PAR, which correlated with enhanced growth (Kurepin et al. 2006a). Authors suggested that low PAR irradiance probably provoke an overall increase in GA biosynthesis pathway in shoots of *S. longipes*. Under low PAR irradiance only the ‘sun’ ecotype had altered (decreased) ethylene production but R/FR ratios had no significant effect on ethylene evolution in both ecotypes (Kurepin et al. 2006b). It was concluded that different growth response of ‘shade’ Stellaria ecotype under low R/FR ratio and irradiance was not mediated by ethylene. Phenotypic responses of the two Stellaria ecotypes seem to be due to different profiles and levels of endogenous CKs in growing tissues (Kurepin et al. 2012a). Under low R/FR ratio ‘shade’ ecotype had increased total CKs (including bioactive *trans*-zeatin and dihidrozeatin) and LONELY GUY enzyme activity (converting nucleotide CKs to free base CKs), which was associated with the most rapid shoot growth and increased flowering compared to moderate R/FR ratio. In contrast, ‘sun’ ecotype plants grown under low R/FR ratios had mainly *cis*-pathway CKs causing no growth or reduced growth. Further, low PAR was found to provoke higher biomass accumulation for both ecotypes, but the response of ‘shade’ ecotype was faster and stronger than the one of the sun ecotype (Kurepin et al. 2012b). Low PAR led to reduction in salicylic acid concentration, which was more pronounced for ‘shade’ ecotype. It was not surprising that under low R/FR ratio endogenous salicylic acid levels were changed (increased) only for ‘shade’ ecotype, which showed noticeable change in biomass. Exogenous salicylic acid treatment supported the abovementioned results. Therefore, it was summarized that endogenous salicylic acid levels might be regulated via alterations in R/FR ratio and PAR levels.

Brassica rapa seedling (7d) subjected to low R/FR shade had gradually increasing concentrations of IAA in basal to apex sections and showed that cotyledon-generated auxin controls hypocotyl elongation (Procko et al. 2014). Authors found that seed oil composition was not affected by simulated shade in mature plants. Compared to normal light, low R/FR increased stem height and ethylene production as well as transpiration and stomatal conductance in evening primrose (*Oenothera bienis* L.) but decreased flavonoids content, photosynthetic pigments and plant biomass (Qaderi et al. 2015).

An increase in spermine concentration was found in turmeric rhizome grown under 70 and 50% of screen shading (Ferreira et al. 2016). In addition, turmeric plants grown under 50% shading had higher yield of curcuminoid, curcumin, demethoxycurcumin and bisdemethoxycurcumin in their rizhomes compared with those grown under full sun.

Concluding Remarks

Current review is an attempt to summarize investigations reporting how an alteration of one specific environmental factor, i.e., light, might be used as elicitor treatment to enhance biosynthesis of bioactive compounds in plants. In addition, UV and PAR depending on their quality and intensity may induce ROS and oxidative stress, affecting plant growth, biomass and biosynthesis of primary and secondary metabolites, as well as phytohormones (Wani et al. 2016). Like UV-B (Jansen et al. 2008), other types of light or shade also induce very complex metabolic acclimation response, including synthesis of new secondary metabolites, which were not found under control conditions and may have positive or negative effect on human health. Therefore, it is important to investigate not only the compounds of interest for particular plant but a maximal set of secondary metabolites in order to find out if there is an induction of harmful metabolites. Unfortunately, such complex studies of the effect of light and shade on secondary metabolites are very rare.

There is vast number of papers discussing the effect of UV-B exposure on secondary metabolites in medicinal plants, while information about the effects of visible light (including narrow band of wavelengths) and shade is rather scarce. Light quality (monochromatic or polychromatic; visible or UV) and quantity as well as plant species used in different studies are various and it is difficult to make a reliable conclusion how they might affect biosynthesis of secondary metabolites and/or phytohormones. Moreover, biosynthesis of both plant secondary metabolites and phytohormones depends on genotype, genetic regulation and environmental factors. Optimal habitat of the plant and complexity of environmental factors also influence biosynthesis of plant secondary metabolites and plant growth regulators (Reifenrath and Müller 2007; Xenophontos et al. 2008; Kurepin et al. 2015). Thus, different plant species have their own optimal light set (quality and quantity) which manage to elicit maximal yield of secondary metabolites (Sun et al. 2010; Rai et al. 2011; Kokotkiewicz et al. 2014; Alvarenga et al. 2015; Zhou et al. 2016). These authors suggested that improving the yield and medicinal properties of plants might be achieved by appropriate adjustment of light quality and quantity. Sometimes light quality and intensity which yield higher accumulation of secondary metabolites have negative impact on growth of medicinal plants but it is well known that plant growth and development is affected by phytohormones. Plant growth regulators like ABA, jasmonic acid and salicylic acid are related with stress response. Phytohormones like cytokinins, auxins, gibberellins are known to manipulate positively plant growth and biomass, which influence the yield of secondary metabolite production. Cytokinins and ethylene play role in ageing process and should influence quality and quantity of secondary metabolites. In general, the effect of any light type on the plant growth regulators was underestimated in comparison with secondary metabolites, which are the active substances in medicinal plants. For example the survey on the literature does not found any recent information about the effect of UV radiation, light or shade on the endogenous brassinosteroid content in medicinal plants. Therefore, it is necessary to expand the research on phytohormones in medicinal plants exposed to different light quality and quantity.

As mentioned above, the changes of stress-induced metabolites are too complex and researchers face the necessity to study them in more details to be able to assess if the altered nutritional and/or pharmaceutical characteristics of medicinal plants acclimated to UV, light or shade would benefit to the consumers. Recently more detailed research appeared in regard to metabolite but not phytohormone profile of plants subjected to UV, artificial light or shade, but the question for medicinal effect on plants still needs to be answered (Kim et al. 2012; Carvalho et al. 2016; Estell et al. 2016). Secondary metabolites accumulation is highly important from biotechnological and pharmacological point of view and further studies including phytohormone profiling with different doses and qualities of light exposure are essential in order to obtain higher yield of these valuable compounds.

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Effect of Climate Change on Algae Valuable Source of Medicinal Natural Compounds

Morteza Yousefzadi and Mohammad Hossein Mirjalili

Abstract The word algae represent a large group of different organisms from different phylogenetic groups, representing many taxonomic divisions. They are distributed worldwide in the sea, in freshwater and in moist situations on land. Algae grow rapidly, produce useful products, and provide environmental benefits. Algae have potential as foods, and vitamins, bioactive substances, polysaccharides and other valuable commercial products and also are useful as raw material for future biofuel production and liquid fertilizer. Algae can be classified into two main groups; first one is the microalgae, which includes blue green algae, dinoflagellates, bacillariophyta (diatoms) etc., and second one is macroalgae (seaweeds) which includes green, brown and red algae. Changes in global temperature and ocean chemistry associated with increasing greenhouse gas concentrations are forcing widespread shifts in biological systems. In response to warming, species ranges are shifting toward the poles, up mountainsides, and to deeper ocean depths. Concern for the environment and global climate change has increased in recent years, and algae can provide a number of significant environmental benefits. They remove carbon dioxide from the atmosphere, helping to reduce the harmful effects of the gas on climate change and the health of the environment. The aim of this chapter is to provide an overview of the current knowledge on these photosynthetic organisms regarding their environmental and pharmaceutical benefits.

Keywords Seaweed · Microalgae · Natural product · Greenhouse gas

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Introduction

In the last decades, the attention of the natural product researchers has been focused on the marine environment. It is a wealthy source of plants, animals and micro-organisms, which due to their adaptation to this unique habitat, produce a wide variety of bioactive molecules, which can be developed as nutraceuticals and pharmaceuticals for human nutrition supplementation and disease therapy. The specific habitat where an organism is growing has influence on the chemical nature of the marine primary and secondary metabolites. The intraspecific variation can produce differences in the chemical structures of compounds and their concentrations in different marine environments (Fan et al. 2014; Kiuru et al. 2014).

The word algae represent a large group of different organisms from different phylogenetic groups, representing many taxonomic divisions. In general algae can be referred to as plant-like organisms that are usually photosynthetic and aquatic, but do not have true roots, stems, leaves, vascular tissue and have simple reproductive structures. They are distributed worldwide in the sea, in freshwater and in moist situations on land. Most are microscopic, but some are quite large, e.g., some marine macroalgae that can exceed 50 m in length. Algae grow rapidly, produce useful products, and provide environmental benefits. Algae have potential as foods, and vitamins, bioactive substances, polysaccharides and other valuable commercial products and also are useful as raw material for future biofuel production and liquid fertilizer. Furthermore, algal products can be produced sustainably. With the great diversity of algae, there is great promise that algal features may serve to enhance a large variety of current products, and produce new ones. As the currently expanding market for algae-based goods indicates, the future is bright for the research and development of algal products. Algae also contribute to increased sustainable practices. In the last 500 years, agricultural production processes on land have advanced, and there are now new and well-developed approaches to conventional farming operations. Significant progress also stems from the production of commercial products in water. As an aquatic organism, algae can be grown to produce useful products without using very much land (Hudek et al. 2014).

Algae can be classified into two main groups; first one is the microalgae, which includes blue green algae, dinoflagellates, bacillariophyta (diatoms)… etc., and second one is macroalgae (seaweeds) which includes green, brown and red algae.

Microalgae are important sources of commercially produced high-value unique chemicals including carotenoids, antioxidants, fatty acids, enzymes, polymers, peptides, toxins and sterols. They are also the first step in the aquatic food chain. Moreover, microalgae are considered as the actual producers of some highly bioactive compounds found in marine resources. Attempts to develop commercial products from microalgae are not new, with the earliest being the proposal to use microalgae as a source of lipids, carotenoids and the extensive work done in the 1970s on microalgae as a source of single-cell protein (Borowitzka 2013).

Macroalgae are used for great number of application by man. The principal use of seaweeds as a source of human food and as a source of gums (phycocollides).

Gums like agar, alginic acid and carrageenan are primarily constituents of brown and red algal cell walls and are widely used in industry. An increasing attention to macroalgae metabolites of industries from different branches (textile, fuel, plastics, paint, varnish, cosmetics, pharmaceutical and food) was noticed in the last years. Seaweeds are being used in cosmetics, and as organic fertilizers. They have the potential to be much more widely used as a source of long- and short-chained biochemical with medicinal and industrial uses. From human health point, macroalgae rich in unique bioactive compounds not present in terrestrial food sources, including different proteins (lectins, phycobili proteins, peptides, and amino acids), polyphenols, and polysaccharides and a novel source of compounds with potential to be exploited in human health application. Macroalgae are part of the diet in different countries and constitute a source of beneficial nutrients, such as vitamins, trace minerals, lipids, amino acids and dietary fibers, its use as a low-calorie food should be investigated, as it might be important in body weight control and cardiovascular health. The metabolites found in macroalgae are described as having anti-inflammatory, antimutagenic, antitumor, antidiabetic, and antihypertensive properties. In addition, they are hepato protections, and can also inhibit the lipoxygenase, aldose reductase and cholinesterase's (Zubia et al. 2009; Lopes et al. 2012; Andrade et al. 2013).

Changes in global temperature and ocean chemistry associated with increasing greenhouse gas concentrations are forcing widespread shifts in biological systems. In response to warming, species ranges are shifting toward the poles, up mountainsides, and to deeper ocean depths. Factors including warming and ocean acidification are causing the reorganization of local communities as species are added or deleted and as interactions among species change in importance. Because greenhouse gas emission rates continue to accelerate, the climatically forced ecological changes that have been documented over the past half century will likely pale in comparison to changes in the coming decades (Harley et al. 2012).

Concern for the environment and global climate change has increased in recent years, and algae can provide a number of significant environmental benefits. They remove carbon dioxide from the atmosphere, helping to reduce the harmful effects of the gas on climate change and the health of the environment.

Climate Change and Marine Organisms

Rising atmospheric carbon dioxide (CO_2) is one of the most critical problems because its driving a number of important physical and chemical changes. The primary direct consequence of global warming is thermal expansion of water and global-scale trends ocean acidification, Climbing temperatures create a host of additional changes, such as sea level increase, increased ocean stratification, decreased sea-ice extent, and change patterns of ocean circulation, precipitation. Both warming and altered ocean circulation act to reduce subsurface oxygen (O_2) concentrations, along with regionally specific increases or decreases in wave heights, upwelling, terrigenous nutrient runoff, and coastal salinity, all of which in turn impact the health of marine species, ecosystems, and our coastal communities (Harley et al. 2012; Keeling et al. 2010; Zacharioudaki et al. 2011; Doney et al. 2012).

Studies on the global response of a wide variety of marine and terrestrial species to climate change conclude that the planet is facing drastic ecosystem shifts and numerous extinctions. Species that fail to acclimatize physiologically or evolve genetically to increasing temperatures will either move northwards into cooler habitats or become extinct (Jueterbock et al. 2013).

Marine ecosystems cover 71% of the Earth's surface, our knowledge of the effects of climate change in oceans is limited compared to terrestrial ecosystems.

Responses to climate change are particularly rapid and strong in marine ecosystems, especially in the marine intertidal where species often live at their upper temperature tolerance limits. Global warming-related range shifts of marine species exceed those of terrestrial species by an order of magnitude. Furthermore, climate-change induced range shifts are more predictable for marine than for terrestrial species, since the distributional limits of marine species are usually directly correlated with their thermal tolerance limit. Range shifts of key or foundation species are of central importance, since by definition these species play a crucial role in, and can trigger changes throughout, the entire web of interactions within an ecological community (Jueterbock et al. 2013; Sunday et al. 2012).

In recent decades, the rates of change have been rapid and may exceed the current and potential future tolerances of many organisms to adapt. Further, the rates of physical and chemical change in marine ecosystems will almost certainly accelerate over the next several decades in the absence of immediate and dramatic efforts toward climate mitigation. However, there is increasing evidence suggesting that marine plants and animals could respond as fast or faster than their terrestrial counterparts, from both observations and theory (Keeling et al. 2010; Doney et al. 2012).

Cyanobacteria in a Changing Climate

Cyanobacteria are the Earth's oldest known oxygen evolving photosynthetic microorganisms, and they have had major impacts on shaping our current atmosphere and biosphere. Their long evolutionary history has enabled cyanobacteria to develop survival strategies and persist as important primary producers during numerous geochemical and climatic changes that have taken place on Earth during the past 3.5 billion years (Paerl and Huisman 2009a, b).

Cyanobacteria are predicted to increase due to climate and land use change. biospheric environmental perturbations, including nutrient enrichment and climatic changes strongly affect cyanobacterial growth and bloom potentials in freshwater and marine ecosystems. Since the 1960s, however, there has been a dramatic global increase in the number of publications and reports of cyanobacterial blooms. While increased reports may to some extent be due to increased monitoring efforts, there is substantial evidence that blooms are increasing not only in frequency, but also in biomass, duration and distribution. Furthermore, it has been hypothesised that cyanobacteria may continue to increase in response to global climate change (Paerl et al. 2011; Carey 2012).

Cyanobacterial blooms present major challenges for the management of rivers, lakes and reservoirs. Blooms have adverse impacts on aquatic ecosystems and human health, with wide-ranging economic and ecological consequences. Toxic cyanobacterial blooms present a considerable risk to drinking water and have major public health, ecological, and economic effects. The increased frequency and intensity of blooms have been attributed to changing precipitation patterns, melting glaciers and intensifying storms result in the escalation of nutrient's input and promote eutrophication. Eutrophication is a process induced by increased nutrients, especially nitrogen and phosphorus, in water bodies resulting in growth of algae and plants. More recently, it has been predicted that a changing climate associated with rising levels of atmospheric CO_2 will increase the occurrence of blooms, or at least favour cyanobacterial dominance of phytoplankton communities (Carey 2012; Paerl and Huisman 2009a, b).

Climate change, including global warming, is causing changes to regional rainfall and hydrology, which will have cumulative effects with nutrient-over-enrichment in modulating cyanobacterial harmful algal blooms. Regional and global warming enhances the initiation, magnitude, duration, and distribution of cyanobacterial harmful algal blooms. Furthermore, increasing variability in rainfall patterns impacts nutrient and sediment delivery, sediment-water exchange and metabolism, flushing and water residence time, and vertical stratification, which, in turn, may affect cyanobacterial harmful algal blooms dominance and persistence (Paerl et al. 2016).

Temperature is an all-pervasive environmental parameter that affects the metabolism, growth, reproduction, and survival of living organisms, as well as the interactions among species. As a consequence, climate warming will result in shifts in phytoplankton community composition, including shifts between cyanobacteria. In natural systems, it has been shown that warmer water temperatures do favour cyanobacterial dominance in phytoplankton communities. Similarly, it has been proposed that warmer temperatures will mean earlier and longer potential bloom periods, as well as lead to possible range expansions. warming on cyanobacteria are increased occurrence of cyanobacterial blooms with ongoing climate warming (Carey 2012; Kingsolver 2009).

Many species of planktonic cyanobacteria produce gas vesicles, which provide buoyancy and allow access to well-lit surface waters. The regulation of buoyancy, which allows migration in stratified lakes between illuminated surface waters and nutrient rich bottom waters, occurs as cells accumulate carbohydrates when exposed to light and respire these products of photosynthesis in the dark. Changes in climate, such as increased temperatures, prolonged droughts and longer water residence times, are predicted to promote cyanobacterial bloom establishment by increasing the strength and duration of stratification. Increased stratification will favor the fast-migrating buoyant cyanobacteria. As nutrient loading and stratification increase, there will tend to be a shift towards buoyant species that can access both the well-lit surface waters and hypolimnetic nutrient pool (Carey 2012; Rinke et al. 2010).

Fundamentally, cyanobacteria are an extremely diverse group with different sets of traits, and will respond to different aspects of climate change (e.g., increased stratification, altered nutrient availability). The ability of cyanobacteria to response to environmental conditions associated to climate change has been documented since the first occurrence of cyanobacteria around 3.5 million years ago. Thus, looking into the evolutionary history, cyanobacteria has been adapted to extreme conditions such as temperature fluctuation, exposure to high UV radiation, abundance and scarcity of nutrients. cyanobacterial blooms are favored not by an individual environmental factor, but by a combination of multiple interacting physical, chemical, and biotic drivers. It is evident that although cyanobacterial blooms can be enhanced by increasing temperature, there must be sufficient phosphorus and nitrogen to sustain high populations. There will most likely also be regional differences in which cyanobacterial taxa dominate, depending on how future climate, hydrology, and nutrient loading vary geographically (Carey 2012).

In general, Climate change alters local hydrologic and biogeochemical processes, including rainfall and runoff (amount and temporal dynamics), nutrient export from watersheds, mixing regimes, internal nutrient cycling, and food web dynamics. These changes present a significant challenge to resource managers aiming to control cyanobacterial harmful algal blooms in a future favoring bloom occurrence. A research program that focuses on how extant strategies will be influenced by climate change is needed to support effective cyanobacterial harmful algal blooms control programs (Paerl et al. 2016).

Climate Change Effects on Phytoplankton

Every day, more than 100 million tons of carbon dioxide are drawn from the atmosphere into the ocean by billions of microscopic ocean plants called phytoplankton during photosynthesis. In addition to playing a big role in removing greenhouses gases from the atmosphere, phytoplankton are the foundation of the ocean food chain (Behrenfeld et al. 2006).

Phytoplankton account for <1% of the photosynthetic biomass on Earth, but are nevertheless responsible for nearly 50% of global net primary production and are the primary energy source for aquatic ecosystems. Climate modifications, such as the rise in atmospheric CO_2 and warming, affect the marine biosphere through modifications in pH, carbonate availability, water column stability, nutrient and light regimes. These changes directly impact small-sized (*ca.* <1–>100 μm) phytoplankton organisms, whose short-term life cycles make them amenable to quickly respond to subtle environmental variations. Phytoplankton response can be both directly through physiology and indirectly mediated through effects on environmental factors limiting primary production most notably light and nutrients.

Changing climatic conditions can modify these environmental factors and alter phytoplankton structure and taxonomic composition. Therefore, tracking changes in the phytoplankton community structure can be an accurate indicator of ecosystem perturbations (Winder and Sommer 2012; Guinder and Molinero 2013).

Interactions between climate and phytoplankton are complex, because other factors such as resource availability, density dependence, and predation strongly control the abundance, distribution, and size structure of the community. Despite these complexity of interacting processes, some widespread climate related responses have emerged, and the mechanisms involved in climate-related changes are becoming better understood. Impacts of climate change on plankton are mainly manifested as shifts in seasonal dynamics, species composition, and population size structure (Winder and Sommer 2012; Adrian et al. 2009).

Temperature is a key parameter that directly affects physiological rates of phytoplankton at multiple scales, e.g., enzymatic reactions, respiration, body size, generation time, ecological interactions, community metabolism. Phytoplankton experience an increase in enzymatic activity and growth rates over a moderate range of temperature rise with an average $Q_{10} = 1.88$ (photosynthesis and respiration rate increase resulting from a 10 °C rise in temperature). As a result, a temperature increase should lead to greater phytoplankton growth rates and biomass accumulation under adequate resource supply. Enhanced growth of primary producers is expected under future trends of temperature increase. The projected scenario, however, becomes complex when considering indirect effects of warming, such as grazing acceleration, which play key modulating roles of phytoplankton biomass accumulation. However, compared to photosynthesis rates, the metabolism of heterotrophic organisms is more sensitive to temperature Consequently, warming should increase consumption by herbivores more strongly than primary production. This can strengthen top-down control over primary production by increasing grazing rates and thus affect phytoplankton production and taxonomic composition (Winder and Sommer 2012; Guinder and Molinero 2013; Sommer and Lewandowska 2011). Salinity closely co-varies with temperature and also has important implications in plankton physiology, affecting germination of resting stages, growth rates and development of blooms in coastal waters (Guinder and Molinero 2013).

The most significant climatic effects on phytoplankton species composition will very likely be mediated through changes in thermal stratification patterns such as the extent of the growing season and vertical mixing processes. Vertical mixing is one of the key variables that conditions the growth performance of phytoplankton within the water column. because mixing processes are usually accompanied by changes in resource availability of light and nutrients (Winder and Sommer 2012).

Increasing evidence of changes in plankton size structure has been reported worldwide in relation to global warming. Temperature effects on the size structure of the phytoplankton community composition toward a dominance of small species The reduction in cell/body size of planktonic organisms and displacements of species ranging to higher latitudes have been mainly ascribed to global warming. Phytoplankton cell size follows biophysical rules (e.g., nutrient uptake, motion, sinking rates, kinetics of metabolism) that affect growth rates, the biogeochemical cycling and trophodynamics. The replacement of large cells by smaller ones under warming conditions is likely related to shifts of the species' environmental optimum growth and the higher competitive skills of small cells (Winder and Sommer 2012; Beaugrand et al. 2010).

Finally, Climate-driven modifications in marine water physics and chemistry impact phytoplankton from the individual to ecosystem levels through alterations in both bottom-up and top-down controls, namely resources availability-light and nutrients- and zooplankton grazing pressure and selectivity. At the individual level phytoplankton is affected via changes in physiology, morphology and behavior and the community level is affected by changes in structure (size, composition, diversity), phenology and the biogeographical distribution of species that derive in new interspecific interactions and trophodynamics. Among the most common direct and indirect universal responses of phytoplankton to climate change are alterations in growth and photosynthesis rates and in calcification processes related to CO_2 rise and ocean acidification, dominance of smaller species under warmer conditions (related to both water stability and/or grazing pressure) and changes in the phenology, magnitude and species composition of phytoplankton spring bloom due to earlier thermal stratification, overwintering and/or enhanced zooplankton activity (Guinder and Molinero 2013).

Effects of Climate Change on Seaweed

Seaweeds are ecologically important primary producers, competitors, and ecosystem engineers that play a central role in coastal habitats ranging from kelp dforests to coral reefs. Although seaweeds are known tobe vulnerable to physical and chemical changes in the marine environment, Less attention has been paid to seaweed even though they constitute some of the most significant biogenic producers of calcium carbonate ($CaCO_3$) and contribute to deep-sea productivity. Seaweeds are key habitat structuring agents that harbor incredible biodiversity. Seaweeds form the base of productive food webs that include economically valuable species and extend well beyond the shallow waters in which seaweeds dwell. Seaweeds are intimately linked to human cultural and economic systems via the provision of ecosystem goods and services ranging from food to medicine to storm protection (Harley et al. 2012; Christie et al. 2009; Koch et al. 2012).

Seaweed survival, growth, and reproduction are known to vary with numerous climatically sensitive environmental variables including temperature, desiccation, salinity, wave heights, nutrient supply via upwelling and run-off, pH, and carbon dioxide concentration itself (Harley et al. 2012; Chu et al. 2012).

Temperature determines the performance of seaweeds, and indeed all organisms, at the fundamental levels of enzymatic processes and metabolic function. Seaweeds have evolved biochemical and physiological adaptations, including variation in the identity and concentration of proteins and the properties of cell membranes, that enable them to optimize their performance with respect to the temperatures they encounter. In response to increase temperature, seaweeds can produce heat shock proteins that repair or remove damaged proteins However, protein thermal physiology is not well understood in macroalgae. and the upregulation of heat shock protein production is only one of many transcriptional changes that occur in seaweeds during periods of thermal stress. As a result of non-stressful conditions at intermediate temperatures and stress at the extremes, the relationship between temperature and most subcellular, tissue-level, or whole-organism processes is described by a hump-shaped thermal performancecurve. From colder to warmer, these curves generally rise exponentially as rates of biochemical reactions increase, peak at some optimum temperature, and then fall rapidly as the biological components of the system become less efficient or damaged. The effect of a small increase in thallus temperature will be beneficial when the initial temperature is cooler than optimal and detrimental when it is warmer than optimal, and the precise change in performance can be predicted from the starting and ending temperature values along the curve. Unfortunately, the shapes of thermal performance curves and the positions of their optima are poorly described in most seaweeds (Harley et al. 2012; Kordas et al. 2011; Kim et al. 2011; Eggert 2012).

Increased temperature is generally thought to have negative effects on spore production, germination, recruitment, and sporophyte growth and context-specific effects on gametogenesis depending on the source population and degree of warming has also been linked to mortality of spores, gametophytes, eggs, and sporophytes. Much less is known about the effects of increasing CO_2 concentrations. On the basis of current knowledge, we can expect positive effects on gametogenesis and variable effects (e.g., positive effect of increasing CO2, but negative effect of decreasing pH) on germination (Harley et al. 2012; Buschmann et al. 2004).

Carbon dioxide concentrations in seaweed habitats are increasing with anthropogenic emissions and, in some regions, with intensified upwelling of CO_2-enriched water. As with terrestrial plants, it is tempting to predict that sea weedswill benefit from the increase in inorganic carbonconcentration. Because CO_2 diffusion rates are much higher in air than in water, seaweeds that are exposed at low tide and those with floating canopies at the sea-air interface have greater access to CO_2. However, aerial exposure does not necessarily reduce the probability of carbon limitation, as exposure at low tide can dramatically reduce rates of carbon acquisition and even immersed seaweeds can benefit from increasing atmospheric CO_2 concentrations. Most green and brown algae (and many red algae) can also utilize bicarbonate by converting it to CO_2 intracellularly Just as terrestrial C_3 plants are more likely to be CO_2 limited and therefore more likely to benefit from elevated CO_2 than C_4 plants, seaweeds lacking CCMs are more likely to be carbon-limited and thus more likely to benefit from additional CO_2 (Harley et al. 2012; Long et al. 2004; Williams and Dethier 2005).

In addition to providing carbon for photosynthesis, anthropogenic CO_2 emissions reduce seawater pH and the saturation state of calcium carbonate. As this increases the cost of calcification and the likelihood of dissolution, calcifying organisms are particularly sensitive to elevated CO_2 in seawater. Ocean acidification is consistently related to reduced growth rates in calcified macroalgae (Harley et al. 2012; Kroeker et al. 2010).

All of the anthropogenically forced changes in the physical and chemical environment are occurring simultaneously, and in many cases, the impact of any particular stressor on the physiology and performance of marine macrophytes will depend upon the presence and magnitude of additional limiting or disruptivestressors. the enhancement of photosynthesis by elevated CO_2 varying with nutrient availability. The percent cover of algal turfs decreased with increasing CO_2 under ambient nutrients, but the reverse was true under elevated nutrients. There are also many interactions among disruptive stressors, including temperature, desiccation, pH, salinity, and ultraviolet radiation. For example, in tropical and warm-temperate crustose coralline algae, the negative effect of warmer temperatures on bleaching, growth rates, calcification rates, and survival were significantly greater under conditions of elevated CO_2/reduced pH. As of yet, it is difficult to predict when one stressor will increase or decrease the effect of another. There are also no known biases toward synergistic or antagonistic effects; in a meta-analysis of multi-stressor studies on Fucus spp., synergistic, additive, and antagonistic outcomes were all equally prevalent. We desperately need to incorporate more ecophysiological research into a multi-stressor framework to improve our understanding of when, where, and why important context-dependent outcomes emerge (Harley et al. 2012).

Linkages Between Harmful Algal Blooms and Climate Change

Increasing human settlements in the near-shore modify the environment through eutrophication and pollution processes, and significantly affect the marine biota and human health. The synergistic effects of anthropogenically driven temperature rise and eutrophication can enhance the occurrence of harmful algae blooms (HABs) in the coasts. HABs have significant negative implications for the marine ecosystem functioning, as they can cause detriment to biodiversity and eventual death of predators, causing severe impacts on fisheries and resource availability with serious repercussion for human health and economy (Guinder and Molinero 2013).

Temperature is one of the main environmental factors affecting physiological processes in phytoplankton, acting at many different stages of growth and bloom development. It not only is one of the most important environmental drivers expected to change with climate, but also is the least contentious, since there already has been measurable warming of the surface mixed layer and the upper several hundred meters of the oceans (Wells et al. 2015).

The link between increasing water temperatures and phytoplankton growth rates has long been recognized, and it is expected that increased potential growth rates will accompany warming. Temperature influences motility, germination, nutrient uptake, photosynthesis, and other physiological processes. Optimal and inhibitory threshold temperatures differ among metabolic processes. The optimal temperature for photosynthesis is generally greater than the optimum for growth. Indeed, the biogeography of phytoplankton species boundaries is determined in large part by temperature regimes, as is almost certainly is true for HAB species. But phytoplankton, including HAB species, can show strong intraspecific differences in temperature tolerance and responses. The chemical composition of a species' (e.g., lipids, fatty acids, and toxicity) also is a function of temperature. While higher toxicity (i.e., toxin accumulation) of some species can occur with slowing growth, temperature and toxin production appear to be directly linked in some species but not others. Much of the basic information needed to generate a preliminary forecast of which regions or habitats (poles vs. tropics, estuaries vs. coasts) HAB species will be the most resilient or susceptible to temperature change likely is available (Wells et al. 2015; Bissenger et al. 2008; De Boer 2005).

Temperature is a keystone parameter differentially affecting a range of metabolic processes, and thus is anticipated to have a strong influence on phytoplankton community compositions and trajectories. While increasing annual temperatures should broaden the windows of some HAB activity it will not affect others or even lessen other harmful algal blooms. Ecosystem interactions, strain variability within HAB and non-HAB species, and concurrent hydrographic or oceanographic changes all complicate even this seemingly straightforward expectation. It is logical that HAB habitats should expand to higher latitudes, but there is little clear evidence that this has happened and the time series observation systems needed to verify this change currently are lacking. There is little evidence to date that changing temperatures directly affect toxin production in HAB species although these findings are by no means comprehensive. It is unlikely that temperature alone will drive competitive selection of HAB species over non-HAB species, but it is probable that temperature effects on metabolic rates will magnify or lessen the influence of other climatic pressures on HAB prevalence (Wells et al. 2015).

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Metabolic and Hormonal Responses of Plants to Cold Stress

Joanna Lado and Matías Manzi

Abstract The adaptation and survival of plants in challenging environments involves changes at cellular and molecular levels. Plants inherently possess sophisticated mechanisms to timely perceive environmental stimuli and respond accordingly. Temperature and water availability are the major environmental factors that significantly influence geographical distribution of plants. In particular, cold acclimation includes structural and morphological modifications, changes in cell membrane composition and the accumulation of compatible solutes among others cryoprotective compounds. In order to survive in such non-optimal conditions, plants have developed complex mechanisms to perceive external signals and trigger crucial responses. These phenomena are mediated by complex phytohormone networks that are also involved in the adaptation to other abiotic and biotic stresses. Through hormonal signaling, cold stress modifies biomass and bioactive compounds accumulation in medicinal plants, both of special interest regarding their biological activity and therefore, their pharmacological potential. In this chapter we review the latest information regarding cold signaling in plants, phytohormones and how integrated crosstalk underpins cold tolerance together with a special mention of cold effect on bioactive compounds accumulation, with emphasis in medicinal and aromatical plants.

Keywords Abscisic acid · Bioactive compounds · Brassinosteroids · Chilling · Ethylene · Jasmonic acid · Salycilic acid

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Introduction

Plants have developed a striking set of different mechanisms to adapt and survive in challenging environments facing biotic and abiotic stresses. Cold is one of the most harmful abiotic stresses for plants due to its notable impact on different developmental processes and its huge influence on crop productivity and geographic distribution (Chinnusamy et al. 2007; Rahman 2013). Cold stress directly affects plant growth and development, inducing several physiological, metabolic and genetic mechanisms (Ruelland et al. 2009). Under cold conditions plants rapidly arrest plant growth and modify plant morphology which are believed to underpin the reallocation of resources to other processes aiming to increase cold tolerance (Eremina et al. 2016). Structural and celular changes associated with cold response involve the alteration of membrane conformation, leading to a decrease in membrane fluidity (Sevillano et al. 2009; Upchurch 2008) together with a rapid generation of reactive oxygen species (ROS) which could cause oxidative stress if plants are not able to detoxify them by activation of the antioxidant system (Ruelland et al. 2009; Sevillano et al. 2009). In addition, low temperatures induce a rise in the content of cryoprotective proteins and metabolites such as soluble solids and amino acids. In order to adapt and survive, plants have also developed diverse cellular enzymatic and non-enzymatic mechanisms to cope with cold stress, the vast majority of which are mediated by phytohormones (Ruelland et al. 2009; Eremina et al. 2016).

Plants ability to minimize and survive oxidative stress depends on the effectiveness of enzymatic and non-enzymatic antioxidants, a complex system that is triggered in response to low temperature (Shi et al. 2014; Chinnusamy et al. 2010). As mentioned, many of these processes are mediated by phytohormones, involving the participation of stress-related hormones such as abscisic acid (ABA), etylene (ET), salicylic acid (SA) and jasmonic acid (JA) and a tight interaction with growth-promoting cytokinins (CKs), auxins (AUXs), gibberellins (GAs) and brassinosteroids (BRs). Phytohormones act as secondary signals initiating a cascade of events that may follow almost a generic pathway with the involvement of different components (Shi et al. 2014; Ahammed et al. 2015; Lado et al. 2016; Peleg and Blumwald 2011). Signal perception is followed by the generation of second messengers such as calcium, ROS or nitric oxide (NO), a generic signal transduction pathway that eventually involves a protein phosphorylation cascade to initiate transcription of specific sets of stress-regulated genes as well as biochemical changes at tissue and celluar levels (Fig. 1). The proper spatial and temporal coordination of all signaling molecules is essential for successful cold acclimation or adaptation (Ahammed et al. 2015).

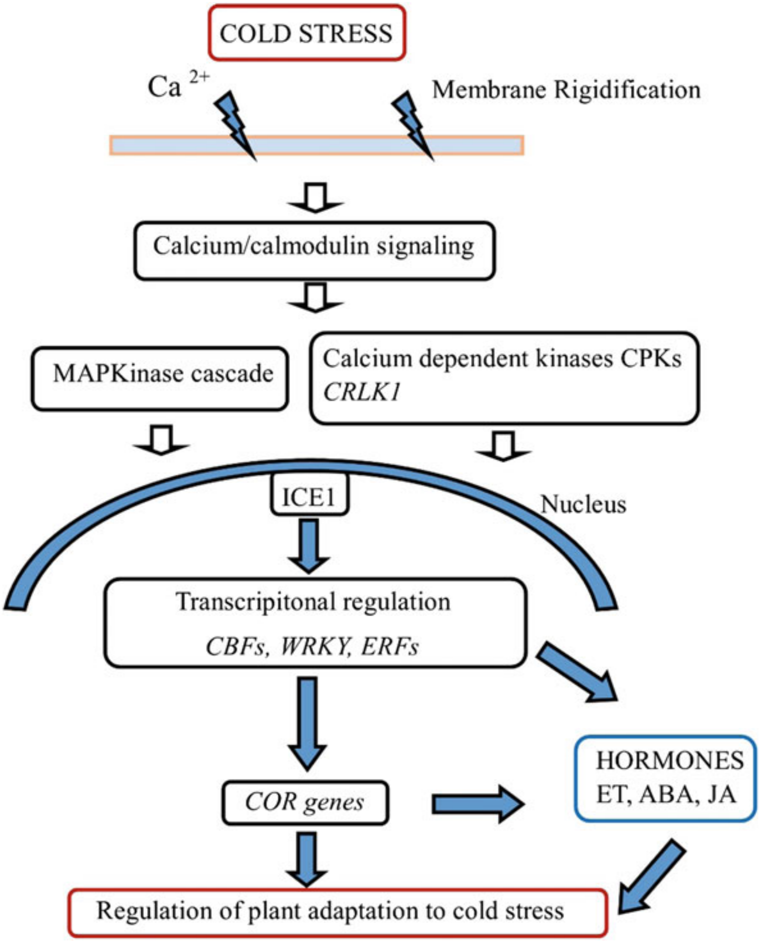

Fig. 1 Schematic representation of main pathways and interactions towards cold response and adaptation in plants

Physiological, Biochemical and Molecular Responses to Cold Stress

Low temperature causes morphological and structural changes, alters membrane fluidity, triggering changes the activity of macromolecules and osmotic potential in the cell. Cold-induced damage in photosynthetic apparatus includes inhibition of CO_2 assimilation, photoinhibition of photosystem I (PSI) and PSII, and inhibition of diverse enzymatic activities. There is also an enhancement in the production of ROS which induces damage to membranes, proteins, lipids, DNA and RNA, among other cell constituents (Sevillano et al. 2009). Transgenic plants overexpressing

ROS scavengers or mutants with enhanced ROS scavenging capacity usually exhibit increased tolerance to cold stress (Ahammed et al. 2015).

It is still no clear how low temperatures are perceived in plants, however, the involvement of membranes appears to be crucial in this process (Shi et al. 2014). Lipid composition is a key factor in the stabilization of membranes and therefore plays a role in the enhancement of cold tolerance. Unsaturated fatty acids have been associated with cold tolerance since a greater amount was determined in the plastid membrane of cold-resistant compared to cold-sensitive plants (Upchurch 2008). The fluid mosaic physical state of the plasma membrane is vital for the structure and function of the cells, as well as to sense temperature variations. At low temperatures plant membranes undergo transition from a liquid crystalline to a gel-like phase with reduced fluidity while ion leakage and deactivation of membrane-integrated proteins occurs (Upchurch 2008), where free, esterified and glycosylated sterols play a crucial function (Valitova et al. 2016; Grunwald 1971). Lipid changes detected only in the cold-acclimating wild potato (*Solanum comersonii*) species included an increase in phosphatidylethanolamine, a decrease in sterol to phospholipid ratio, an increase in linoleic and a decrease in linolenic acid (Palta et al. 1993). Sterol acylglycosides are present in plant cells in minor amounts but it was demonstrated that relative changes in their concentration in the plasma membrane, along with other lipids, are associated with plants adaptation to low temperatures (reviewed in Valitova et al. 2016).

Calcium-mediated signaling is believed to play a pivotal role in plant responses to cold stress. Currently, a widely accepted hypothesis states that the reduction in membrane fluidity appears to be a primary event of cold perception, activating Ca^{2+} channel in higher plants. Ca^{2+} acts as secondary messenger in this process, triggering responses at the nuclear level (Shi et al. 2014). It was also described that receptor-like kinases (RLKs) are involved in this perception, amplification and transmission of environmental stimuli via signaling cascades that finally modulate gene expression, protein activation and cell adjustment (Ye et al. 2017; Wang et al. 2017). *CRLK1*, a calcium/calmodulin-regulated RLK is mainly localized in the plasma membrane and its protein levels increase notably after cold exposure, suggesting that it is involved in a cold-related oxidative stress signal transduction pathway (Fig. 1). Numerous reports have indicated that low and high temperature as well as hormones induce specific changes in the expression of *CDPK* genes in many plants (Ye et al. 2017; Wang et al. 2017; Xiao et al. 2017). Moreover, *CRLK1*-knockout mutant plants exhibited increased sensitivity to cold and had delayed induction of cold-responsive genes compared to wild type plants. These results indicated that *CRLK1* is a positive regulator of cold tolerance in plants and acts as a bridge in calcium signaling and cold signaling (Ye et al. 2017; Yang et al. 2010). Similarly, it was recently described that *MaCDPK7* gene is involved in regulating banana fruit ripening and chilling resistance (Wang et al. 2017) whereas its orthologue in rice (*OsCDPK7)* is induced in response to cold and its overexpression confers an enhanced cold tolerance (Saijo et al. 2000).

In the same line, the LRR receptor kinases are one of the largest and most renowned classes of RLKs, playing important functions towards cold stress tolerance. The perception of cold stimuli by receptors can activate large and complex intracellular signaling cascades, leading to the generation of secondary signaling molecules including $Ca^{2+,}$ ROS and inositol 1, 4, 5-trisphosphate (reviewed in Ye et al. 2017). Therefore it can be suggested that plant cells can perceive cold stress via membrane rigidification (Shi et al. 2014) and interestingly, histidine kinases (HKs) are also an important class of hormone receptors (Ha et al. 2012), being a possible point of convergence between cold sensing and phytohormone responses (Shi et al. 2014).

In addition to the plasma membrane, chloroplasts are among the most severely impacted organelle and could be contributing to ambient temperature sensing (Kratsch and Wise 2000). The maintenance of polyunsaturated fatty acid levels in chloroplast membranes has been shown to contribute to plant survival under low temperatures and to the normal formation of chloroplastic membrane in plants under cold stress (Upchurch 2008). Saturated phosphatidylglycerol (PG) content may be related to the phase transition temperature and thus related to the low-temperature adaptability of plants, being its proportion 40% or higher in cold-sensitive plants and lower in cold tolerant species (Upchurch 2008). Notable changes also occur at the photosynthetic apparatus; under low temperature, the ability to use the absorbed light energy decrease leading to an overreduction of PSII and consequently, to enhance the generation of ROS (Janda et al. 2014). Furthermore, cold-acclimated plants usually show less photoinhibition of PSII than cold-sensitive plants being therefore a good indicative of cold tolerance (Janda et al. 2014). Further, protein folding is influenced by temperature changes. Therefore, plant cell can sense cold stress and act through membrane rigidization, protein/nucleic acid conformation, and/or metabolite concentration, either a specific metabolite or the redox status (Chinnusamy et al. 2010).

Regarding molecular changes in response to cold stress, *Lotus japonica* exposed during 24 h to low temperatures upregulated genes related to lipid, cell wall, phenylpropanoid, sugar, and proline metabolism, while downregulated genes involved in photosynthesis and chloroplast development (Calzadilla et al. 2016). Moreover, the same authors described a total of 41 cold-inducible transcription factors, including members of the AP2/ERF, NAC, MYB, and WRKY families. In this sense, the best understood cold signaling pathway is mediated by *ICE1/CBF/COR* transcriptional cascade. The *C-repeat* (CRT)-*binding factors* (CBFs)/*dehydration-responsive elements* (DREBs) are induced by cold, binding to the promoter of *cold responsive* (*COR*) genes, activating their transcription. There are evidence showing that this pathway is tightly regulated at transcriptional, postranscriptional and postranslational levels (Shi et al. 2014; Kurepin et al. 2013). *CBF14* gene expression is considered as a marker of cold-treatment in wheat and was induced in response to cold treatment in wheat plants, in proportion with their freezing-sensitivity/tolerance being its basal levels higher in cold-tolerant genotypes (Ba et al. 2016). It is noteworthy that *CBF* genes are rapidly and transiently induced by cold while induction of *COR* genes by cold is much slower. A peak of *CBF1* and *CBF3* genes expression after 1 h of cold expossure was measured but expression levels drop off further on while no relevant changes were observed in *CBF2*

(Calzadilla et al. 2016). A higher expression of these TFs in different herbaceous and woody plants has been associated with an increased tolerance to low temperatures (Chinnusamy et al. 2007; Ba et al. 2016; Zhou et al. 2011; Champ et al. 2007; Zhou et al. 2014). Therefore CBFs appear to play a pivotal role in cold stress signaling and response in different plants showing a fine specializaton for the different isogenes. Key components of the ET, GA and JA signaling pathways have been shown to also modulate CBFs in different extents (Achard et al. 2008). Moreover, certain *CBF* genes are induced by exogenous application of ABA, contributing to sustain cold tolerance (Knight et al. 2004).

In Arabidopsis, the CBF/DREB1 pathway is controlled by an MYC-type factor known as ICE1 (Fig. 1). Overexpression of *ICE1* confers increased freezing tolerance whereas *ice1* mutant exhibited high chilling and freezing sensitivity (Chinnusamy et al. 2003). Similarly, in chilling-tolerant tomato (*Solanum chilense*) a higher constitutive expresson of *ICE1* was described (Nosenko et al. 2016). Around 40% of *COR* genes and nearly 46% of cold-regulated transcription factors genes are regulated by ICE1 which suggests that ICE1 functions as a master regulator controlling *CBF3/DREB1A* and many other *COR* genes (Lee et al. 2005). Interestingly, it was recently demostrated that CALMODULIN-BINDING TRANSCRIPTION ACTIVATOR 3 (CAMTA3) and CAMTA5, which bind to the CGCG-box in ICEr2 in order to activate the expression of *DREB1B* and *DREB1C*, only respond to a rapid but not to a gradual decrease in temperature. Moreover, plants appear to differentially regulate daily temperature changes, since CAMTA acts during both day and night whereas usual *DREB1*-regulating circadian components such as CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL 35 (LHY) only function during the day. These findings demostrate that the adquisition of tolerance to low temperatures could follow different pathways depending on the speed of temperature decrease (Kidokoro et al. 2017). In addition to the above mentioned CBF-dependent pathway, some CBF-independent components have a function in cold signaling. In fact, transcriptome analysis, indicated that only ~12% of the *COR* genes are controlled by CBFs (Fowler and Thomashow 2002). The Arabidopsis *esk1* mutant shows constitutive freezing tolerance that is independent of the CBF regulon (Xin and Browse 1998). Loss of *HOS9*, a homeobox transcription factor, causes reduced freezing tolerance without affecting the expression of CBFs and their target genes (Zhu et al. 2004). Also, *GIGANTEA* (*GI*), which encodes a nuclear-localized protein involved in flowering and the circadian clock, is induced by low temperature. The *gi-3* mutant shows both decreased constitutive cold tolerance and impaired cold acclimation ability without affecting *CBF* expression (Cao et al. 2005). It was also suggested that ABA, GA and CK could also regulate cold responses via CBF-independent pathways (Shi et al. 2014; Kosová et al. 2012). Thus, mechanisms dependent and independent of CBF signaling cascade appear to mediate in plant responses to cold and both could be behind the interaction with different phytohormones.

Key Phytohormones Involved in Plant Cold Tolerance

Biological activity of hormones depends on its availability which is controlled by its synthesis, transport and conjugation and catabolism as well as by the efficiency of signal perception and transduction. This processes are also integrated with tight cross-talks among different phytohormones and the interaction of other biological process and signaling molecules (reviewed in Deb et al. 2016). Then, the final plant response to low temperature is not determined by the specific activity of a single hormone but rather by a complex interactive network of diverse signaling molecules. Nearly half of the genes differentially expressed in response to a one-day cold shock in wheat were related to hormone metabolism (Ba et al. 2016), illustrating their key relevance in plant's cold stress response.

Growth-Promoting Phytohormones

Despite what could be first guessed based on their promoting role towards plant development, the phytohormones GAs, AUXs and CKs also display relevant functions in plants cold stress response. In general, growth rate decline at the beginning of cold stress meanwhile plant architecture is modified generally in response to a decrease in GA, AUX and CKs metabolism (Rahman 2013; Vanková et al. 2014).

A decrease in GA content in mostly all organs of wheat plants has been described in response to cold stress (Vanková et al. 2014) mainly due to a rise in the expression of GA-catabolizing *GA2ox* gene, and a repression of GA-biosynthetizing GA20ox enzyme (Kurepin et al. 2013). Besides, *CBF1/DREB1B*-overexpressing plants showed growth retardation, explained in part by the accumulation of DELLA proteins (negative regulators of GA signaling), a phenotype that could be easily rescued by the exogenous application of GAs (Achard et al. 2008). Therefore, it was suggested that *CBF1* enhances the accumulation of DELLA proteins by reducing GA content through the stimulation of *GA2ox* genes expression (Achard et al. 2008). These data indicate that *CBF/DREB1*-dependent signaling pathway partially regulates plant growth through GAs modulation by the regulation of DELLA proteins. It was also shown in tobacco that *CBF* overexpression enhances cold tolerance and causes growth inhibition through the interaction with GAs and cell cycle pathways. Moreover, the observed dwarfism as well as the cold-induced *CBF* expression and its increased cold tolerance were arrested by exogenous GAs application (Zhou et al. 2014), showing that a reduction in the level of this phytohormone is necessary to develop cold tolerance in plants. Additionally, a GIBERELLIC ACID INSENSITIVE (*gai*) mutant showed an impaired GA production and a higher cold tolerance (correlated with elevated levels of CBF1, CBF2 and COR15A proteins), a response mediated by the action of the GATA TFs (Eremina et al. 2016).

The knowledge about the exact role of AUXs in cold stress signaling is still limited. An increase in endogenous IAA level was reported in rice seedlings in response to cold stress (Du et al. 2013a) however cold tolerance was higher in mutants with a lower IAA level (Du et al. 2013b). It is generally considered that intracellular AUX transport and hence, AUX gradient, plays a major role in controlling hormonal crosstalk with other phytohormones and therefore, in the regulation of plant growth and development under cold stress conditions (Rahman 2013). It was described that cold stress inhibit certain processes governed by this phytohormone, such as root gravity response (Shibasaki et al. 2009) which suggests that cold adaptation involves a decrease in internal levels of indol-3-acetic acid (IAA). However, since AUX signaling mutants (*axr1* and *tir1*) showed a reduced gravity response but a normal cold tolerance, it was suggested that cold stress affects more AUX transport rather than AUX signaling (Shibasaki et al. 2009). It was also demonstrated by direct transport assays that cold stress inhibits AUX transport, suggesting that its effect is linked to the inhibition of intracellular transport (Rahman 2013; Shibasaki et al. 2009). Besides that, a crosstalk among IAA, JA and ABA has been reported since low temperature induce JA and IAA accumulation in rice seedlings (Du et al. 2013a) whereas lines overexpressing an enzyme (OsGH3-2), that catalyzes IAA conjugation into amino acids (with the concomitant reduction in free IAA content), finally derived in a rise in ABA levels and in a higher cold tolerance (Du et al. 2012).

As described for GAs and AUX, there is also a drop in CKs content as well as in its direct precursors in response to cold stress (Ba et al. 2016). There is also a differential regulation of genes from CK metabolism under cold stress in wheat plants (Ba et al. 2016) while CK receptors function as negative regulators in plant responses to low temperatures through an still unknown mechanism (Shi et al. 2014; Jeon et al. 2010). CK signaling is based in a multistep two-component system involving the membrane sensor Arabidopsis histidine kinase CKs receptors (AHK2-AHK4), Arabidopsis histidine phosphotransfer proteins (AHP1-AHP5) and Arabidopsis response regulators (ARRs), being the last localized in the nucleus (Jeon et al. 2010). Among ARRs (DNA binding TFs) two types (A and B) have been descibed, being the former negative and the second positive regulators of CKs signaling. It was demonstrated that *AHK2* and *AHK3* and the cold-inducible ARRs (A-type) play a negative regulatory role in cold stress signaling via inhibition of ABA response, occurring independently of the cold acclimation pathway (Jeon et al. 2010).

Cytokinin response factors (CRFs) are transcripiton factors localized in the nucleus, that belong to the APETALA2/ETHYLENE RESPONSIVE FACTOR (AP2/ERF) superfamily, being transcriptionally regulated by CKs and it is proposed that they dislpay an integrative role between environment and hormonal cues (Kim 2016). ARR1, ARR10 and ARR12 were described to upregulate a *CRF2* expression by binding direclty to its promoter in response to cold stress. However, *CRF3* expression remains unaltered in the two-component system mutants, suggesting that cold response of *CRF3* occurs independently of this two-component system (Kim 2016). Both CKs response factors (*CRF2* and *CRF3*) integrate the environmental cold signal into lateral root development, contributing root adaptation to cold stress (Jeon et al. 2016). Similarly, *CRF6* was described to mediate a connection between CKs and oxidative stress response in part by repressing the expression of CK-related genes (Zwack et al. 2016). Apart from CKs singnaling, *CRF*s have been described to be involved in the regulation of other phytohormones such as ABA, SA, JA, AUXs and BRs. Moreover, *CRF*s mediate a subset of CKs responses, acting not only through CK signaling but also independently of CKs (reviewed in Kim 2016). Certain CRFs like *CRF2* (but not *CRF3*) are also capable of transcriptionally regulating AUX efflux carriers (*PIN FORMED-PIN* genes), participating also in the fine-tunning of auxin transport (Šimášková et al. 2015). It was therefore proposed by these authors that *CRF*s represent a missing cross-talk component that regulate auxin transport capacity downstream of CKs signaling to control plant development, exerting a special function under cold stress conditions.

Brassinosteroids are a class of plant steroid hormones whose general effects are the promotion of cell elongation, cell division, differentiation, disease resistance, stress tolerance, exerting also a role in plant senescence. Thus, BRs possess significant growth-controlling activity as well as a function in reproductive and vascular development, membrane polarization and proton pumping. They also influence various other developmental processes like seed germination, rhizogenesis, and flowering (Bartwal et al. 2012). A key point is that BRs function strictly depends on tissue concentration, since opposite effects can be observed at high or low levels (i.e. stomatal aperture or photosynthesis regulation (Ahammed et al. 2015)).

A positive role of BRs in chilling stress tolerance was described in certain crops such as maize, cucumber, tomato and rice (Divi and Krishna 2009). Exogenous application of BR induces the expression of stress-related genes, leading to the maintenance of photosynthetic activity, activation of antioxidant enzymes, accumulation of osmoprotectants and induction of other hormones responses. The former suggest a role for BRs in promoting cold tolerance in Arabidopsis seedlings (Divi and Krishna 2010). Furthermore, mung bean epicotyls whose growth was initially suppressed by chilling partly recovered their ability to elongate after treatment with 24-epibrassinolde (a bioactive BRs). In this experiment, 17 proteins involved in methionine assimilation, ATP synthesis, cell wall construction and the stress response were down-regulated by chilling but re-up-regulated in response to exogenous application of BRs (Huang et al. 2006). The same bioactive BR was applied to young grapevine seedlings causing an enhancement in antioxidant enzymes activities (CAT, SOD and APX) and consequently, a reduction in ROS and lipid peroxidation. In addition, the exogenous treatment greatly increased the contents of free proline, soluble proteins and soluble sugars. These results indicated that exogenous BRs treatment could enhance the antioxidant defense system and reduce oxidative damage caused by ROS and lipid oxidation in plants (Xi et al. 2013). It was also suggested that BRs participate in the enhancement of cold acclimation in winter ryegrass by regulation of stress-related signaling compounds such as JA and ET but not SA (Pociecha et al. 2017).

BRs have been also suggested to underpin cold tolerance in the medicinal plant *Withania somnifera,* an effect that could be linked to the stimulation of ABA or ET biosynthesis (Bartwal et al. 2012). There was also reported that the increased cold tolerance of BRs-treated plants may be attributed to the induced changes in membrane stability, a key process affected by chilling (Bartwal et al. 2012). Recent studies described the existence of several BRASSINAZOLE-RESISTANT (BZR) transcription factors (positive regulators of BRs signaling) which are upregulated in response to exogenous ABA treatment and showed differential expression in response to low temperature stress in *Brassica rapa* plants. These TFs were proposed to activate CBF-mediated cold responses in this species, displaying a key regulatory function in cold tolerance (Saha et al. 2015). In addition to various stimulatory effects on growth, development and stress tolerance in field plants, BRs were described to improve post-harvest quality of fruits and vegetables through extension of shelf life under chilling stress (Ahammed et al. 2015). However other studies support the idea that downregulation of these compounds could be also part of certain plant responses to low temperatures, therefore BRs role in cold tolerance is still elusive (Calzadilla et al. 2016).

Stress-Responsive Phytohormones

ABA regulates many physiological and developmental processes in plants and it is also considered as the most important hormone in the response to abiotic stress (Gómez-Cadenas et al. 2015). In fact, nearly 10% of the protein encoding-genes are regulated by ABA (Nemhauser et al. 2006). ABA accumulation in plant tissues constitutes a common response in order to cope with abiotic stresses, including low temperature (Eremina et al. 2016). However, there are many genes which are induced in response to cold stress but remain irresponsive to ABA (Shinozaki et al. 2003), indicating the existence of both ABA-independent and ABA-dependent signal transduction pathways (Yamaguchi-Shinozaki and Shinozaki 2006). Exogenous application of ABA before the onset of stress generally favors cold tolerance in different plants (Li et al. 2014; Fu et al. 2017). Most of these responses are related to an enhancement of antioxidant defense system, reducing the harmful effect of ROS and other molecules such as NO (Fu et al. 2017; Liu et al. 2011), a key system that is considered the major factor involved in the tolerance to cold stress (Du et al. 2013b). In this sense, both, enzymatic and non-enzymatic antioxidants are stimulated by ABA application during chilling exposure (Wang et al. 2013).

There is still some controversy regarding the precise involvement of ABA in cold stress. Plant stress responses have been divided into ABA-independent, such as the above mentioned CBF and ICE1-regulated; and ABA-dependent responses, including bZIP transcription factors known as ABA Responsive Element Binding Protein/Factors (AREB/ABF) (Nakashima et al. 2014). Moreover, a parallel effect of dehydration/osmotic stress occurs during cold stress, being water deficit also common consequence of low temperatures, inducing therefore overlapping responses between dehydration and low temperatures (Fu et al. 2017). The implication of ABA on the tolerance to water deficit is well-known (Gómez-Cadenas et al. 2015; Gómez-Cadenas et al. 2014) and involves ABA-dependent and ABA-independent mechanisms (Nakashima et al. 2014), whereas cold stress mostly triggers and ABA-independent pathway (Yamaguchi-Shinozaki and Shinozaki 2006; Fu et al. 2017; Vishwakarma et al. 2017). However, ABA has been recently proposed to control *COR* genes expression through the regulation of CBFs (Eremina et al. 2016). Thus, there appears to be an intricate interaction among genes which is regulated by dehydration and low temperatures, evidenced by a reduced expression of cold and drought stress related genes in an Arabidopsis *los5* mutant (negatively affected in ABA biosynthesis) which also showed an extreme sensitivity to low temperatures (Xiong et al. 2001). ABA content increases during plant exposure to cold being the more tolerant genotypes which showed a faster and higher ABA accumulation compared to the sensitive ones (Kosová et al. 2012; Janowiak et al. 2002). Moreover, key enzymes (β-carotene-3-hydroxylase-βCHX, zeaxanthin epoxidase-ZEP and 9-*cis*-epoxycarotenoid dioxygenase-NCED1) involved in ABA biosynthesis were induced in wheat plants in response to low temperature, being this rise more intense in cold-tolerant varieties (Ba et al. 2016). ABA catabolism was also down-regulated in those cold tolerant plants through the repression of a cytochrome P450 8-hydroxylase gene (*CYP707A2*), which catalyzes the first step of ABA catabolism toward phaseic and dihydrophaseic acids (Ba et al. 2016). Furthermore, a negative interaction between ABA and CKs signaling was described in rice seedlings under cold stress, showing that the cold-induced ABA increase is linked to CKs signaling (Maruyama et al. 2014).

Interestingly, exogenous application of certain molecules increases the ABA levels leading to an alleviation of cold stress. In this sense, melatonin, which was recently discovered in plants and it is present in high amounts in several medicinal plants such as feverfew (*Tanacetum parthenium*), St John's wort (*Hypericum perforatum*), and Huang-qin (*Scutellaria baicalensis*) (Bajwa et al. 2014), was shown to induce the antioxidant defense via ABA-dependent and ABA-independent pathways in the grass *Elymus nutans* (Fu et al. 2017). Melatonin increases ABA levels but there were no evidence of a reciprocal effect of ABA on melatonin endogenous content, suggesting that this compound should be acting upstream of ABA (Fu et al. 2017). Similarly, celastrol, a triterpenoid isolated from *Tripterygium wilfordii* which is used to treat some systemic illnesses, was demonstrated to enhance cold tolerance in cucumber by inducing the activities of antioxidant enzymes, reducing the oxidative stress and increasing the ABA levels by upregulating its biosynthesis through the expression of *NCED2* gene (Zhu et al. 2017).

ET is involved in abiotic stress resistance and specifically in cold stress response however, the question of whether it exerts a positive or negative role remains still elusive. Some works report changes in ET production in response to low temperatures (Kazan 2015). In Arabidopsis, an increase in ET production was related to a higher activity of biosynthetic enzymes ACC synthase and ACC oxidase in response to cold while the application of the ET precursor 1-aminocyclopropane-1-carboxylic acid (ACC) enhanced cold tolerance (Catala et al. 2014). Similarly, the application of the ET biosynthesis inhibitor 1-methylcyclo- propene (1-MCP) reduces cold tolerance in tomato, suggesting a positive ET effect on cold tolerance (Zhao et al. 2009).

Ethylene perception and response in plants is carried out by a two-component receptor kinases system. ET binds to its receptors ETHYLENE-RESPONSIVE1 (ETR1) which are a superfamily of negative regulators of this signal and activates the signaling cascade through CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1), a Ser/Thr kinase that dimerizes when active and suppresses the ethylene response. CTR1 controls ethylene-insensitive 2 (EIN2) cleavage, a membrane integral protein that induces downstream nuclear TFs such as ETHYLENE INSENSITIVE 3 (EIN3) and ein3-like 1 (EIL1) which stimulates ethylene response genes (Merchante et al. 2013). In the absence of ET, active receptors negatively regulate EIN2 through phosphorylation via the protein kinase CTR1. Upon ethylene perception, the receptors become inactivated, which in turn facilitates EIN2 activation to mediate ethylene signaling through the nucleus, inducing the activity of EIN3/EIL1 and ERFs (Merchante et al. 2013).

ERFs are TFs located downstream in the signaling pathways of many hormones such as ET, JA and ABA, and are possibly a cross-talk point among these signaling pathways. ERFs family is huge and each member responds to different biotic and abiotic stresses, regulating many biosynthetic pathways (Mizoi et al. 2012). The overexpression of soybean *GmERF9* in tobacco enhanced the accumulation of proline and soluble carbohydrates as well as cold tolerance compared to wild type, indicating a positive role of this TF cold tolerance (Zhai et al. 2017). Tomato *ERF2* showed a role in regulating ethylene biosynthesis and a higher expression was determined under cold stress, implying that this gene may be involved in cold response through ethylene modulation. Moreover, overexpression of this gene in tobacco showed a higher expression of *COR* genes, an enhanced cold tolerance and a reduced membrane damage (Zhang and Huang 2010). ET can also induce the expression of calcium dependent kinases (*CDPK* genes), which positive role in cold tolerance has been previously described (Ye et al. 2017) in many different plants including banana (Wang et al. 2017), tomato (Chang et al. 2009), grape (Zhang et al. 2015) and rubber tree (Xiao et al. 2017). However this family is integrated by at least 30 different members which exhibit different expression patters, suggesting that their roles are distinct during plant development and stress response (Xiao et al. 2017). *LeCFB1* expression and cold tolerance in tomato plants and fruits was induced by ET as well as by ethephon application, underpinning a positive role of this hormone on cold tolerance (Zhao et al. 2009).

Contrastingly, other studies determined at least a 90% reduction in ET content in *Medicago truncatula* plants after cold exposure together with a lower ACC oxidase activity (Zhao et al. 2014). This reduction is consistent with a negative role of ET towards cold tolerance in accordance with the fact that its exogenous application (or the application of its direct precursor ACC) causes a significant drop in *M. truncatula* freezing tolerance. Moreover, the application of ET inhibitors promoted this tolerance through *CBF3* expression regulation (Zhao et al. 2014). Supporting also this negative role of ET, cold tolerance was reduced in Arabidopsis *ethylene overproducer1* as well as after the application of ethylene precursor ACC whereas it was increased with the application of an inhibitor of ET biosynthesis or perception (Shi et al. 2012). Moreover, ET insensitive mutants (*etr1-1, ein4-1, ein2-5, ein3-1,* and *ein3 eil1*) showed an enhanced freezing tolerance and they proposed that ET negatively regulates cold signaling at least partially through the direct transcriptional control of *CBF*s and *type-A ARR* genes by EIN3 (Shi et al. 2012). Therefore, it appears that the role of ET on cold tolerance could be species-dependent and that certain differences would exists between cold and freezing tolerance for this phytohormone.

JA positive role in cold and freezing tolerance was recently unveiled by different works, suggesting that cold exposure elevates endogenous JA levels by the induction of biosynthetic genes such as *LOX1, AOS1, AOC1* and *JAR1* in Arabidopsis (Hu et al. 2013) and *OsAOS, OsOPR1* and *OsLOX2* in rice (Du et al. 2013a). Furthermore, JA positively regulates Arabidopsis response to freezing since its exogenous application significantly enhanced freeze tolerance whereas the blockage of its endogenous biosynthesis and signaling rendered cold-hypersensitive plants (Hu et al. 2013). JA is described as a positive regulator of the ICE1 protein transcriptional activity (Zhao et al. 2013), which exerts a key role in cold signaling cascade (Fig. 1) as well as an inducer of CBFs expression in Arabidopsis (Hu et al. 2013), modulating cold stress responses. Therefore, jasmonate function is a critical upstream signal in ICE1-CBFs pathway to positively regulate low temperature tolerance in Arabidopsis (Hu et al. 2013).

In the same line, cold-tolerant tomato species showed a higher expression of the *FAD7* gene (Nosenko et al. 2016), which plays a role in JA biosynthesis, encoding a chloroplast ω-3 fatty acid desaturase involved in temperature-dependent remodeling of the thylakoid membrane fluidity (Upchurch 2008). Similarly, in the leaves of *Camellia sinensis* a higher expression of *FAD7* and *FAD8* genes was associated with a reduced damage in thylakoid membranes after cold exposure (Ding et al. 2016). An induction of JA and its precursor, α-linolenic acid production were described in *Camellia japonica* during cold stress (Li et al. 2016) and the rise in expression of JA biosynthetic genes indicate that JA and its signaling pathway may play critical roles in the early stages of cold acclimation in *C. japonica*. In this plant, genes from GAs, AUXs, ET, ABA, JA, CKs and BRs pathways were also identified to be differentially expressed during cold acclimation (Li et al. 2016). Thus, GA, ET, ABA and JA signaling were predominantly induced while the AUX, BR and CK pathways were mostly inhibited (Li et al. 2016).

A recent and detailed review about cross-talk among different phytohormones in rice suggest a tight interaction towards abiotic stress survival in plants (Deb et al. 2016). CKs conjugation is regulated by ABA and BRs whereas BRs biosynthesis is affected by both, ABA and CKs. JA and ET biosynthesis appears to be modulated by ABA through DREB factors and JA and SA pathways are also co-regulated but unlikely to influence each other production. Thus, a complex regulatory network is spun by multiple hormones which may modulate hormone biosynthesis pathways where biosynthesis of one hormone is affected by several others.

Apart from phytohormones, other compounds were described to exert as messengers among different plant tissues and to trigger mechanisms associated with cold tolerance in plants. Such is the case of melatonin, which local exogenous application in *Citrullus lanatus* confers cold tolerance not only at the application site but also in distant tissues. Such induction is associated with an enhanced antioxidant capacity and optimized defense gene expression such as heat shock proteins, peroxidases and lipoxygenases (Li et al. 2017). Moreover, many of the observed upregulated genes were related to phytohormone signaling; melatonin pretreatment alleviated cold-induced down-regulation *ERFs*, key genes in ET signaling network, and up-regulated other *ERF* transcription factors. Other intermediaries in AUX, CK and GA metabolisms were also regulated by melatonin treatment and ABA receptor (*PYL8*) was notably suppressed by melatonin plus cold application but not by cold alone (Li et al. 2017), suggesting an important role of all these phytohormones in the observed cold tolerance induction by melatonin pre-application.

Medicinal Plants and Cold Stress Response

Many phryganic plants (*Thymus sibthorpii, Satureja thymbra, Cistus incanus, Phlomis fruticosa, Teucrium polium*) are aromatic or medicinal and display the phenomenon of seasonal dimorphism (Lianopoulou and Bosabalidis 2014), developing mechanical and chemical defensive barriers to cope with cold stress. These plants induce morphological, anatomical, cytological, physiological, and biochemical defense mechanisms and most of these changes are mediated by phytohormones. In order to cope with cold conditions, *Origanum dictamnus* was described to induce structural and functional changes affecting leaves shape, size and distribution in favor of small and apical ones. Mesophyll cells develop large intracellular spaces with the aim of accumulating air at higher temperature (Lianopoulou and Bosabalidis 2014). This plant also stimulates the development of a thick and dense layer of non-glandular trychomes as a protective shield together with a thick cuticular/waxy layer on the leaf epidermis in response to cold. Essential oils are more abundantly secreted by glandular hairs under cold, being the major constituent p-Cymene (60%) during the winter and Carvacrol (42%) during the summer (Lianopoulou and Bosabalidis 2014).

Similarly, *Salvia sclarea*, commonly known as clary sage and an important medicinal herb with high market demand, showed a reduction in individual leaf area but an increased length and number of spikes and a longer inflorescence with higher content of essential oils under cold conditions (Kaur et al. 2015). The mountain germander (*Teucrium polium*) and *Thymus sibthorpii* are also structurally affected by chilling stress since winter leaves are smaller and thicker, have more stomata and glandular hairs while epidermal and mesophyll cells contain in their vacuoles dark phenolic and calcium oxalate crystals (Lianopoulou et al. 2014a, b). Thus, in general, winter plants exhibit small, thick, and curled leaves, high densities of glandular and non-glandular hairs, compact mesophyll with developed palisade parenchyma, numerous stomata and sclerenchymatic fibers, and abundant phenolics in the epidermal and mesophyll cells (Fahn and Cutler 1992).

All these morphological and structural changes are regulated by phytohormones, mostly ABA, which is also involved in stomatal closure, resulting in a decline in photosynthesis as occurred in winter leaves of *O. dictamnus* (Lianopoulou and Bosabalidis 2014). However, a higher photosynthetic rate and stomatal conductance was measured in *Teucrium polium* and *Thymus sibthorpii* winter leaves (Lianopoulou et al. 2014a, b), showing that physiological changes also depend on the plant species considered. Chloroplasts showed relevant modifications under cold weather, with the presence a few grana and the appearance of lipid grains in plastoglobuli (Lianopoulou and Bosabalidis 2014). Chloroplastic membranes are strongly affected by cold stress, showing signs of damage and lipid accumulation during low temperature exposure. Thylakoids swell and distort, starch granules disappear, and a peripheral reticulum (vesicles arising from inner membrane of chloroplast envelope) appears, driving finally to chloroplast disintegration after prolonged chilling (Kratsch and Wise 2000).

Apart from structural changes observed in many medicinal and aromatic plants, it was reported that certain environmental stresses are linked to a higher accumulation of antioxidant compounds in different medicinal plants (Lianopoulou and Bosabalidis 2014; Mir et al. 2015; Saema et al. 2016), contributing in most cases to increase their medical or nutritional value (Mir et al. 2015; Nourimand et al. 2012). Therefore, the knowledge of how cold stress is affecting medicinal plants and the possible impact on bioactive compounds accumulation in relevant species is valuable (Mir et al. 2015). Moreover, understanding how plant hormones are mediating in these responses could be a key tool for future manipulation of plants in the search of bioactive molecules.

Cold severely impacts on biomass production and slightly chlorophyll and β-carotene content in fennel (*Foeniculum vulgare*) seedlings growing at 2 °C for 2, 3 and 4 h whereas total antioxidant activity increased in response to cold (Nourimand et al. 2012). Relevant differences in membrane damage among 11 fennel varieties have been described (Xiao et al. 2011), showing that tolerance to cold and therefore, adaptation to different environments, is also dependent on the variety.

The Indian ginseng or ashwagandha (*Withania somnifera*) showed an increase in withanolide (steroidal lactones, key bioactive compound from this species) accumulation in the leaves in response to cold stress (Mir et al. 2015; Kumar et al. 2012) which also was linked to a better recovery after cold exposure in *W. somnifera* transgenic plants (Saema et al. 2016). Cold also caused a concomitant rise in antioxidant enzymatic activity of CAT, SOD, APX and GR in Indian ginseng (Mir et al. 2015) as well as in *Thymus sibthorpii* leaves (Lianopoulou et al. 2014b). This is part of the antioxidant system in charge of protecting aerial tissues from cold damage. Contrastingly, the root tissue showed a slight reduction in withanolides concentration during cold stress (Mir et al. 2015), suggesting that different plant organs have developed specific mechanisms towards cold survival and that an internal balance in the distribution of these compounds could be crucial upon survival. The medicinal properties of *W. somnifera* have been attributed to these withanolide compounds, synthesized in leaves and roots, which biosynthesis and accumulation are directly influenced by environmental factors. In this plant, seasonal low temperature appears to exert a key role in increasing the accumulation of bioactive withanolides (Kumar et al. 2012).

Certain enzymes have been studied in order to understand possible mechanisms behind cold tolerance in *W. somnifera.* Sterol glycosyltransferases (SGT) are enzymes that glycosylate sterols, which are crucial in plant adaptation to low temperatures. The *WsSGTL1* gene is specific for 3β-hydroxy position and has a catalytic specificity to glycosylate withanolides and sterols. Glycosylation not only stabilizes the products but also alters their physiological activities and governs intracellular distribution (Ullmann et al. 1993). The clonation of *WsSGTL1* gene in *Arabidopsis thaliana* showed an increase in cold tolerance in transgenic plants linked to a faster formation of sterol glycosides as well as a higher enzymatic activity (Mishra et al. 2013). Furthermore, the overexpression of this gene enhanced tolerance to cold together with a better photosynthetic performance and quenching regulation of PSI and PSII as well as a higher stomatal conductance after cold exposure (Saema et al. 2016). These enzymes are able to glycosylate also steroidal hormones, such as BR which function as growth promoters and development regulators (Bartwal et al. 2012). In this sense, the activation of SGTs in response to cold could be slowing down plant growth in order to prioritize cold survival through the regulation of BR metabolism (Mishra et al. 2013). SGTs in plants are also involved in sensitivity to stress-related hormones and in differences found in the relative tolerance to biotic and abiotic stresses (Chaturvedi et al. 2012).

Concluding Remarks

In this chapter, the latest literature about cold responses and phytohormones, with special emphasis in medicinal and aromatic plants was reviewed. Most mechanisms triggered by plants in order to cope with cold stress are conserved among different species involving changes in architecture and morphology as well as in primary and secondary metabolism. Therefore, examples about model plants and different crops were included to illustrate the complexity of these phenomena. Phytohormones act together as an orchestrated network in order to finely tune main changes in response to low temperatures, being their regulation crucial for plant acclimation and survival. Further studies should be focused on the modification of cold-signaling and response pathways with the aim of stimulating desired responses (i.e. accumulation of health-related phytochemicals that are induced by cold in medicinal plants) and plant adaptation to challenging environments.

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The Impact of Drought Stress on the Quality of Spice and Medicinal Plants

Dirk Selmar, Maik Kleinwächter, Sara Abouzeid, Mahdi Yahyazadeh and Melanie Nowak

Abstract This review addresses the well known phenomenon that spice and medicinal plants grown under semi-arid conditions generally reveal significantly higher concentrations of relevant natural products than identical plants, which however are cultivated in moderate climates. Unfortunately, only limited information on this intriguing phenomenon is available. The corresponding data are compiled, the relevant aspects are outlined, and the metabolic background is presented. Based on these reflections, it becomes obvious that drought stress and the related metabolic changes are responsible for the higher natural product accumulation in plants grown in semi-arid regions. In principle, there are three causes: first, the effect might be a consequence of a reduced production of biomass in the stressed plants. Even without an enhancement of biosynthesis of natural products, their concentration on dry or fresh weight basis simply will be elevated. Secondly, the drought stress enhances the actual rate of biosynthesis of natural products due to a passive shift or thirdly, due to an active up-regulation of the enzymes involved in natural product biosynthesis. The latter both options are related to the strongly enhanced reduction status of the leaves exposed to drought stress. The over-reduction, which goes along with a strongly enhanced concentration of $NADPH+H^+$, directly results in a passive increase of all processes consuming $NADPH+H^+$, including the biosyntheses of highly reduced secondary plant products. Alternatively, the enzymes responsible for the biosynthesis of natural products could be actively up-regulated. The corresponding increment in $NADPH+H^+$ consumption significantly contributes to the dissipation of the stress related surplus of energy and thus, is part of the machinery that prevents the generation of toxic oxygen radicals.

Keywords Drought stress · Secondary metabolites · Over-reduced state · Energy surplus · Medicinal plants

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Introduction

One of the most stated assertions with respect to the main issue of this book undoubtedly is: "*Aromatic and spice plants grown in semi-arid areas such as the Mediterranean regions generally are much more aromatic than identical plants of the same species, which however have been cultivated in moderate climates*". Frequently, this well known phenomenon is explained by the trivial statement that plants grown in Southern Europe "*…are exposed to much more sunlight, resulting in enhanced rates of biosynthesis*". Indeed—at least on the first sight—such assertion appears to be consistent. However, sunlight is not at all a limiting factor for plant growth; even in Central Europe—especially, when the plants grow in open areas without any shade. Accordingly, they absorb much more light energy in the leaves than is required and utilized for photosynthetic CO_2-fixation (Wilhelm and Selmar 2011). Consequently, there must be another explanation for this phenomenon, which generally is stated to be a matter of common knowledge (Selmar and Kleinwächter 2013a). This chapter is aimed to illustrate this syndrome and to elucidate the corresponding scientific background. As a matter of fact, in this context we have to consider various levels of contemplation, which are treated consecutively.

We all are aware that—at least in the semi-arid and arid subtropics—high irradiation often is co-occurring with water deficiencies. In consequence, under these conditions, plants frequently suffer drought stress. Since stress related reactions extensively impact the entire metabolism, the synthesis and accumulation of secondary metabolites also should be affected. Whereas in the past these coherences have not been considered adequately, meanwhile this topic is well established (Kleinwächter and Selmar 2014, 2015).

The Concentration of Natural Products Frequently Is Enhanced in Drought Stressed Plants

It is well known that various environmental conditions, such as the temperature, the light regime, the nutrient supply, etc., strongly influence the synthesis and accumulation of secondary plant products (for review see, e.g., Gershenzon 1984; Falk et al. 2007; Das and Bhattacharya 2016). Consequently, much more severe environmental influences, such as typical stress situations, which are known to strongly impact on the entire general metabolism (Sampaio et al. 2016; Bohnert et al. 1995), also must alter the secondary metabolism. By now, a tremendous lot of information on the impact of various biological stresses, e.g., pathogen or herbivore attack, on elicitation of the synthesis of natural products is available (for review see, e.g., Harborne 1988; Hahlbrock et al. 2003; Hartmann 2007; Namdeo 2007; Wink 2010). Further on, many papers deal with the impact of abiotic stresses on the secondary metabolism; nonetheless the knowledge about the related biological background is still limited (for review see, e.g., Ramakrishna and Ravishankar 2011; Selmar and Kleinwächter 2013a, b; Kleinwächter and Selmar 2014).

Meanwhile, a large number of studies manifested that plants exposed to drought stress indeed accumulate higher concentrations of secondary metabolites than those cultivated under well watered conditions (Table 1). Obviously, the drought stress related enhancement in the concentration is a common feature, which concerns all different classes of natural products. Corresponding increases are reported for simple as well as for complex phenols, and also for the various classes of terpenes (Table1). In the same manner, the concentrations of nitrogen containing substances, such as alkaloids, cyanogenic glucosides, and glucosinolates, are also positively impacted by drought stress (Table 1). There is no doubt that drought stress consistently enhances the concentration of secondary plant products. However, in this context, we have to consider that the drought stressed plants generally are reduced in their growth. Accordingly, the stressed plants reveal a far lesser biomass than the well watered controls.Drought stress increases the concentration of natural products

Due to this reduced production of biomass—even without any enhancement in the rate of biosynthesis of natural products—their concentration on dry or fresh weight basis simply will be elevated. Corresponding explanations frequently are reported in the literature for review see Kleinwächter and Selmar (2014). As most of the related studies focus on the concentration of active compounds and do not consider the overall plant production, very often data on the overall biomass per plant are lacking. A further reason for this deficit of information might be due to the fact that mostly only one certain plant part or plant organ was studied, e.g., roots, leaves, or seeds. Nonetheless, in few papers the total contents of secondary plant products per entire plants are given or could be calculated from the data published.

The Overall Amount of Natural Products Is Increased by Drought Stressed

In sage (*Salvia officinalis*) drought stress results in a massive increase in the concentration of monoterpenes (Nowak et al. 2010). As this enhancement is far higher than the corresponding reduction in biomass, the entire amount of monoterpenes present in the sage plants suffering drought stress is significantly higher than in the well watered controls. In consequence, in this particular case, the actual biosynthesis of monoterpenes is increased in response to drought stress. Corresponding experiments with parsley (*Petroselinum crispum*) revealed that the drought stress related concentration enhancement of monoterpenes in the leaves is more or less completely compensated by the accompanied loss in biomass. Thus, the overall contents of essential oils are quite similar in drought stressed and in well watered plants (Petropoulos et al. 2008). Corresponding results for Greek oregano (*Origanum vulgare*) have been elaborated by Ninou et al. 2017, which showed that the overall content of essential oils per plant remained constant although the

Table 1 Drought stress increases the concentration of natural products

Simple Phenols			
Helianthus annuus	chlorogenic acid	massive increase (tenfold)	del Moral 1972
Prunus persica	total phenols	higher contents	Kubota et al. 1988
Thymus capitatus	phenolics	higher contents	Delitala et al. 1986
Echinacea purpurea	total phenols	strong increase (67 %)	Gray et al. 2003
Crataegus spp.	chlorogenic acid	massive increase (2 - 6fold)	Kirakosyan et al. 2004
Hypericum brasiliense	total phenols	strong increase (over 80 %)	de Abreu & Mazzafera 2005
Trachyspermum ammi	total phenols	strong increase (100 %)	Azhar et al. 2011
Labisia pumila	total phenols	significant increase (50 %)	Jaafar et al. 2012
Complex Phenols			
Pisum sativum	flavonoids	strong increase (45%)	Nogués et al. 1998
Pisum sativum	anthocyanins	strong increase (over 80 %)	Nogués et al. 1998
Crataegus spp.	catechins / epicatechins	massive increase (2 - 12fold)	Kirakosyan et al. 2004
Hypericum brasiliense	rutine / quercetin	massive increase (fourfold)	de Abreu & Mazzafera 2005
Hypericum brasiliense	xanthones	strong increase (over 80%)	de Abreu & Mazzafera 2005
Camellia sinensis	epicathecins	higher contents	Hernández et al. 2006
Salvia miltiorrhiza	furoquinones	significant increase	Liu et al. 2011
Prunella vulgaris	rosmarinic acid	slight increase	Chen et al. 2011
Labisia pumila	anthocyane / flavonoids	significant increase	Jaafar et al. 2012
Monoterpenes / Essential Oils			
Mentha x piperita ssp.	essential oils	significant increase	Charles et al. 1990
Cymbopogon pendulus	geraniol & citral	strong increase	Singh-Sangwan et al. 1994
Pinus halepensis	α-pinen, carene	strong increase	Llusià & Penuelas, 1998
Cistus monospeliensis	caryophyllene	enormous increase	Llusià & Penuelas, 1998
Satureja hortensis	essential oils	increase	Baher et al. 2002
Picea abies	monoterpenes	strong increase	Turtola et al. 2003
Pinus silvestris	monoterpenes	strong increase	Turtola et al. 2003
Petroselinum crispum	essential oils	strong increase (double)	Petropoulos et al. 2008

(continued)

Table 1 (continued)

Salvia officinalis	essential oils	massive increase (2 - 4fold)	Beattaieb et al. 2009
Salvia officinalis	monoterpenes	strong increase	Nowak et al. 2010
Scrophularia ningpoen.	iridoid glycosides	increase	Wang et al. 2010
Nepeta cataria	essential oils	significant increase	Manukyan, 2011
Ocimum basilicum	essential oils	significant increase	Forouzandeh et al. 2012
Thymus vulgaris	monoterpenes	increase	Kleinwächter et al. 2015
Petrosilinum crispum	essential oils	massive increase	Kleinwächter et al. 2015
Origanum vulgare	essential oils	increase	Ninou et al. 2017
Origanum vulgare			
subsp. gracile	essential oils	significant increase	Morshedloo et al. 2017
subsp. virens	essential oils	no significant increase	Morshedloo et al. 2017
Di- and Triterpenes			
Solanum tuberosum	steroid alkaloids	strong increase	Bejarano et al. 2000
Hypericum brasiliense	betulinic acid	strong increase	de Abreu et al. 2005
Bupleurum chinense	saikosaponin	significant increase	Zhu et al. 2009
Prunella vulgaris	triterpenes	slight increase	Chen et al. 2011
Glycyrrhiza glabra	glycyrrhizinc	massive increase	Nasrollahi et al. 2014
Alkaloids			
Senecio longilobus	pyrrolizidine alkaloids	strong increase	Briske & Camp, 1982
Lupinus angustifolius	quinolizidin alkaloids	strong increase	Christiansen et al. 1997
Solanum tuberosum	steroid alkaloids	strong increase	Bejarano et al. 2000
Glycine max	trigonelline	higher contents	Cho et al. 2003
Papaver somniferum	morphine alkaloids	strong increase	Szabó et al. 2003
Catharanthus roseus	indole alkaloids	strong increase	Jaleel et al. 2007
Phellodendron amurense	benzylisoquinolines	strong increase	Xia et al. 2007
Senecio jacobaea	pyrrolizidine alkaloids	massive increase	Kirk et al. 2010
Nicotiana tabacum	Nicotiana-alkaloids	strong increase	Çakir & Çebi, 2010
Chelidonium majus	benzylisoquinolines	increase	Kleinwächter et al. 2015
Various Classes			
Manihot esculenta	cyanogenic glucosides	strong increase	de Bruijn, 1973
Triglochin maritima	cyanogenic glucosides	strong increase	Majak et al. 1980

(continued)

Table 1 (continued)

Brassica napus	glucosinolates	massive increase	Jensen et al. 1996
Coffea arabica	γ-aminobutyric acid	massive increase (tenfold)	Bytof et al. 2005
Brassica oleracea	glucosinolates	significant increase	Radovich et al. 2005
Brassica carinata	glucosinolates	significant increase	Schreiner et al. 2009
Phaseolus lunatus	cyanogenic glucosides	higher content in stressed plants	Ballhorn et al. 2011
Tropaeolum majus	glucotropaeolin	higher content in stressed plants	Bloem et al. 2014

concentrations significantly increased. On the first sight, it seems that in these cases no change in the rate of monoterpene biosynthesis occurs. However, if we consider that the equal amount of monoterpenes is produced by a far lower biomass, it becomes obvious that the biosynthetic activity per dry weight must strongly be elevated. This problem to use an appropriate reference value was exemplarily outlined for the stress related increase of monoterpenes in thyme (Paulsen and Selmar 2016). As already mentioned for parsley, also in drought stressed thyme plants a lower gain of biomass seemed to be responsible for the increase in monoterpene concentration. Accordingly, no change in the total content of monoterpenes and thus in their overall biosynthesis could be pointed out. However, a detailed and thorough consideration revealed that the rate of biosynthesis on dry weight basis was indeed different in stressed and well watered plants. Whereas the biosynthetic activity (on dry weight basis) in the first phase of the experiment was much higher in the stressed plants in comparison to the well watered controls, the situation was reverse, when the stress situation was prolonged (Paulsen and Selmar 2016). For clarification, this complex situation—with special emphasis on the importance of the employment of appropriate reference values—is displayed in Fig. 1. These intricate coherences suggest that—over the course of time—an adaption to the drought stress occurred, which finally leads to a reduction of the formerly enhanced rate of biosynthesis. Unfortunately, due to the large efforts required, these multifactorial coherences cannot be studied and considered adequately in routinely investigations. Consequently, the interest and aim of a certain study determine the focus on appropriate reference values, such as concentration, total amount, overall production, or composition.

Similar situations have been observed with respect to phenolic compounds. In *Hypericum brasiliense* plants, both, concentration and the total amount of the phenolic compounds, are drastically enhanced under drought stress (de Abreu and Mazzafera 2005). Although the stressed *H. brasiliense* plants were quite smaller than the well watered controls, due to their tremendous increase in the concentration of phenolic compounds, their overall content (product of biomass and concentration) was significantly higher in the stressed plants (de Abreu and Mazzafera 2005). In the same manner, the overall amount of anthocyanins was about 25% higher in stressed peas (*Pisum sativum*) than in plants cultivated under standard conditions

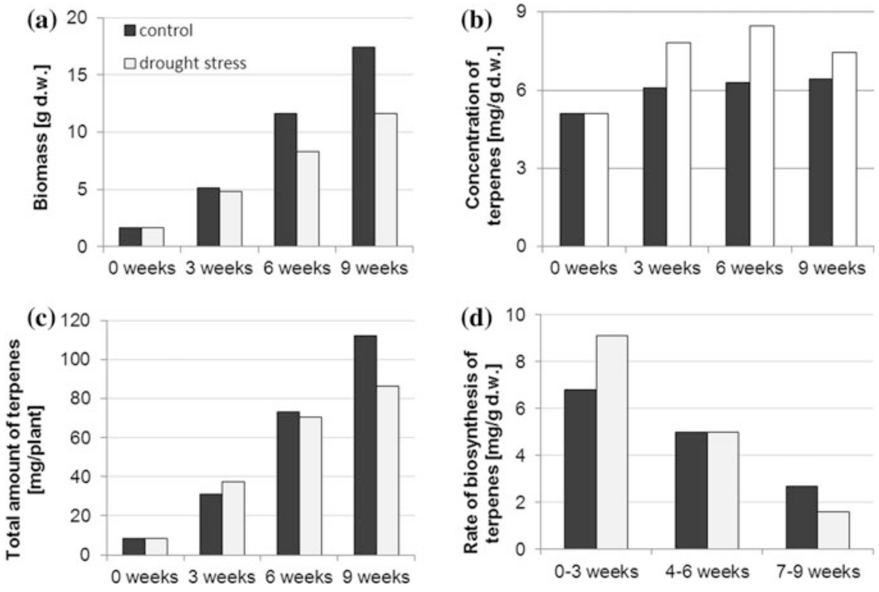

Fig. 1 Juxtaposition of the relevant parameters in well-watered and drought stressed thyme plants. The comparison of biomass (**a**), concentration of terpenes (**b**), total amounts of terpenes (**c**) and rates of bio-synthesis of terpenes (**d**) in drought stress thyme (Paulsen and Selmar 2016) illustrates the complex problematic of choosing the correct reference value as well the multilayered metabolic processes that change in the course of the prolongation of the stress situation

(Nogués et al. 1998). Jaafar et al. (2012) reported that in *Labisia pumila* plants not only the concentration, but also the overall production of total phenolics and flavonoids per plant was increased in plants suffering drought stress.

Unfortunately, nearly all of the studies dealing with the impact of drought stress on nitrogen containing natural products focus just on the concentrations of these compounds in certain organs. In most of the cases no data on the biomass and thus on the total amount of natural products per entire plant are available. Kleinwächter et al. (2015) demonstrated that the stress related increase in the concentration of coptisine is overcompensated by the strongly reduced gain in biomass of the stressed plants, resulting in significant lower overall alkaloid content in the stress plants. Up to now, no conclusion about the impact of drought stress on the biosynthesis of these natural products could be drawn.

In summary: in nearly all plants analyzed, the concentrations of secondary plant products are significantly elevated under drought stress conditions. Unfortunately, only in few cases, also a corresponding increase of the total content of the natural compounds per plant is reported. This could be either due to the lack of data on the biomass of the corresponding plants, or to the fact that the stress related decrease in biomass frequently overcompensates the increase in the concentration of relevant natural products.

Metabolic Background

Due to our recurring experience in daily life, we all have internalized that energy saving represents one of the most important issues in our subsistence. Indeed—on the first sight—it seems reasonable to transfer such statements and considerations also into plant biology. However, when beholding more closely, it becomes obvious that—in contrast to heterotrophic organisms—plants have no problem to cover their energy requirements. By contrast, in general, they absorb much more energy than being required for photosynthetic CO_2-fixation (Wilhelm and Selmar 2011). This basic statement easily can be deduced from a fact we all are familiar with: elevation of ambient CO_2 concentration results in a massive enhancement of photosynthesis—the corresponding rate of photosynthesis easily could be doubled or even tripled (Wilhelm and Selmar 2011). This however means that the tremendous oversupply of energy has to be dissipated in order to avoid massive damages by oxygen radicals, which otherwise would be generated by an overflow of the electron transport chain (Reddy et al. 2004; Szabó et al. 2005). During evolution, plants have developed various mechanisms for effective energy dissipation, i.e., non-photochemical quenching, photorespiration, or xanthophyll cycle (Fig. 2). Indeed, this protecting system operates properly under regular environmental conditions, however, any stress situation induces massive imbalances. This especially accounts for the drought stress. Water shortage induces stomata closure, which massively diminishes the CO_2-influx into the leaves. In consequence, far less reduction equivalents ($NADPH+H^+$) are consumed and re-oxidized, respectively via the Calvin cycle. Although the various energy dissipating mechanisms are up-regulated, the reduction status of the chloroplasts increases massively (Selmar and Kleinwächter 2013a, b; Kleinwächter and Selmar 2015). As result, electrons from the photosynthetic electron transport chain will directly be conveyed to oxygen, generating superoxide radicals, which subsequently have to be detoxified by superoxide dismutase (SOD) and ascorbate peroxidase (APX). As a further consequence of this over-reduction, the ratio of $NADPH+H^+$ to $NADP^+$ is strongly enhanced. According to the law of mass action, all processes consuming $NADPH+H^+$, e.g. for the biosyntheses of highly reduced secondary plant products, such as isoprenoids, phenols or alkaloids (Selmar and Kleinwächter 2013b; Kleinwächter and Selmar 2015) will be favoured even without changing any enzyme activity (Fig. 2). These straightforward coherences easily but conclusively explain why the rate of biosynthesis of certain natural products might be enhanced under stress conditions although the corresponding enzyme activities had not been changed at all.

Alternatively, to the increase in natural product biosynthesis due to the passive shift mentioned above, the biosynthesis might also be elevated by a stress related up-regulation of the corresponding enzymes. Unfortunately, up to now, only limited data are available on this very interesting issue. In contrast to the tremendous lot of information dealing with the impact of biological stress (e.g., pathogen or herbivore attack) on the expression of enzymes involved in natural product biosynthesis, only

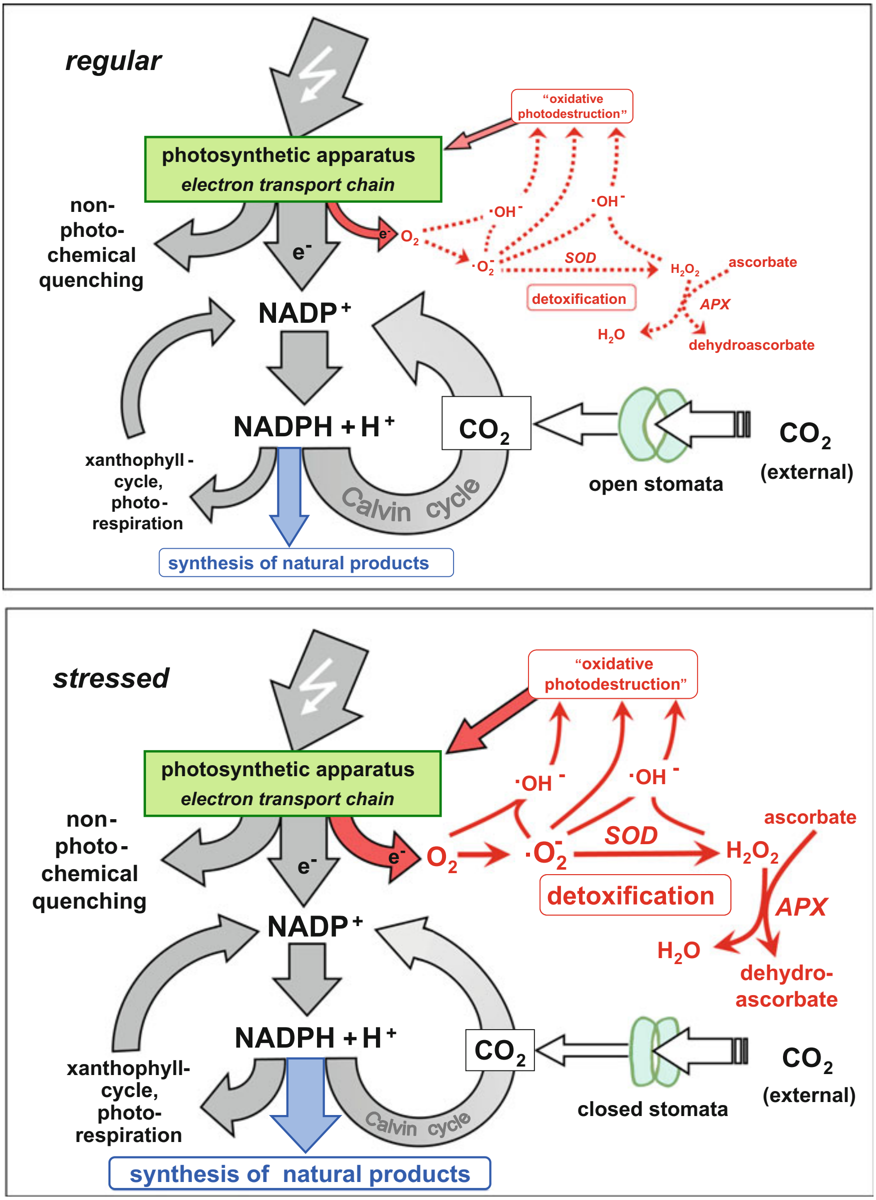

Fig. 2 Drought stress induced metabolic changes. The model scheme according to Kleinwächter and Selmar (2014) outlines the coherences between drought the stress induced increase in reduction status and the enhancement of natural product biosynthesis

very few analogous investigations with respect to drought stress have been performed. Moreover, we have to consider that plants with different genotypes might behave differentially under drought stress. In this context two native subspecies of *Origanum vulgare* (*subsp. virens and subsp. gracile*) were studied under drought stress. It turned out that the subspecies exhibited significant differences in essential oil content, compositions, and patterns of gene expression under drought stress conditions (Morshedloo et al. 2017).

Just recently, expression studies on monoterpene synthases (Radwan et al. 2017) demonstrated that the stress related enhancement of monoterpenes in sage is not only due to a passive shift as outlined above, but might also be caused—at least in part—by an elevated expression of the enzymes responsible for the biosynthesis of the essential oils. In the same manner, the stress induced increase in the glycyrrhizin concentration in *Glycyrrhiza glabra* is accompanied by an up-regulation of genes encoding the major enzymes responsible for the triterpenoid biosynthesis (Nasrollahi et al. 2014). Using semi-quantitative RT-PCR assays the authors showed that the expression of the genes encoding a squalene synthase and a ß-amyrin synthase is enhanced under drought stress. There is a massive need for further research in order to elucidate, whether or not these findings represent a quite general issue. If however, forthcoming studies will confirm that such stress related up-regulation of genes responsible for natural product biosynthesis will occur frequently, the question on the biological significance of this effect will arise. In general, the interaction with pathogens or herbivores should not be affected by drought. Accordingly, apart from this coherency, there has to be a different and additional relevance for the observed up-regulation of terpene biosynthesis. In this context, the strong isoprene emission of numerous plants (e.g., Fall 1999; Sharkey and Yeh 2001) attracts special attention: under standard conditions the isoprene emission is neglectable and the energy consumption for its biosynthesis accounts for less than 1% of the entire photosynthetic energy. However, isoprene synthesis and emission increases drastically, when the plants suffer stress. Magel et al. (2006) outlined that at elevated temperatures, the amount of energy dissipated by the strongly enhanced isoprene emission might rise up to 25% of the energy used for net photosynthesis. From these coherences it could be deduced that the energy demand and the re-oxidation of reduction of $NADPH+H^+$ for the biosynthesis of the isoprene contribute significantly to the dissipation of the excess of photosynthetic energy. Moreover the emission of isoprene will cool down the stressed leaves as well (Behnke et al. 2007). Hence an increase in the biosynthesis of highly reduced natural products seems to be necessary to enhance the amount of energy dissipation, which strongly has to be elevated under stress conditions.

We have to realize that—apart from all the well established ecological functions—the drought stress related increase in the biosynthesis of highly reduced natural products might also have a relevance as an additional mechanism for energy dissipation (Grace and Logan 2000; Wilhelm and Selmar 2011).

It is a matter of fact that drought stress generally has to be filed as negative factor, being responsible for severe yield losses in agriculture. With respect to spice and medicinal plants, however, the situation seems to be different. Since the content of active natural products determines the quality of the plant derived commodities, any approaches to increase this quality could be favourable. Based on the novel insights displayed in this review, we have to recognize that the concentration of natural products and thus the product quality of spice and medicinal plants could be enhanced by deliberately applying moderate drought stress during their cultivation. However, as drought stress also reduces the biomass production, special emphasis has to be put on the interference of these two stress related effects and the decision what is required, a high quality, i.e. high concentrations of relevant compounds or a high yield, i.e. a large amount of these compounds.

Case Study: Change in the Composition of Indole Alkaloids by Stress

Apart from the desired high concentrations of active compounds also their chemical composition influences the quality of spices and medicinal plants. Up to now—with special emphasis on the impact of drought stress—only limited data on this issue are available. To display the tremendous possibilities in modulating the composition of active compounds of certain medicinal plants, a model experiment with lesser periwinkle (*Vinca minor*) was performed. In order to preferably perceive the most distinctive effects, not a real drought situation was applied, but the stress situation was mimicked by spraying of methyl jasmonic acid (MeJA). MeJA and jasmonic acid, respectively are potent regulators of genes involved in cell growth and in most biotic and abiotic stress responses, including drought stress (Turner et al. 2002; Kazan and Manners 2008) and, moreover these growth regulators promote senescence (Cree and Mullet 1997). Accordingly, a treatment with MeJA should promote most severe and comprising stress situations that also impact secondary metabolism (Kim et al. 2006; Bloem et al. 2014; Kleinwächter et al. 2015). A corresponding approach using *Catharanthus roseus* revealed that the content and the composition of indole alkaloids strongly were affected by MeJA application (Aerts et al. 1994).

The indole alkaloids extracted from the leaves of *V. minor* were analyzed by HPLC-DAD. Although a certain difference in the alkaloid pattern of well watered control and stressed plants had been predicted, the outcome of this experiment was surprising and unexpected (Abouzeid et al. 2017). Whereas the alkaloid spectrum of the control plants was in accordance with the literature (Proksa and Grossmann, 1991; D´Amelio et al. 2012), and vincamine (B) and vincadifformine (D) had been identified as major components, the alkaloid composition of the MeJA treated plants was drastically different (Fig. 3). As response to the MeJA treatment, the concentration of vincamine and vincadifformine was strongly decreased whereas two other alkaloids (A and C) massively increased in their concentration. It can be assumed that vincamine and vincadifformine are converted to the two other, so far unidentified alkaloids. To verify this assumption, the structures as well as the

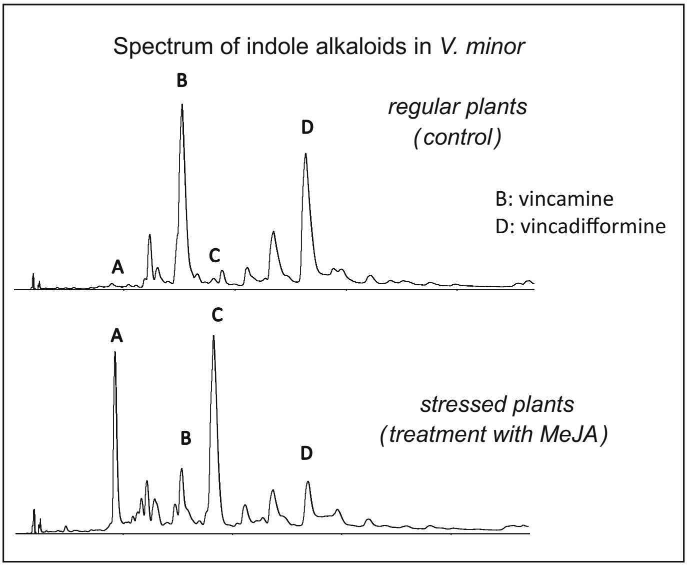

Fig. 3 Drought stress induced changes in the indole alkaloid spectrum of *V. minor*. HPLC chromatograms of the alkaloids extracted from leaves of *V. minor* according to Abouzeid et al. (2017). The upper graph shows the pattern of indole alkaloids extracted from the well watered controls, the lower one corresponds to those from *V. minor* plants which have been treated with MeJA fraction. Details of the treatment are outlined by Abouzeid et al. (2017). The identification and structure elucidation, respectively, of the A and C, whose concentrations are tremendously enhanced in the plants treated with MeJA, is under way

enzymes putatively responsible for such conversion must be identified. Right now, these compounds were highly purified to ensure the required NMR studies for their characterization and structure elucidation.

This example vividly displays that stress not only induces quantitative changes in natural product biosynthesis but also may alter the composition of the relevant substances. Much more research is required to elucidate the molecular coherences of this fascinating issue.

Conclusion

Apart from the tremendous negative effects of drought situations on growth and development, the corresponding stress situations frequently lead to an increase in the concentration of natural products. In principle, there are three possibilities to explain this enhancement (Fig. 4). First, it could be the consequence of a reduced production of biomass in the stressed plants: when the rate of biosynthesis of natural products is not changed or only slightly reduced, their concentration on dry

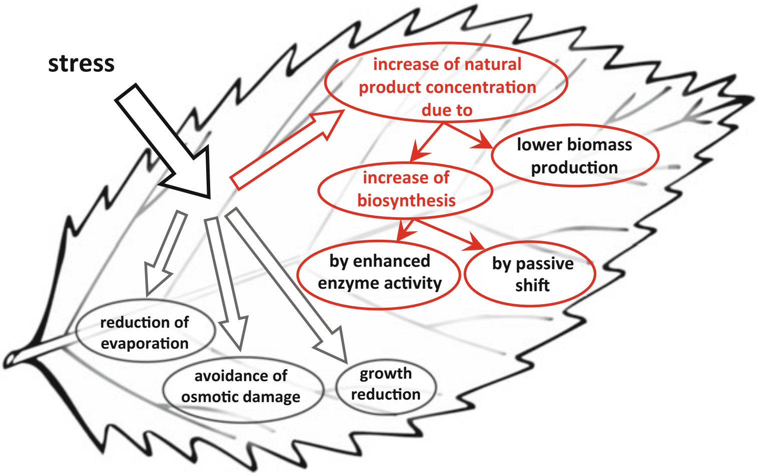

Fig. 4 The impact of drought stress on natural product concentration. The scheme displays the three different causes, why the natural product concentration is enhanced in plants suffering drought stress

or fresh weight basis simply will be elevated. Alternatively, the drought stress enhances the actual rate of biosynthesis. This could be due either to a passive shift or an active up-regulation of the enzymes involved in natural product biosynthesis. Both options are related to the strongly enhanced reduction status of the leaves exposed to drought stress. In the first case (Fig. 4), the over-reduction, which goes along with a huge increase in concentration of NADPH+H^+ and results - according to the law of mass action—in an acceleration of all processes consuming NADPH +H^+, including the biosyntheses of highly reduced secondary plant products, such as isoprenoids, phenols or alkaloids. In the second case, the enzymes responsible for the biosynthesis of natural products are actively up-regulated. The corresponding increment in NADPH+H^+ consumption significantly contributes to the dissipation of the stress related surplus of energy contributes to prevent the generation of toxic oxygen radicals (Fig. 2).

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Effect of Salinity Stress on Growth and Physiology of Medicinal Plants

Aditya Banerjee and Aryadeep Roychoudhury

Abstract Cultivation of medicinal plants has been economically beneficial for medicine and drug industries. These plants are storehouses of precious active principles which can be developed into a variety of drugs, some of which are life-saving. However, it is difficult to initiate large scale cultivation of these plants as most of the cultivable lands are dominantly used for the growth of essential food crops. The other non-cultivable lands are often affected with various kinds of abiotic stresses, salinity being the predominant amongst them. This chapter focuses upon the growth and physiology of different medicinal plants in response to salinity stress. We highlight the physiological processes as well as primary and secondary metabolism of medicinal plants during salt stress that will promote a global cultivation map for the medicinal plants under such challenging or hostile situations.

Keywords Medicinal plants · Salt stress · Physiology · Primary metabolites · Proteomic investigations · Secondary metabolites

Introduction

Salinity stress has been regarded as a worldwide limiting factor for agricultural production. About 20% of the global irrigated land is affected with high salt content, toxic enough for sustaining agriculture (Flowers and Yeo 1995). Salt stress tolerance in plants is induced by multilevel changes in molecular responses accompanied with alterations in the plant transcriptome, metabolome and proteome.

Since ancient times, India has been well known for being the storehouse of traditional medicinal knowledge. The ancient text of Ayurveda is completely based on herbal medicine effective in treating complex diseases which sometimes cannot be tackled with conventional medicines. As much as 25% of the total drugs in

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developed countries like USA constitutes of plant drugs, whereas it is close to 80% in developing countries like China and India. The cultivation and maintenance of medicinal plants thus forms a profitable business throughout the world and must be protected. However, cultivating such plants in salt contaminated soils has imposed severe abnormalities in their overall growth and physiology (Thornber et al. 1977). Hence, it is inevitable to understand the correlation between medicinal plants and salt stress in terms of yield and sustainability.

Correlation Between Salt Stress and Developmental Morphology in Medicinal Plants

Germination

Salt stress affects germination by destroying the embryo or drastically decreasing the soil potential to such an extent that water uptake gets hampered. Germination was retarded in case of *Ocimum basilicum*, *Eruca sativa*, *Petroselinum hortense*, chamomile, sweet marjoram and *Thymus maroccanus* when the seeds were sown in salt contaminated soil (Miceli et al. 2003; Ramin 2005; Ali et al. 2007; Belaqziz et al. 2009).

Growth of Seedlings

Seedling has been considered as the most vulnerable stage of the plant's life cycle. It was reported that salinity caused a significant reduction in the seedling growth of *Thymus maroccanus* by inhibiting reserve food mobilization, suspending cell division, enlarging and injuring hypocotyls. Similar reports are available for basil, chamomile and marjoram (Said-Al Ahl and Omer 2011). Salinity decreased the number of foliages, root growth and dry matter in *Aloe vera* plants exposed to 2, 4, 6 and 8 $\mathrm{ds\ m^{-1}}$ salinity. Such limitations were mainly due to the reduced levels of total soluble solids (TSS). This also resulted in 30% lower sprout production in the salt-stressed plants compared to the control (Moghbeli et al. 2012). Chauhan and Kumar (2014) reported that high salinity levels significantly reduced the number of tillers in *Citronella java* plants. The vegetative and reproductive stages in cumin were found to be the most sensitive towards 5 $\mathrm{ds\ m^{-1}}$ salt stress (Hassanzadehdelouei et al. 2013).

Developmental Characteristics and Survival

Salinity stress has been reported as a major inhibitor of plant growth in case of medicinal plants like *Majorana hortensis*, peppermint, pennyroyal, apple mint, *Aloe vera*, *Matricaria recutita*, *Thymus maroccanus*, geranium, *Thymus vulgaris*, sweet fennel, sage and *Mentha pulegium* (Said-Al Ahl and Omer 2011; Aziz et al. 2008). A significantly lower number of leaves, leaf area and leaf biomass was noted in *Mentha piperita* var. officinalis and *Lipia citriodora* var. verbena exposed to salt stress (Tabatabaie and Nazari 2007). Exposure to greater than 9 ds m^{-1} salt stress reduced plant height, number of leaves per plant, capitula number per plant and the diameter of the main shoot capitulum in milk thistle (Ghavami and Ramin 2008). Growth parameters were found to be repressed under salt stress in *Withania somnifera*, *Catharanthus roseus*, *Achillea fragratissima*, *Salvia officinalis*, thyme, *Nigella sativa*, *Chamomilla recutita* and basil (Jaleel et al. 2008a, b; Abd EL-Azim and Ahmed 2009; Ben Taarit et al. 2009; Hussain et al. 2009; Ghanavati and Sengul 2010).

Nutrient Uptake

Salinity directly inhibits plant growth by disrupting the nutrient uptake equilibrium maintained by the plant system. Nutrient availability, partitioning and transport are mostly affected. This is due to the competition of Na^+ and Cl^- ions with the nutrient ions like K^+, Ca^{2+} and $NO_3{}^-$. Such ionic imbalances caused due to the abundance of Na^+ and Cl^- ions directly acts upon the biophysical and/or metabolic components of the plant system (Banerjee and Roychoudhury 2016). Increases in Na^+ and Cl^- during salt stress have resulted in decreased levels of N, P, K^+, Ca^{2+} and Mg^{2+} in fennel, *Trachyspermum ammi*, peppermint, lemon verbena, *Matricaria recutita*, *Achillea fragratissima* (Abd El-Wahab 2006; Queslati et al. 2010; Abd EL-Azim and Ahmed 2009). Ali and Hassan (2013) showed that the N, P and K^+ percentages were influenced by different salt concentrations in the leaves of *Simmondsia chinensis* (jojoba). Salt concentration over 17.2 mM drastically lowered the three elements. Similar decrease was reported for the Ca^{2+} content in the salt stressed leaves.

Productivity

Increasing salt concentration inevitably affected the productivity in medicinal plants like fennel, cumin, *Ammi majus, Trachyspermum ammi* and milk thistle. The fruit yield per plant as well as the number of umbels significantly decreased in such stress-exposed plants (Ashraf and Orooj 2006).

Correlation Between Salt Stress and Photosynthesis in Medicinal Plants

The primary target of any abiotic stress is the photosynthetic machinery of plants. Photosynthesis is the most important and crucial physiological process essential for plant growth and survival. This process demands a huge expenditure of energy for maintenance and hence is vulnerable to stress conditions which disrupt the metabolic equilibrium within the cell (Roychoudhury et al. 2007; Banerjee and Roychoudhury 2017). Both chlorophyll a and b along with the total chlorophyll content were reduced in centaury, *Teucrium polium*, *Thymus vulgaris*, *Zataria multiflora*, *Ziziphora clinopodioides* and *Satureja hortensis* (Said-Al Ahl and Omer 2011). Such overall decrease in chlorophyll content is mainly due to inhibition of chlorophyll synthesis coupled with increased degradation of chlorophyll. The imbalance in chlorophyll metabolism leads to suppressed photosynthesis and ultimately causes plant death due to the dearth of energy equivalents. The disturbance in ion absorption occurring during salt stress negatively affects the development of chloroplasts and protein translation machinery within plastids. These are sometimes accompanied with plastid degradation in fennel (Abd EL-Wahab 2006).

Correlation Between Salt Stress and Primary Metabolites in Medicinal Plants

Amino Acids

Salt stress increases the levels of alanine, arginine, glycine, serine, leucine, valine and most importantly proline. Apart from these, the non-protein amino acids, citrulline and ornithine along with the amides like glutamine and arginine also accumulate in plants subjected to salt stress. Proline accumulation occurs at much higher levels compared to other amino acids in *Salvia officinalis*, *Trachyspermum ammi*, spearmint, chamomile, sweet marjoram, *Catharanthus roseus, Achillea fragratissima*, *Matricaria chamomilla*, sweet fennel and *Satureja hortensis.* This may be attributed to the lower proline oxidase activity under saline conditions. Proline has been reported to act as compatible solute which maintains the osmotic equilibrium within the cell even under sub optimal conditions like salinity (Roychoudhury et al. 2015). Higher levels of total free amino acids have been reported in *Catharanthus roseus* and *Matricaria chamomilla* exposed to salt stress (Osman et al. 2007; Cik et al. 2009).

Proteins

An increase in free amino acid content in plants subjected to salt stress has also been explained on the basis of degradation of intact proteins (Roychoudhury et al. 2011). A decrease in protein content during salt stress was reported in *Catharanthus roseus* (Osman et al. 2007). Levels of soluble protein decreased in salt stressed chamomile and sweet marjoram. This indicates that salt stress induces protein aggregation within cells and hence the soluble protein fraction gets reduced (Ali et al. 2007). Depressed synthesis of crude proteins was reported in *Achillea fragratissima* exposed to salt concentration of 4000 ppm (Abd EL-Azim and Ahmed 2009). In other studies, stimulation of protein synthesis correlated with the salinization degree has also been observed. Such spike in protein accumulation might be to provide nitrogen in the stored form that could be re-utilized during the post-stress recovery phase in plants (Roychoudhury and Chakraborty 2013).

Carbohydrates

Salinity stress induces nutritional imbalances, hyper-osmotic stress and reduced photosynthesis. This disrupts the total carbohydrate balance as has been reported in fennel (Abd El-Wahab 2006). However, a contrasting increase in carbohydrates was found in *Salvia officinalis* and *Satureja hortensis* with increasing concentrations of salt stress (Hendawy and Khalid 2005; Najafi et al. 2010).

Lipids

The cellular fatty acid content is an important indicator of plant susceptibility to salt stress. Oil and fatty acid synthesis of plants are influenced by abiotic stresses (Banerjee and Roychoudhury 2014). Oil yield was reported to be lowered in the roots of *Ricinus communis* exposed to salt stress. However, the oil content increased in the plant shoots (Ali et al. 2008). Total fatty acid content in *Coriandrum sativum* leaves was significantly reduced due to salt stress. Increasing NaCl concentrations also led to lowered levels of α-linolenic and linoleic acids (Neffati and Marzouk 2008).

Correlation Between Salt Stress and Secondary Metabolites in Medicinal Plants

Study of Alkaloids Through Proteomic and Other Approaches

Technological developments in proteomics research have opened novel perspectives for ethno botanical and phytomedical research purposes. The regulation of secondary metabolism at the protein level plays essential role in the growth of medicinal plants. The natural yield of secondary products in medicinal plants is generally low. However, it is the secondary metabolite which actually determines the medicinal and economic utility of the plant. Complicated biochemical structures of these metabolites have been a problem to further investigate their properties. Cell suspension cultures and metabolic engineering techniques, facilitating the overexpression of rate limiting enzymes involved in the biosynthesis of these secondary metabolites, have been proposed. Hence, a proteomic approach has been adopted to identify the proteins participating in the biosynthesis of such secondary metabolites (Banerjee et al. 2016).

A model system for secondary metabolites in medicinal plants is *Catharanthus roseus* (Verpoorte et al. 1997). The plant has been reported to produce effective anti-cancer alkaloid drugs like vinblastine and vincristine. Two-dimensional gel electrophoresis was used to investigate the influence of zeatin and 2, 4-dichlorophenoxyacetic acid (2, 4-D) on protein patterns and alkaloid production in *C. roseus*. It was reported that the proteins which were decreased by 2, 4-D but increased by zeatin exposure could function in the regulation of alkaloid biosynthesis (Jacobs et al. 2000).

In another proteome analysis of *C. roseus*, differential expression of 88 protein spots was detected. Mass spectrometric analyses identified two isoforms of strictosidine synthase, tryptophan synthase and 12-oxophytodienoate reductase. Strictosidine synthase catalyzes the formation of strictosidine in alkaloid biosynthesis. Tryptophan synthase is essential for the supply of the alkaloid precursor, tryptamine and 12-oxophytodienoate reductase catalyses the last step in the synthesis of the non-traditional plant growth regulator, jasmonic acid (JA). JA has been considered as an important signalling molecule which regulates plant defence during biotic stress responses and promotes the accumulation of alkaloids (Jacobs et al. 2005).

The concentration of reserpine in *Rauvolfia tetraphylla* increased during salt stress. However, the ricinine alkaloids were reduced in the roots of salt stressed *Ricinus communis*. Significant increase in the alkaloid levels was reported in *Solanum nigrum* (solasodine) and *Achillea fragratissima* plants grown in high saline soils (Aghaei and Komatsu 2013). Hung et al. (2010) investigated the effects of the secondary metabolites in *Salvia miltiorrhiza* on atherosclerotic lesions and cancer. Ginsenoids are important alkaloids produced in the roots of *Panax ginseng*. The root proteome from a culture of *P. ginseng* hairy roots was studied to identify the proteins involved in the production of ginsenoids. The putative functions of 91 out of the 159 detected proteins were determined from the root proteome studies. It was found that about 20% of the proteins (with assigned functions) regulated energy metabolism during stress responses. Some proteins like enolase, glyceraldehyde-3-phosphate dehydrogenase and aldolase were detected as isotypes. However, their relation with secondary metabolism could not be deciphered (Nam et al. 2005).

Two dimensional gel electrophoresis of milky sap from *Chelidonium majus* identified 21 proteins related to plant defence, nucleic acid binding and stress signalling (Nawrot et al. 2007). Decker et al. (2000) attempted to identify proteins involved in morphine production in opium poppy (*Papaver somniferum*), using two dimensional electrophoresis of poppy latex. The most important detected protein was codeinone reductase which might play a crucial role in morphine synthesis during stress.

Phenolic Compounds During Stress

Phenolics have been regarded as a sensor for salt stress. This group consists of about 9000 compounds having diverse biological functions in plants, some phenolic compounds can scavenge the reactive oxygen species (ROS) produced during abiotic stresses. Salt stress reduces the electron flow in the photosynthetic electron transport system and promotes oxidative stress via ROS production (Das and Roychoudhury 2014). Higher plants produce compounds like phenolic acids, flavonoids and proanthocyanidins to scavenge the harmful ROS. Several reports in crop plants have shown the accumulation of phenolics during salt stress (Waskiewicz et al. 2012).

Phenolic acid and tannin concentration increased in the salt stressed spearmint and *Achillea fragratissima* plants (Abd EL-Azim and Ahmed 2009). Phenolic compounds like protocatechuic, chlorogenic and caffeic acids significantly increased in *Matricaria chamomilla* plants grown in salt stress (Cik et al. 2009). Accumulation of phenolics with increasing salt concentration was also recorded in *Nigella sativa* and *Mentha pulegium* (Bourgou et al. 2010; Queslati et al. 2010). The biosynthesis of other phenolic compounds like quercetin, apigenin and trans-cinnamic acid was reportedly enhanced in *Nigella* grown in soil with high salinity content (Bourgou et al. 2010).

Salt Stress and Steroid (Cardenolide) Metabolism

Cardenolide is a steroid derivative found in plants like *Digitalis purpurea*. Extracts of this plant is used to treat heart failures. They mainly occur in the form of glycosides containing structural groups derived from sugars. Morales et al. (1993) reported that the cardenolide level in *D. purpurea* leaves and roots were elevated under moderate salinity conditions. Cardenolide content in the leaves and roots of *D. purpurea* was higher during 100 mM salt stress compared to the plants exposed to 200 mM salt stress and those grown under control conditions.

Effects of Salt Stress on Essential Oil

Essential oil have high economic and industrial values. They are mainly found in mint species like peppermint (*Mentha piperita*), pennyroyal (*Mentha pulegium*) and apple mint (*Mentha suaveolens*). Aziz et al. (2008) reported that salt stress decreased the essential oils in all mint species. Such salt induced reduction in essential oil content compared to control was higher in peppermint and apple mint than in pennyroyal. The major constituent of these essential oils in mint species is monoterpenes. The percentage of menthone (another constituent of essential oils) was found to increase with the increase in salt concentration. However, other constituents decreased when the plant was exposed to the highest concentration of salt stress. Interestingly, the authors found that the alterations in the levels of the constituents in pennyroyal were much subtle during salt stress compared to those of peppermint and apple mint. Lower yield of essential oils during salt stress was also reported in *Trachyspermum ammi*, *Thymus maroccanus*, basil and fennel (Ashraf and Orooj 2006). Abd El-Wahab (2006) showed that salinity stress decreased even the anethole content in fennel. Levels of the main compounds in essential oils of marjoram were differentially affected by salt stress (Baatour et al. 2010). However, several essential oil compounds like α-bisabololoxide B, α-bisabolonoxide A, chamazulene, α-bisabolol oxide A, α-bisabolol and trans-β-farnesene were reportedly increased during salt stress (Baghalian et al. 2008).

Said-Al Ahl and Hussein (2010) observed sharp decreases in essential oil constituents like carvacrol, *p*-cymene and γ-terpinene in *Origanum vulgare* exposed to salt stress. Salt-induced inhibitory effects were profound in lemon balm, *Majorana hortensis*, *Matricaria chamomile*, *Salvia officinalis* and basil (Said-Al Ahl and Omer 2011). Essential oil constituents like (*E*)-2-decenal, (*E*)-2-dodecenal and dodecanal initially increased in coriander during moderate salt stress. However at still higher concentrations, the oil level decreased significantly (Neffati and Marzouk 2008). Major constituents of essential oils in *Ocimum basilicum* under control conditions were eugenol and linalool. Exposure to salt stress decreased the eugenol content, whereas the linalool content was elevated (Said-Al Ahl et al. 2010). In case of *Salvia officinalis*, the main essential oil compound was viridiflorol under control conditions and 25 mM NaCl, whereas 1, 8-cineole accumulated under 50 and 75 mM NaCl stress. However, when the plants were exposed to 100 mM NaCl stress, manool became the predominant compound (Ben Taarit et al. 2010).

An increase in the percentage of essential oils was reported in *Satureja hortensis*, sage, thyme and basil grown in salt contaminated soils (Baher et al. 2002; Hendawy and Khalid 2005; Ezz El-Din et al. 2009). The essential oil constituents like carvacrol increased and γ-terpine decreased in coriander roots significantly with increasing stress (Neffati and Marzouk 2008). The percentage of essential oils and the oil yield (ml per plant) were enhanced in curly leafed parsley under salt stress. Foliar aroma constituents like β-phellandrene, myristicin, β-myrcene and apiole were also affected under the stress conditions (Said-Al Ahl et al. 2016).

Contrasting effects of salt stress on essential oil production have been discussed so far based on the reports available for different medicinally important plant species. It has been proposed that increased oil gland density, accompanied with higher absolute number of gland production during stress, could be the reasons behind accumulation of essential oil in some plant species. Other factors could be the net assimilation or the partitioning of assimilates among growth and differentiation processes. Sometimes, the decline in the primary metabolism of plants during stress could lead to the accumulation of certain intermediate products, which get channelized to form secondary metabolites like essential oil. In plants with decreased levels of essential oil under stress, the lowering could be due to the overall anabolism which gets inhibited on exposure to saline conditions (Said-Al Ahl et al. 2016).

Conclusion and Future Perspectives

Medicinal plants have special economic importance due to the presence of a large number of active principles which can be marketed in the form of even life-saving drugs. These plant products possess high curing values and naturally grow in diverse environmental conditions. This property can be utilised for their widespread cultivation in lands with sub-optimal conditions. However, to enhance such cultivation, elucidating the growth and physiology under stress conditions like salinity is highly significant. Researches correlating plant genotypes with stress responses, are lacking and should be pursued in details. Better farm practices like micro-jet irrigation coupled with fertilization management or leaching can be utilised to recover vast stretches of land affected with toxic salt concentrations. On the transgenic frontline, it can be firmly advised that researches should also focus upon developing salt-tolerance in susceptible plant species. Such tolerance can also be conferred upon by following an extensive plant breeding program to facilitate the introgression of salt-tolerant genes in susceptible species of medicinal plants.

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Heavy Metal-Mediated Changes in Growth and Phytochemicals of Edible and Medicinal Plants

Shohreh Fahimirad and Mehrnaz Hatami

Abstract One of the most important kinds of environmental contaminates is heavy metals pollution. Plants which are exposing to high metal concentrations illustrate down regulated growth and development. Various alterations in the medical plants production of bioactive compounds have been documented. On the other hand, many researches have illustrated the high toxic residuals of heavy metals in several parts of medical plants which are potent to cause hazard to human health. Interestingly, phytoremediation is most effective and promising methods among several strategies already used to clean up the environment from heavy metals. Medical plants with high potential in heavy metal accumulation can be good candidates for soil heavy metal remediation. The cultivation or deliberate usage of medical plants in soil polluted by heavy metals must be managed carefully to diminish the final heavy metal residuals in marketing products. This chapter explains the mechanisms of plants heavy metal tolerance, the studies on transgenic plants tolerant to heavy metals, heavy metal impacts on medical plant growth and metabolites, phytoremediation ability of medical plants and standard heavy metal residuals concentration in medical plants.

Keywords Heavy metals · Medicinal plants · Phytoremediation · Secondary metabolites · Heavy metal residuals concentration

Introduction

Heavy metals are major environmental pollutants, and large amount of them are toxic ultimately at absolutely low concentrations. Fossil fuels burning, mining, municipal wastes, fertilizers and pesticides are primary sources of heavy metals

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Table 1 Types of heavy metals and their effect on human health with their permissible limits (Singh et al. 2011)

Pollutants	Major sources	Effect on human health	Permissible levels (mg/L)
As	Pesticides, fungicides, metal smelters	Bronchitis, dermatitis, poisoning	0.02
Cd	Welding, electroplating, pesticides, fertilizer	Renal dysfunction, lung disease, lung cancer, bone defects, kidney damage, bone marrow	0.06
Pb	Paint, pesticides, smoking, automobile emission, mining, burning of coal	Mental retardation in children, development delay, fatal infant encephalopathy, chronic damage to nervous system, liver, kidney damage	0.1
Mn	Welding, fuel addition, ferromanganese production	Inhalation or contact damage to central nervous system	0.26
Hg	Pesticides, batteries, paper industry	Tremors, gingivitis, protoplasm poisoning, damage to nervous system, spontaneous abortion	0.01
Zn	Refineries, brass manufacture, metal plating	Damage to nervous system, dermatitis	15
Cr	Mine, mineral sources	Damage to nervous system, irritability	0.05
Cu	Mining, pesticide production, chemical industry	Anemia, liver and kidney damage, stomach irritation	0.1

pollutions (Dhir et al. 2008; Wei and Zhou 2008). Any non-biologically degradable metal causes an environmental problem considered to be a "heavy metal". Fifty three elements with an atomic density greater than 6 g cm^{-3} now fall into the category of heavy metal. Common toxic metals are mercury (Hg), lead (Pb), cadmium (Cd), copper (Cu), 35 chromium (Cr), manganese (Mn), zinc (Zn), and aluminum (Al) (Herrera-Estrella and Guevara-Garcia 2009).The Types of heavy metals and their effect on human health with their permissible limits are enumerated in Table 1.

Effect of Heavy Metal Polluted Soil on Plant Growth

Although plants require certain heavy metals for their growth and uptake, excessive amounts of these metals can become toxic to plants. The ability of plants to accumulate essential metals equally enables them to acquire other nonessential metals. Some of the direct toxic effects caused by high metal concentration include inhibition of cytoplasmic enzymes and damage to cell structures due to oxidative stress. The Toxicity of heavy metals to life forms are shown in Table 2 (Chibuike and Obiora 2014). Due to the high prevalence of heavy metals in the environment, their residues also reach and are assimilated into medicinal plants (Sarma et al. 2012). Contamination during cultivation, inadvertent cross-contamination during processing and the purposeful introduction of heavy metals for alleged medicinal purposes are three key mechanisms that have been proposed to explain heavy metal contamination of medical plant-based products (Denholm 2010).

Plant Survival Strategies to Increasing Metal Concentrations

Plants have devoted three various behaviors against heavy metals. First group named as metal excluders avoid heavy metals to enter their aerial parts. Second group, known as metal indicators, accumulate metals in their above-ground tissues and the metal levels in the tissues of these plants generally reflect metal levels in the soil.

The third and most important group of plants includes around 500 plant species is hyper accumulators which concentrate metals in their above-ground tissues to levels far exceeding those present in the soil. Localization of metal ions in roots and shoots in nontoxic forms, binding of toxic metals in cell walls of roots and leaves and sequester metals into the vacuoles or compartments of the cytosol are three major procedure for heavy metal accumulation and keep them away from active metabolic sites in plant cells in tolerant plants (Cosio et al. 2004) (Fig. 1).

Novel approaches such as transcriptomics, proteomics, and metabolomics clear the function of the plants cells in heavy metal area. Heavy metal accumulators are increasing steadily and some are presented in Table 3 (Memon and Schroder 2009).

In the last few decades many scientists in different parts of the worlds has worked out the metals bioaccumulation potential of various species and some are presented in Table 4.

Molecular Basis of Metal Uptake

ESTs expressed sequence tags analysis and Comparing EST sequences of the target species with the appropriate reference model species or additional public databases is one of important performance to survey gene expression pattern and determining major gene involved in heavy metal tolerance for species whose complete genome sequence information is not available (Roosens et al. 2008). Analysis of quantitative trait loci (QTLs) involved in metal tolerance is a perfect method for identifying

Table 2 Toxicity of heavy metals to different plant species (Chibuike and Obiora 2014)

Heavy metal	Plant species	Effects	References
As	Rice (*Oryza sativa*)	Reduction in seed germination; decrease in seedling height; reduced leaf area and dry matter production	Abedin et al. (2002)
	Tomato (*Lycopersicon esculentum*)	Reduced fruit yield; decrease in leaf fresh weight	Barrachina et al. (1995)
	Canola (*Brassica napus*)	Stunted growth; chlorosis; wilting	Cox et al. (1996)
Cd	Wheat (*Triticum* sp.)	Reduction in seed germination; decrease in plant nutrient content; reduced shoot and root length	Yourtchi and Bayat (2013)
	Garlic (*Allium sativum*)	Reduced shoot growth; Cd accumulation	Jiang et al. (2001)
	Maize (*Zea mays*)	Reduced shoot growth; inhibition of root growth	Wang et al. (2007)
Co	Tomato (*Lycopersicon esculentum*)	Reduction in plant nutrient content	Jayakumar et al. (2013)
	Mung bean (*Vigna radiata*)	Reduction in antioxidant enzyme activities; decrease in plant sugar, starch, amino acids, and protein content	Jayakumar et al. (2008)
	Radish (*Raphanus sativus*)	Reduction in shoot length, root length, and total leaf area; decrease in chlorophyll content; reduction in plant nutrient content and antioxidant enzyme activity; decrease in plant sugar, amino acid, and protein content	Jayakumar et al. (2007)
Cr	Wheat (*Triticum* sp.)	Reduced shoot and root growth	Sharma and Sharma (2003)
	Tomato (*Lycopersicon esculentum*)	Decrease in plant nutrient acquisition	Moral et al. (1995)
	Onion (*Allium cepa*)	Inhibition of germination process; reduction of plant biomass	Nematshahi et al. (2012)
Cu	Bean (*Phaseolus vulgaris*)	Accumulation of Cu in plant roots; root malformation and reduction	Cook et al. (1997)
	Black bindweed (*Polygonum convolvulus*)	Plant mortality; reduced biomass and seed production	Kjær and Elmegaard (1996)
	Rhodes grass (*Chloris gayana*)	Root growth reduction	Sheldon and Menzies (2005)

(continued)

Table 2 (continued)

Heavy metal	Plant species	Effects	References
Hg	Rice (*Oryza sativa*)	Decrease in plant height; reduced tiller and panicle formation; yield reduction; bioaccumulation in shoot and root of seedlings	Du et al. (2005)
	Tomato (*Lycopersicon esculentum*)	Reduction in germination percentage; reduced plant height; reduction in flowering and fruit weight; chlorosis	Shekar et al. (2011)
Mn	Broad bean (*Vicia faba*)	Mn accumulation shoot and root; reduction in shoot and root length; chlorosis	Arya and Roy (2011)
	Spearmint (*Mentha spicata*)	Decrease in chlorophyll a and carotenoid content; accumulation of Mn in plant roots	Asrar et al. (2005)
	Pea (*Pisum sativum*)	Reduction in chlorophylls a and b content; reduction in relative growth rate; reduced photosynthetic O_2 evolution activity and photosystem II activity	Doncheva et al. (2005)
	Tomato (*Lycopersicon esculentum*)	Slower plant growth; decrease in chlorophyll concentration	Shenker et al. (2004)
Ni	Pigeon pea (*Cajanus cajan*)	Decrease in chlorophyll content and stomatal conductance; decreased enzyme activity which affected Calvin cycle and CO_2 fixation	Sheoran et al. (1990)
	Rye grass (*Lolium perenne*)	Reduction in plant nutrient acquisition; decrease in shoot yield; chlorosis	Khalid and Tinsley (1980)
	Wheat (*Triticum* sp.)	Reduction in plant nutrient acquisition	Pandolfini et al. (1992)
	Rice (*Oryza sativa*)	Inhibition of root growth	Lin and Kao (2005)
Pb	Maize (*Zea mays*)	Reduction in germination percentage; suppressed growth; reduced plant biomass; decrease in plant protein content	Hussain et al. (2013)
	Portia tree (*Thespesia populnea*)	Reduction in number of leaves and leaf area; reduced plant height; decrease in plant biomass	Kabir et al. (2009)
	Oat (*Avena sativa*)	Inhibition of enzyme activity which affected CO_2 fixation	Moustakas et al. (1994)

(continued)

Table 2 (continued)

Heavy metal	Plant species	Effects	References
Zn	Cluster bean (*Cyamopsiste tragonoloba*)	Reduction in germination percentage; reduced plant height and biomass; decrease in chlorophyll, carotenoid, sugar, starch, and amino acid content	Manivasagaperumal et al. (2011)
	Pea (*Pisum sativum*)	Reduction in chlorophyll content; alteration in structure of chloroplast; reduction in photosystem II activity; reduced plant growth	Doncheva et al. (2001)
	Rye grass (*Lolium perenne*)	Accumulation of Zn in plant leaves; growth reduction; decrease in plant nutrient content; reduced efficiency of photosynthetic energy conversion	Bonnet et al. (2000)

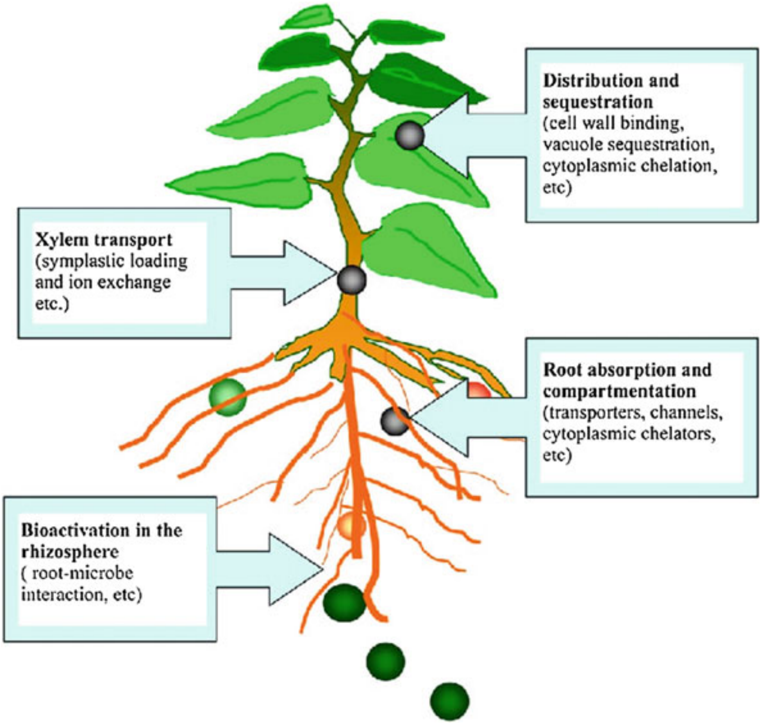

Fig. 1 Major processes proposed to be involved in heavy metal hyperaccumulation by plants (Yang et al. 2005)

Table 3 Examples of some plants and metals they can remediate (Sarma 2011)

Plant species	Metal	Reference
Alyssuim wulfenianum	Ni	Reeves and Brooks (1983)
Azolla pinnata, Lemna minor	Cu, Cr	Jain et al. (1990)
Brassica Juncea	Cu, Ni	Ebbs and Kochian (1997)
Arobiadopais hallerii	Cd	Kupper and Kochian (2010)
Pteris vittata	Cu, Ni, Zn	Ma et al. (2001)
Psychotria douarrei	Ni	Davis et al. (2001)
Pelargonium sp.	Cd	Dan et al. (2002)
Thlaspi caerulescens	Cd and Ni	Assuncao and Schat (2003)
Arabidapsis hallerii	Cd	Bert et al. (2003)
Amanita muscaria	Hg	Falandysz et al. (2003)
Arobis gemmifera	Cd and Zn	Kubota and Takenaka (2003)
Pistca stratiotes	Ag, Cd, Cr, Cu, Ni, Pb	Odjegba and Fasidi (2004)
Piptathertan miliacetall	Pb	Garcia et al. (2004)
Astragulus bisukatus, Brassica Juncea	Selenium	Ellis et al. (2004)
Sedum alfredli	Pb	Xiong et al. (2004)
Sesbania drummondi	Pb	Sharma et al. (2004)
Lemn agibba	As	Mkandavire and Dude (2005)
Pteris vittata	As	Dong (2005)
Thlaspi caerulescens	Zn, Pb, Zn, and Cd	Sun et al. (2005)
Sedum alfredii	Pb	Banasova and Horak (2008)
Chengiopanax sciadopkvltoides	Mn	Mizuno et al. (2008)
Tamarix smyrnensis	Cd	Manousaki et al. (2008)
Brassica napus	Cd	Selvam and Wong (2008)
Arabidopsis thaliana	Cd/Zn	Saraswat and Rai (2009)
Crotalaria juncea	Ni and Cr	Saraswat and Rai (2009)
Rorippaglobosa	Cd	Sun et al. (2010)

major genes in plants which their genome maps are trolley identified (Deniau and Pieper 2006). Many researchers have tried to introduce new hyperaccumulating transgenic plants after finding the major genes involved in heavy metal tolerance. The responses of transgenic plants and its biosynthetic pathway genes against heavy metal stress are listed in Table 5 (Yang et al. 2005). A summary of the most effective transgenes and the effects of their expression on tolerance, accumulation, and volatilization of metals in plants is given in Tables 5 and 6.

Researches on gene expression pattern during heavy metal stress have demonstrated that genes coding membrane transporters responsible for the uptake, efflux, translocation, and sequestration of mineral nutrients overexpressed under adaptive situations. Plants ability in take up and translocate of metals to avoid their direct

Table 4 Examples of some metal hyperaccumulator (Sarma 2011)

Plant species	Metal	Bioaccumulation	References
Sebertia acuminate	Ni	25% by wt, dried sp	Jaffre et al. (1976)
Ipomea alpine	Cu	12,300 mg kg^{-1}	Baker and Walker (1989)
Eichornia crassipes	Ni	6000 mg kg^{-1}	Lytle et al. (1998)
Alternanthera sessilis	Cv	1017 mg kg^{-1}	Sinha et al. (2002)
Zea mays L. Cv Ganga 5	Cr	2538 mg kg^{-1}	Sharma and Sharma (2003)
Pteris vittata	As	2300 mg kg^{-1}	Dong (2005)
Sesbania drummondi	Cd	1687 mg kg^{-1}	Israr et al. (2006)
Thlaspi caerlescens	Zn	19,410 mg kg^{-1}	Banasova and Horak (2008)
Thlaspi caerlescens	Cd	80 mg kg^{-1}	Banasova and Horak (2008)
Myriophyllurn heterophyllum	Cd	21.46 μg g^{-1}	Sivaci et al. (2008)
Potamogetan crispus	Cd	49.09 μg g^{-1}	Sivaci et al. (2008)
Sorghum sudanense	Cu	5330 mg kg^{-1}	Wei et al. (2008)
Phragrnites australis	Cr	4825 mg kg^{-1}	Calheiros et al. (2008)
Arabis paniculata	Cd	1127 mg kg^{-1}	Zeng et al. (2009)
Atriplex halimus	Cd	60,651 μg g^{-1} DW	Nedjimi and Daoud (2009)
Sedum alfredii	Cd	2183 mg kg^{-1}	Jin and Liu (2009)
Sedum alfredii	Zn	13,799 mg kg^{-1} DW	Jin and Liu (2009)
Brassica juncea	Ni	3916 mg kg^{-1} DW	Pollard et al. (2009)
Potentilla griffithii	Zn	19,600 mg kg^{-1} DW	Saraswat and Rai (2009)
Rorippa globosa	Cd	218.9 μg g^{-1} DW	Hu et al. (2009)
Thlospi praecox Wulfen	Cd	>1000 μg g^{-1} DW	Sun et al. (2010)

toxicity for cells are consequence of powerful heavy metal transportation systems. Transport proteins and intracellular high-affinity binding sites mediate the uptake of metals across the plasma membrane. The overview of the metal transporters and their tissue-specific expression in plants is summarized in Table 7 (Memon and Schroder 2009).

Phytoremidation

Remediation is the main strategy to protect the environment from heavy metal toxic effects. Phytoremediation is one of most promising technologies that is used for remediation of vast quantities of heavy metals. The potential of heavy metal phytoremediation depends on the capability of a plant to accumulate excessive concentrations of metals (Ullah et al. 2015).

Table 5 Genes introduced into plants and the effects of their expression on heavy metal tolerance, accumulation, or volatilization (Yang et al. 2005)

Gene	Target	Product	Source	Maximum observed effect
merA	Hg(II) reductase	Gram-negative bacteria	*Liriodendron tulipifera*	50 mmol 11 $HgCl_2$; 500 mg $HgCl_2$ kg^{-1}
merA	Hg(II) reductase	Gram-negative bacteria	*Nicotiana tabacum*	V: Hg-volatilization rate increase 10-fold
			Arabidopsis thaliana	T: 10 mmol 11 CH_3HgCl (440-fold)
merB	Organomercurial lyase	Gram-negative bacteria	*A. thaliana*	V: Up to 59 pg Hg(0) mg^{-1} fresh biomass min^{-1}
APS1	ATP sulfurylase	*A. thaliana*	*B. juncea*	A: Two-fold increase in Se concentration
MT-I	MT	Mouse	*N. tabacum*	T: 200 $mmol^{-1}$ $CdCl_2$ (20-fold)
CUP1	MT	*Saccharomyces cerevisiae*	*B. oleracea*	T: 400 $mmol^{-1}$ $CdCl_2$ (approximately 16-fold)
gsh2	GSH synthase	*E. coli*	*B. juncea*	A: Cd concentrations 125%
gsh1	g-Glu-Cys synthase	*E. coli*	*B. juncea*	A: Cd concentrations 190%
NtCBP4	Cation	Channel	*N. tabacum*	A: Pb concentrations 200%
				T: 250 $mmol^{-1}$ $NiCl_2$ (2.5-fold), Pb-sensitive
ZAT1	Zn	Transporter	*A. thaliana*	T: Slight increase
TaPCS1	PC	Wheat	*Nicotian aglauca* R. Graham	A: Pb concentrations 200%

Relative values refer to control plants not expressing the transgene. References are given in the text. *A* accumulation in the shoot; *GSH* glutathione, *MT* metallothionein; *T* tolerance; *V* volatilization

Phytoremediation involves accumulation of heavy metals in the roots and shoots of plants. Plants used for phytoextraction usually possess the following characteristics: rapid growth rate, high biomass, extensive root system, and ability to tolerate high amounts of heavy metals (Chibuike and Obiora 2014). Phytoremediation of contaminated soils is generally believed to be effective through one or more of the following mechanisms or processes: phytoextraction, phytostabilization, phytodegradation, phytovolatilization, and rhizodegradation are phytoremediation mechanisms of contaminated soils. These mechanisms are described briefly in Table 8 (Oh et al. 2013). Numerous kindes of medical plants have been explored for phytoremediation (Padmavathiamma and Li 2007; Sarma 2011) however investigation to prevent elevated concentrations of heavy metals in medicinal plants should be done before marketing (Sharma et al. 2009; Steenkamp et al. 2000).

Table 6 Transgenic plants and heavy metal stress (Singh et al. 2016)

Antioxidant and/or its Source biosynthetic pathway gene (s)	Source	Target transgenic	Response of transgenic plants and/or organisms	References
CAT3	*Brassica juncea*	*Nicatiana tabacum*	Cd stress tolerance, better seedling growth, and longer roots	Gichner et al. (2004)
CAT	*Brassica juncea*	*Nicatiana tabacum*	Zn and Cd stress tolerance, 2.0-fold higher CAT activity than wild type, lower H_2O_2 level, and cell death	Guan et al. (2009)
CAT1 and CAT2	*Brassica oleracea*	*Arabidopsis*	Low level of H_2O_2 and enhanced stress tolerance	Chiang et al. (2013)
Cu/Zn SOD and/or CAT	*Zea mays*	*Brassica compestris*	Less reduction in photosynthetic activity than wild type under SO_2 stress SOD activity was 1.5–2.5-fold greater than wild type and enhanced Al tolerance	Tseng et al. (2007)
Mn SOD	*Trificumaestivum*	*Brassica napus*	SOD activity was 1.5–2.5-fold greater than wild type and enhanced Al tolerance	Basu et al. (2001)
cylGR/cpGR	*Bacterial*	*Brassica juncea*	cpGR transgenic showed lower Cd accumulation and 50 times higher GR activity than wild type plants	Pilon-Smits et al. (1999)
GR	*Brassica rapa*	*Eschenthia coli*	Increased tolerance against H_2O_2 induced by Cd, 2n, and Al due to an enhanced OR activity	Kim et al. (2009)
DHAR/GR/GST	*Escherichia coil*	*Nicatiana tobacum*	Overexpression enhanced metal tolerance due to maintained red-tax couples such as ascorbate and glutathione	Le Martret et al. (2011)
DHAR	*Oryza sativa*	*Eschenthia coli*	DHAR-overexpressing E. colt strain was more tolerant to oxidant and metal-mediated stress conditions than the control E coil strain	Shin et al. (2008)
MDHAR/DHAR	*Arabidopsis*	*Nicatiana tobacum*	DHAR but not MDHAR enhanced Al tolerance by maintaining ascorbate level	Yin et al. (2010)

(continued)

Table 6 (continued)

Antioxidant and/or its Source biosynthetic pathway gene (s)	Source	Target transgenic	Response of transgenic plants and/or organisms	References
GST	*Tericoderm aviens*	*Nicatiana tobacum*	Enhanced Cd tolerance simultaneously no Cd accumulation, increased activity of SOD, CAT, GST, APX, and GPX than wild type	Dixit et al. (2011)
Sulfite oxidase (SO)	*Zea mays*	*Nicatiana tobacum*	Increased tolerance against S due to enhanced OAT-mediated H_2O_2 scavenging	Xia et al. (2012)
TcPCS1	*Thlaspi caerulescens*	*saccharcrnycescerevisiae and Nicotianatabacurn*	Increased tolerance to Cd due to the decreased lipid peroxiclation and enhanced activities of SOD, POD, and CAT	Liu at at. (2011)
Serin acetyl transferase	*Thlaspi goesingense*	*Escherichia coil*	Imparts Ni and Co tolerance due to involvement of glutathione	Freeman et al. (2005)

Table 7 Some of the identified metal transporters and their expression patterns in plants

Plant species	Protein families	Gene name	Metals	Tissue expression	References
A. thaliana, *A. halleri,* *L. esculentum*	P-Type ATPase	AtHMA1-8, AhHMA3-4, TcHMA4, GmHMA8, OsHMA9	Cu, Zn, Cd, Co, Pb	Shoots and roots	Bernard et al. (2004), Xing et al. (2008), Talke et al. (2006)
A. thaliana, *A. halleri,* *T. caerulescens,* *G. max,* *O. sativa*	Nramp	AtNRAMP1-6, LeNRAMP1-3, AhNRAMP3	Fe, Cd	Shoots and roots	Bereczky et al. (2003), Lanquar et al. (2005)
A. thaliana, *O. sativa*	ZIP	AtZIP1-12, OsZIP4	Zn	Shoots and roots	Filatov et al. (2006), Ishimaru et al. (2005), Roosens et al. (2008)
A. thaliana, *T. caerulescens,* *L. esculentum,* *O. sativa,* *N. tabacum*	IRT	AtIRT1, OsIRT1-2, LeIRT1-2, TcIRT1-2, NtIRT1	Cd, Zn	Shoots and roots	Kerkeb et al. (2008), Plaza et al. (2007)
A. thaliana, *A. halleri,* *T. goesingense,* *N. tabacum,* *P. trichocarpa,* *P. deltoids*	CDF	AtMTP1, TgMTP1, AhMTP1, PtdMTP1, NtMTP1	Zn	Roots	Kawachi et al. (2008), Shingu et al. (2005), Willems et al. (2007)

Table 8 Phytoremediation mechanisms for treatment of contaminated soils (Oh et al. 2013)

Mechanisms	Description
Phytoextraction	Plants absorb contaminants and store in above-ground shoots and the harvestable parts of roots
Phytostabilization	Roots and their exudates immobilize contaminants through adsorption, accumulation, precipitation within the root zone, and thus prevent the spreading of contaminants
Phytodegradation	Plant enzymatic breakdown of organic contaminants, both internally and through secreted enzymes
Rhizodegradation (phytostimulation)	Plant roots stimulate soil microbial communities in plant root zones to break down contaminants
Phytovolatilization	Contaminants taken up by the roots through the plants to the leaves and are volatized through stomata where gas exchange occurs

Effects of Heavy Metals on Growth and Metabolic Status of Medical Plants

Heavy metal accumulation rate in different parts of medical plants have been reported in Table 9.

Heavy metal stress cause lipid oxidation processes and led oxylipins generation. Oxylipins starts signal transduction process for plant defense mechanism. The induction of biosynthesis and accumulation of secondary metabolites such as phenylpropanoids, terpenoids, and alkaloids are one of the major defense mechanisms of plants (Mithofer et al. 2004).

Table 9 Heavy metal accumulation potency of some medical plants (Nasim and Dhir 2010)

Plant species	Heavy metal	Values	References
Amaranthus dubius	Cd	150 ppm	Chunilall et al. (2005)
Amaranthus hybridus	Hg	336 ppm	Chunilall et al. (2005)
Agave amaniensis	Cd	900 μg g^{-1} dry wt	Kartosentono et al. (2002)
	Pb	1390 μg g^{-1} dry wt	Kartosentono et al. (2002)
Costus speciosus	Cd, Pb	530 μg g^{-1} dry wt	Kartosentono et al. (2002)
Matricaria chamomilla	Zn	271 mg kg^{-1} dry wt	Grejtovsky et al. (2001)
Ocimum tenuiflorum	Cr	419 μg g^{-1} dry wt	Rai et al. (2004)
Matricaria chamomilla	Zn	271 mg kg^{-1} dry wt	Grejtovsky et al. (2001)
Phyllanthus amarus	Cd	82 ppm	Rai et al. (2005)
Hypericum sp.	Cd	0.5 mg kg^{-1} dry wt	Chizzola and Lukas (2006)
Cuminum cyminum	Fe	1.4 mg g^{-1} dry wt	Maiga et al. (2005)
Bombax costatum	Fe	1.5 mg g^{-1} dry wt	Maiga et al. (2005)
Hibiscus sabdariffa	Mn	243 μg g^{-1} dry wt	Maiga et al. (2005)
Spilanthes oleracea	Zn	62.8 μg g^{-1} dry wt	Maiga et al. (2005)
Bombax costatum	Zn	67.1 μg g^{-1}	Maiga et al. (2005)
Aesculus Hippocastanum	Pb	1480 μg g^{-1}	Caldas and Machado (2004)
Tilia sp.	Zn	13.8–32.5 mg kg^{-1}	Celechovska et al. (2004)
Sambucus nigra	Zn	30.8–49.9 mg kg^{-1}	Celechovska et al. (2004)

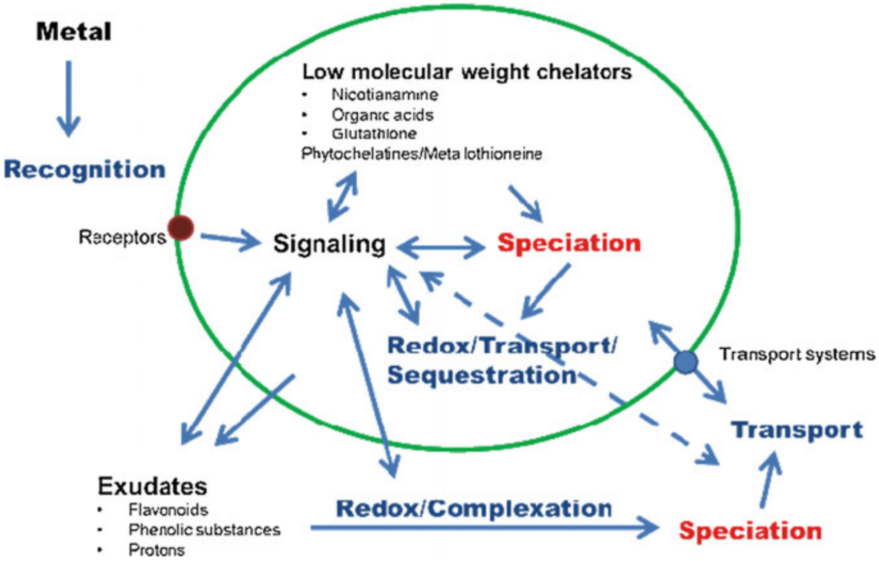

Fig. 2 Short overview about some important aspects of cellular metal interaction (Viehweger 2014)

The general view of heavy metal signal transduction pathway in plants and role of oxylipins and secondary in detoxification process are summarized in Fig. 2.

Table 10 provides an insight in the complex signaling network induced by various environmental stress conditions and their similarity patterns (Viehweger 2014).

Several researches have showed plants exposed to heavy metal stress show varying degrees of secondary metabolite response (Table 11).

Plants exposed to heavy metal stress show differential responses in synthesis and accumulation of pharmacologically active molecules. Such responses range from negative effects on secondary metabolite production in a few plant species, viz., *Matricaria recutita*, to stimulatory effects that result in enhanced metabolite production in other species. Increases in heavy metal-induced secondary metabolite biosynthesis have been reported to occur in some medicinal plant species (Table 12) (Nasim and Dhir 2010). Utilizing heavy metal as inducers for higher production of secondary metabolites depend on the plant part used as consumer safety needs (Street 2012).

Table 10 Overview of some heavy metal triggered signals in comparison to other environmental stresses

Heavy metal	Signal	Other stress conditions	Cellular responses	References
Cu	Calcium fluxes	Cold, drought, salinity	Phosphoprotein cascades, 2nd signalling molecules	Nielsen et al. (2003)
Cd, Cr	Mitogen activated protein kinase (MAPK) pathways	Osmotic stress, pathogen contact	Activation of transcription factors and stress-responsive genes	Liu et al. (2010)
Fe	pH shifts	Pathogen contact	Induction of secondary metabolism	Viehweger et al. (2006)
Co, Zn	Plant hormones like abscisic acid or ethylene	Cold, drought, salinity	Calcium signaling, guard cell regulation (water balance)	Zengin (2006)
Cd, Cu	Jasmonic acid	Pathogen contact, sugar, drought, salinity	Defence/stress response, development, induction of secondary metabolism	Howe and Schilmiller (2002)
Redox-active metals like Fe, Cu; almost all heavy metals at higher concentrations	Reactive oxygen species	Pathogen contact, cold, drought, salinity, high light intensity	Phosphoprotein cascades, activation of transcription factors and stress-responsive genes, activation of antioxidative defence	Ahmad et al. (2008)

Table 11 Heavy metal stress affecting secondary metabolite production (Street 2012)

Plant species	Main findings relating to secondary metabolites	References
Hypericum perforatum L.	In the presence of Ni, the plant completely lost the ability to produce or accumulate hyperforin and demonstrated a 15–20-fold decrease in the concentration of pseudohypericin and hypericin	Murch et al. (2003)
Ocimum tenuiflorum L.	Cr stress induced the production of eugenol	Rai et al. (2004)
Dioscorea bulbifera L.	The occurrence of Cu stimulated diosgenin production	Narula et al. (2005)
Phyllanthus amarus Schum and Thonn	Phyllanthin and hypophyllanthin was enhanced by Cd stress	Rai et al. (2005)
Bacopa monnieri L.	The level of bacoside-A increased due to increased Fe in the media	Sinha and Saxena (2006)
Trigonella foenum-graecum L.	Cd and Co increased diosgenin levels however Cr and Ni inhibited its production	De and De (2011)

Table 12 List of plants secondary metabolites increment under heavy metal stress (Nasim and Dhir 2010)

Plant species	Heavy metal	Compound	Medicinal properties	References
Catharanthus roseus	Cd 0.05–0.4 mM	Ajmalicine	Anticancer, antidiabetic	Zheng and Wu (2004)
Phyllanthus amarus	Cd (0.1–1 mM)	Hypophyllanthin	Hepatoprotective, diuretic, stomachic	Rai et al. (2005)
Matricaria chamomilla	CuC12 2%, Cd 60–120 µM Zn 50 mg kg^{-1} soil	Herniarin, Umbelliferone essential oil, sesquiterpenes	Anti-inflammatory, spasmolytic effect	Eliasova et al. (2004)
Salvia miltiorrhiza	Ag, Cu, Zn, Fe, Mn15–40 µM	Tanshinon	Broad spectrum bactericide, dilating coronary artery	Zhang et al. (2004), Guo et al. (2005)
Ononis arvensis	Ni, Co, Cr 6.3 µmol	Flavonoid	Anti-inflammatory, antiproliferative, anticancer, antioxidant, cardioprotective	Tumova et al. (2001), Tumova and Blazkova (2002)
Rheum palmatum	Cd 10 µM	Anthracene derivatives	Antioxidant	Kasparova and Siatka (2004)
Thalictrum rugosum	Cu 20–500 µM	Berberine	Antimicrobial in the treatment of dysentery and infectious diarrhea	Kim et al. (1991)
Hibiscus sabdariffa sabdanffa	Co 20 mg kg^{-1} soil Ni 25 mg kg^{-1} soil	Anthocyanins Flavons	Diaphoretic, hepatic and gastric disorders, arteriosclerosis, intestinal infections	Eman et al. (2007)
Ocimum tenuiflorum	Cr 20 µM	Eugenol	Antiseptic, antispasmodic, antibacterial	Rai et al. (2004)
Hypericum perforatum	Cr(VI) 0.1 µM	Hypericin Pseudohypericin	Minor burns, wounds, skin inflammations, nerve pain	Tirillini et al. (2006)
Datura stramonium	Cd 1 mM	Hyoscyamine	Peptic ulcers, diarrhea, and bronchial asthma	Furze et al. (1991)
Mentha arvensis	Zn	Essential oil, menthol	Antibacterial, antifebrile, effective in rhinitis, cough sore throat, colic	Misra (1992)
Costus speciosus	Pb 30 mg L^{-1}	Sitosterol	Purgative, febrifuge, expectorant	Kartosentono et al. (2002)
Pluchea lanceolata	200 µM $ZnSO_4$ or 150 µM $CuSO_4$	Quercetin, Tropane alkaloids	Inflammations, bronchitis, psoriasis, piles, antipyretic, analgesic, laxity, Diuretic, sedative, antispasmodic	Kumar et al. (2004)

International Standards of Metals Residuals in Medicinal Plants

There are immense discrepancies between countries regarding regulatory requirements to pledge safety and quality of plant-based products (Diederichs et al. 2006). Several regulations have already been established worldwide for medicinal plants such as the US Pharmacopoeia (USP), Italian Pharmacopoeia (FUI), and European Pharmacopoeia (Ph. Eur.). Moreover, there are legal frameworks at national and/or regional levels that are designed to regulate the quality of plant-based products (Sarma et al. 2012). Several countries, including Canada, China, Malaysia, Singapore and Thailand, have developed their own national guidelines to ensure satisfactory levels of heavy metals in medicinal plants and plant-based products (Table 13) (Street 2012).

Conclusions and General Discussion

The presence of heavy metals in medicinal plants may stimulate production of bioactive compounds in many plant species. Although, the exact mechanism by which this happens remains unclear. Oxidative stress induced by heavy metals triggers signaling pathways that affect production of specific plant metabolites. In particular, reactiveoxygen species (ROS), generated during heavy metal stress, may cause lipid peroxidation that stimulates formation of highly active signaling compounds capable of triggering production of bioactive compounds (Nasim and Dhir 2010).

Heavy metal tolerance is a genetically complex process that involves many components of signaling pathways, multigenic in nature (Vinocur and Altman 2005). Therefore, plant-engineering strategies for heavy metal tolerance depend on the expression of gene(s) whose product(s) are involved either in signaling and regulatory pathways or in the synthesis of functional and structural proteins and metabolites that confer heavy metal stress tolerance. Recently, several efforts are being made to improve heavy metal stress tolerance capacity through genetic engineering with several achievements (Singh et al. 2016).

Phytoremediation holds great potential as an environmental cleanup technology and has been investigated substantially since the last two decades. Considerable interest in phytoremediation exists by both government and industry. The biggest advantage of phytoremediation is its low cost. Phytoremediation can be up to 1000-fold cheaper compared with conventional remediation methods such as excavation and reburial. In general, fast-growing, high-biomass, competitive, hardy, and metal-tolerant plant species could either be selected or could be generated by genetic manipulation and be used for remediation of different polluted sites.

The presence of several hundreds of catabolic enzymes and transporter sequences suggest that plants may have rich potential to mobilize and detoxify toxic

Table 13 National limits for heavy metals in herbal medicinal products (Street 2012)

Country		Arsenic (As)	Lead (Pb)	Cadmium (Cd)	Chromium (Cr)	Mercury (Hg)	Copper (Cu)	Lead (Pb)
Canada	Raw herbal materials	5 ppm	10 ppm	0.3 ppm	2 ppm	0.2 ppm		
	Finished herbal products	0.01 mg/day	0.02 mg/day	0.06 mg/day	0.02 mg/day	0.02 mg/day		
China	Herbal materials	2 ppm	10 ppm	1 ppm		0.5 ppm		20 ppm
Malaysia	Finished herbal products	5 mg/kg	10 mg/kg			0.5 mg/kg		
Republic of Korea	Herbal materials							30 ppm
Singapore	Finished herbal products	5 ppm	20 ppm			0.5 ppm	150 ppm	
Thailand	Herbal material, finished herbal products	4 ppm	10 ppm	0.3 ppm				
WHO recommendations			10 mg/kg	0.3 mg/kg				

contaminants including organic and inorganic in their environment within their tissues and organs. Genomic and proteomic information gained from these sequenced plant species will greatly accelerate the phytoremediation process in situ considerable efforts have been taken by the European Science Foundation, and under this context, a COST 859 Action entitled "Phytotechnologies to promote sustainable land use and improve food safety has been launched since 2004. The main objective of this action is to provide a sound understanding of the absorption, translocation, storage, or detoxification mechanisms of essential or toxic mineral elements, as well as organic contaminants, and to prepare the best use of plants for sustainable land use management and improve food safety. Promotion of cooperation and of data exchange between working groups in this action have been encouraged and the present work is a part of such cooperation (Memon and Schroder 2009).

Further knowledge about metal tolerance in plants is mandatory for several purposes: (1) Predictions about health risk which is caused by metal accumulation in crop plants failing visible symptoms of phytotoxicity. (2) Generation of genetically engineered plants having an enhanced accumulation of metals valuable for nutritional purposes (biofortification). (3) Cleanup of metal contaminated soils (phytoremediation) and mining of rare metals which are accumulated in plant tissues (phytomining) (Viehweger 2014).

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Horizontal Natural Product Transfer: A so Far Unconsidered Source of Contamination of Medicinal Plants

Melanie Nowak, Mahdi Yahyazadeh, Laura Lewerenz and Dirk Selmar

Abstract In this treatise, an actual overview on the newly verbalised phenomenon of “*Horizontal Natural Product Transfer*” is presented. This phenomenon was recently discovered when analyzing the potential sources of contaminations of plant derived commodities by nicotine and pyrrolizidine alkaloids. It turned out that these alkaloids, leached out from decomposing alkaloid containing plant parts (donor plants), are taken up by the roots of acceptor plants. In analogy to the well-known and widespread phenomenon of the uptake of xenobiotics, it can be deduced that a wide array of alkaloids and other natural products are taken up by acceptor plants either. As such uptake generally is due to a simple diffusion of the substances across the biomembranes, the physico-chemical properties of the natural products, especially their hydrophilic as well as their lipophilic character, determines the extent of a corresponding uptake. Latest findings from co-cultivation experiments revealed that natural products are not transferred exclusively from dead and rotting donor plant material, but also from living and vital plants. Yet, up to now, the exact path, i.e., in which manner the natural products are translocated from the living plants into the acceptor plants is unknown. The finding that plants inherently are able to take up most alkaloids from the soil, derived from other plants, necessitates a reconsideration of our understanding of plant-plant-interactions. In this context, also the classical definition of xenobiotics, which up to now are considered as “non-natural” substances, might be extended by including also natural products leached out into the soil.

Keywords Horizontal transfer · Natural products · Xenobiotics · Alkaloids

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Introduction

It is a matter of common knowledge that plants take up substances from the soil by the means of their roots. Apart from inorganic nutrients, such as nitrate, phosphate or various metal ions, also many other compounds are imported via the roots. From chemical ecology (e.g. Inderjit and Duke 2003) we have learned that active compounds, denoted as allelochemicals, i.e., substances inhibiting germination or growth of putative competitors, are frequently exuded from donor plants and exhibiting their effect in the growing plants (Bertin et al. 2003; Kalinova et al. 2007); for this, an uptake by the acceptor plant is indispensable (Willis 1985; Inderjit and Duke 2003). Furthermore, it is well established that various xenobiotics such as systemic herbicides and fungicides (Trapp and Legind 2011) and veterinary medicines (Boxall et al. 2006) are also taken up from the soil and translocated into the shoots. Moreover, salicylic acid, which is responsible for systemic acquired resistance, is known to be imported by the roots (De Meyer et al. 1999; Manthe et al. 1992).

Whereas for any import of most ionic nutrients like nitrate, sulfate or metal ions specific transporters are required (Forde 2000; Buchner et al. 2004; Kobayashi and Nishizawa 2012), most of the xenobiotics are taken up by simple diffusion (Trapp and Legind 2011). Due to their partially hydrophobic character, these molecules are able to diffuse passively through root membranes (Inoue et al. 1998; Nwoko 2010; Sibout and Höfte 2012). Since so many different substances are taken up by plant roots, it seems to be obvious that plant derived natural products should be taken up as well. Amazingly, this obvious issue was not adequately considered so far.

The situation changed not before recent investigations had been conducted, which were aimed to identify the potential sources of various contaminations of plant derived commodities with nicotine (EFSA Reasoned Opinion 2011) and pyrrolizidine alkaloids (EFSA Panel on Contaminations in the Food Chain 2011; Mulder et al. 2015). In this context, it could be shown that nicotine derived from dried tobacco plant material (Selmar et al. 2015a, b), or form cigarette butts, which had been trashed into the fields (unpublished data), is taken up by acceptor plants. Obviously, the alkaloid is leached out from plant remains into the soil and is subsequently taken up by other plants. In the same manner, pyrrolizidine alkaloids (PAs), which are leached out from rotting PA containing weeds, e.g. *Senecio jacobaea*, are also taken up by acceptor plants (Nowak et al. 2016). In other words, this transfer of alkaloids seems to be—at least in part—responsible for the numerous and widespread PA contaminations of spice and medicinal plants reported (EFSA Panel on Contaminations in the Food Chain 2011; Mulder et al. 2015).

The Novel Concept of Horizontal Natural Product Transfer

Based on the findings of nicotine and PA uptake by various acceptor plants, the concept of “*horizontal natural product transfer*” has been introduced by Selmar et al., (2015a, b): when natural products are leached out into the soil from decomposing plants—denoted as donor plants—these compounds could be transferred into the roots of acceptor plants and translocated into their leaves (Fig. 1). In consequence, the substances have to pass the plasmalemma of root cells of the acceptor plants. The required uptake into the symplast may occur within the rhizodermis or, at the latest, in the passage cells of the endodermis. As mentioned above, such uptake could be accomplished either by an active transport, facilitated by the action of carriers, or by passive diffusion through the biomembranes. Indeed, on first glance, due to the great number of transporters known so far (for review see Yazaki 2006; Rea 2007; Remy and Duque 2014), the involvement of carrier proteins seems reasonable. However, we have to be aware that many substances are able to simply diffuse across biomembranes. A precondition for such diffusion is the solubility of the substance in aqueous as well as in organic media. This property roughly can be estimated from the so called K_{OW} value, which describes the distribution of a substance between octanol and water, or its decadal logarithm, denoted as pK_{OW} (e.g., Trapp 2000; Trapp 2009). Accordingly, membrane permeability can be extrapolated from this distribution coefficient (pK_{OW}), which frequently is also termed as log$P$ (e.g., Cronin and Livingstone 2004). Substances revealing log$P$ values between −1 and 3 generally are considered to be able to diffuse easily through biomembranes (Trapp 2000; Trapp and Legind 2011; Limmer and Burken 2014). Albeit these generalizations had been elaborated in studies dealing with an uptake of xenobiotics, they are also relevant for all natural products, e.g. alkaloids. However, in the case of alkaloids, an additional factor has to be considered, i.e. the pH dependent protonation of these natural compounds (Nowak and Selmar 2016). Whereas the free bases of alkaloids like nicotine, or pyrrolizidine alkaloids, are simply able to pass through membranes, their protonated forms, which reveal a positive charge, cannot penetrate so easily through the biomembranes. This is in accordance and approved by the strongly negative log$P$ values of charged alkaloid salts. Consequently, in addition to the log$P$ of the alkaloids, the pH of the medium also determines their membrane permeability.

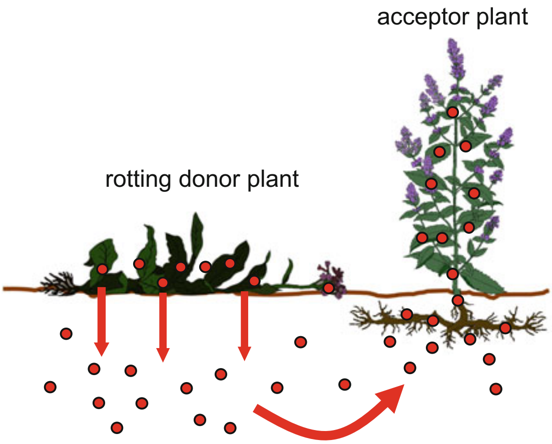

Fig. 1 Horizontal natural products transfer. According to Selmar et al. (2015a, b) substances leached out from rotting plants (donor plants) into the soil could be taken up by acceptor plants

These ambivalent properties of alkaloids are the basis for the accumulation of alkaloids in plant vacuoles. The corresponding ion trap model was already presented by Matile (1976): because of their membrane permeability, unprotonated alkaloids present in the neutral cytosol pass freely through the tonoplast into the vacuole by passive diffusion. Once inside the acidic vacuole, the alkaloids are protonated and the resulting hydrophilic cations are unable to re-permeate the tonoplast and are trapped. Although these relations have been known for a long time, until recently, these coherences have not been considered to explain the uptake of alkaloids from the soil (Nowak and Selmar 2016).

Meanwhile many different classes of alkaloids have been analysed whether they are also transferred from a rotting donor plant into putative acceptor plants. In accordance to their log$P$ values, being between -1 and 3, in addition to nicotine and PAs, also tropane and purine alkaloids as well as benzylisoquinoline and indole alkaloids were taken up by the acceptor plants (Yahyazadeh et al. 2017). In contrast, only quaternary alkaloids such as coptisine, palmatine, or berberine are not able to cross the membrane. On the first sight, these findings seem to be surprising, but they are fully confirmed by the corresponding physico-chemical properties of quaternary alkaloids. Those compounds always carry a positive charge—independently to the pH of the medium. This charge generates a low lipophilicity, which is confirmed and apparent by the quite negative log$P$ values of these alkaloids (Yahyazadeh et al. 2017). In consequence, these alkaloids are not able to pass through biomembranes, and accordingly, they cannot be taken up by the acceptor plant due to simple diffusion.

Apart from the membrane permeability, we have to consider a further aspect: the microbial decomposition of rotting plant materials. It is well known that in principle the entire biomass from rotting plant material is completely decayed by microorganisms. In consequence, the active natural products are also degraded efficiently. Therefore, when discussing the horizontal transfer of natural products, we always have to consider that the actual extent of this phenomenon results from the simultaneous processes between soil microorganisms-degrading these substances—and the acceptor plants, which take them up.

Membrane Permeability of Alkaloids Depends on the pH and its Significance for Transport Processes

Based on the coherences on the pH dependent changes in membrane permeability of alkaloids, it could be deduced that the pH of the soil reveals a significant influence on the extent of the uptake of alkaloids present in the soil. The higher the pH of the soil, the greater the share of unprotonated alkaloids, and consequently, their membrane permeability (Nowak and Selmar 2016). By contrast, in very acidic soils, due to a high degree of protonation, the uptake of alkaloids should be drastically decreased (Trapp and Legind 2011). There is a great demand of future experimental studies, in which these relationships are verified and validated, respectively.

Beyond the significance for the uptake of alkaloids from the soil, the pH related changes in their membrane permeability reveal also a high relevance for the long distance transport of these natural products within the plants. A detailed overview on this issue is given by Nowak and Selmar (2016). In principle, the translocation of alkaloids from roots into the shoots could be performed by either xylem or phloem transport. In case of a xylem transport, translocation would be entrained passively by transpiration flow, whereas a phloem-related translocation requires a *source*-*sink*-allocation. The pattern of imported nicotine in the acceptor plants clearly showed that its concentration was highest in older leaves, i.e., the leaf tissues that exhibit the greatest transpiration surface. In contrast, the smaller young leaves, representing physiological sinks, had far lower nicotine concentrations (Selmar et al. 2015a, b). These coherences indicate that the entered nicotine is translocated via the xylem, driven by the transpiration flow. These findings are supported by Weidner et al. (2005), who determined nicotine in the xylem sap, when the alkaloid was fed to a hydroponic system of *Epipremnum aureum*. Analogously to the xylem based nicotine translocation in acceptor plants, also pyrrolizidine and purine alkaloids are relocated after their uptake. The occurrence of these alkaloids in guttation droplets of acceptor plants (Nowak 2017) as well as the pattern of PA distribution (Nowak et al. 2016) indicates a xylem based translocation, which fully is in accordance with the prediction of Trapp and Legind (2011) based on the log*D* values of substances taken up by the acceptor plants.

In contrast, the translocation of alkaloids in genuine alkaloidal plants follows quite different prerequisites. Depending on the pattern of distribution, either a phloem associated or a xylem transport occurs: in *Senecio vulgaris* the PAs produced in the roots are transported via phloem into the sink tissues such as flowers or seeds (Ehmke et al. 1988; Hartmann and Dierich 1989) whereas in *Nicotina sylvestris* nicotine is translocated from the roots into the leaves via xylem (Baldwin 1989). Details on this entire issue, especially the pH dependent changes in membrane permeability, are given by Nowak and Selmar (2016). These coherences presented a sound explanation for the occurrence of PA-*N*-oxides in the phloem (Fig. 2), a finding which was elaborated in the group of Hartmann several decades ago (e.g., Ehmke et al. 1988; Lindigkeit et al. 1997).

The Horizontal Transfer of Other Classes of Natural Product

It is understood that the coherences on the membrane permeability and log*P* values mentioned above are valid for other classes of natural products, too. Accordingly, phenolic compounds, terpenoids and all other substances are also able to diffuse through biomembranes as long the hydrophilic as well as the lipophilic properties of

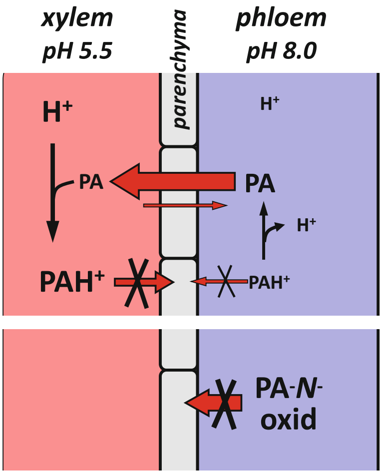

Fig. 2 Passive transfer of pyrrolizidine alkaloids from the slightly alkaline phloem into the acidic xylem. Based on the ion trap model of Matile (1976), it can be deduced that tertiary PAs, due to their membrane permeability, cannot be transported via the phloem. In contrast, the PA-*N*-oxides, which are not able diffuse easily through biomembranes, are translocated (Nowak and Selmar 2016)

the substance are within an appropriate range, i.e., when the log*P* values is between −1 and 3 (Trapp 2000; Trapp and Legind 2011). Unfortunately, up to now, no data on this issue with respect to a putative transfer between donor and acceptor plants are available. Nevertheless, there are various corresponding hints. The analyses of the potential to be taken up by lettuce plants of so-called "*emerging organic contaminants*" (EOCs) revealed that many different classes of such organic compounds are able to pass biomembranes and taken up by acceptor plants (Hurtado et al. 2016). Furthermore, elder studies on the uptake and glucosylation of coumarins revealed that esculetin and scopoletin are taken up efficiently by protoplasts from barley leaves (Werner and Matile 1985). Although there are many indicators that—analogous to the well established uptake of alkaloids—also other classes of natural products might be transferred from donor to acceptor plants, corresponding thorough and comprehensive studies are required.

One of our latest approaches on the further elucidation of the processes involved in the horizontal natural product transfer is aimed to visualize this phenomenon. For this, in principle, colorant like betanidines or its derivatives, have to be applied to acceptor plants. Unfortunately, the green colour of vital leaves overlays all other hues. Accordingly, these red dyes, although they are taken up, cannot be simply detected visually. However, when using chlorophyll free leaves, e.g. etiolated seedlings of peas or barley, the uptake of dyes indeed is visible (Lewerenz 2016). One striking example that manifests this phenomenon is given in Fig. 3. Although this approach demonstrates that these dyes are taken up by the roots of pea seedlings and translocated into their leaves, various ambiguities require further elucidation. In the corresponding experiment, a crude extract from red beet was used, which contains various red coloured substances e.g., betalain, isobetalain, and vulgaxanthin (Attia et al. 2013). Moreover, all these substances are very unstable,

Fig. 3 Uptake of betalains by etiolated pea seedlings. An aqueous extract from red beet tubers containing various red dyed betalains was applied to pea seedlings raised in a hydroponic system in the dark (Lewerenz 2016)

especially in the presence of oxygen (Herbach et al. 2006), or in the course of extraction, they are degraded. Thus, we have to consider that a whole array of different red-coloured substances has been applied to the etiolated seedlings. Consequently, it is not known, which particular compounds have finally been taken up and translocated into the leaves, where it caused the red colour in the acceptor plants. Forthcoming experiments, in which pure dyes are applied to etiolated seedlings, will be a promising approach in understanding the fascinating phenomenon of the natural product transfer.

Broadening of the Concept of Horizontal Natural Product Transfer—Co-cultures

In allelopathy it is well accepted that the release of typical allelochemicals into the environment might be due to three different processes (e.g. Nakano et al. 2003). Apart from their leaching out of decomposing plants residues, these active compounds also could be derived from living plants either via an active exudation by the roots (Bertin et al. 2003; Kalinova et al. 2007) or by the leaves (Tukey 1970; Nakano et al. 2003). In analogy to the transfer processes described above, it seems natural to assume that a horizontal natural product transfer could also occur based on living donor plants. To verify such assumption, so called co-culture experiments have been conducted. For this, a potential vital donor, e.g., pyrrolizidine alkaloid containing *Senecio jacobaea* plants were cultivated in one single pot together with the acceptor plants, e.g. parsley. After two month co-cultivation, the plants where harvested and the pyrrolizidine alkaloids quantified. Surprisingly, all parsley plants co-cultivated with the *Senecio jacobaea* plants contained high concentrations of PAs, in average more than 200 µg/kg (Fig. 4). As consequence of these amazing results, further experiments will be conducted, especially corresponding field trails

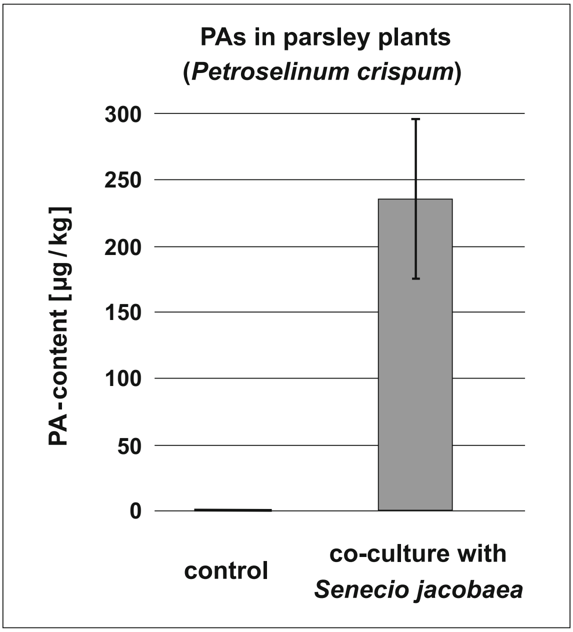

Fig. 4 Uptake of pyrrolizidine alkaloids in parsley plants. Parsley plants (*Petroselinum crispum*) as potential acceptor plants had been cultivated in one single pot together with *Senecio jacobaea* donator plants. Directly after harvest, the PAs were extracted and quantified

on co-cultivation of *Senecio jacobaea* with various different acceptor plants are under way.

Indeed, the presence of a high quantum of PAs in the acceptor plants testifies that the alkaloids are transferred from living and vital donor plants. But, up to now, there is no indication about the path of such transfer. On the first glance, it seems to be obvious that—in analogy to an active exudation of allelochemicals (Tukey 1970; Nakano et al. 2003; Bertin et al. 2003; Kalinova et al. 2007) the PAs (and maybe other alkaloids) might also be exsuded either by the roots or by the leaves. However, we have to take into consideration that vital plants frequently exhibit numerous injuries and damages, e.g. caused by pathogens or herbivores. Furthermore, in many areas in the roots, e.g. the calyptra, regularly large numbers of cells are destroyed. Accordingly, it could not be excluded that the transfer of PA observed is due to natural injuries of the donator plant, enabling the transfer from plant to plant, via the soil. Much further research is required to elucidate the actual paths of transfer. The fact that alkaloids indeed are transferred from living and vital donor plants into acceptor plants requires a corresponding broadening of the concept of horizontal natural product transfer, which is outlined in Fig. 5.

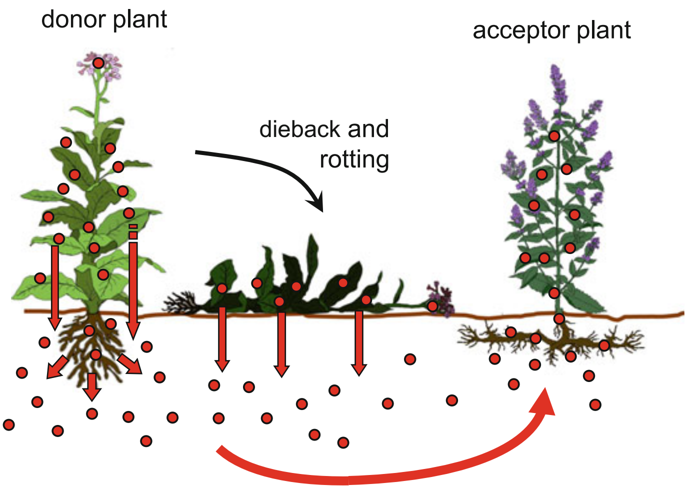

Fig. 5 Broadening of the concept of horizontal natural product transfer. As the results of co-cultures of acceptor and donator plants demonstrated that alkaloids also could be transferred from vital and living donator plants, further and additional paths of the transfer of natural products have to be considered, e.g. their active exudation by leaves and roots, or their leaching in consequence of injuries or damages, e.g., due to pathogen or herbivore attack

Conclusion

We have to approve that the phenomenon of horizontal transfer of natural products is far more prevalent than initially assumed. Obviously, not only alkaloids, but other natural products as well are transferred from donor to acceptor plants. In this context, we have to reconsider the classical definition of xenobiotics, which up to now always were regarded as “non-natural” substances (e.g. Iovdijová and Bencko 2010; Godheja et al. 2016).

The finding that plants inherently are able to take up many alkaloids from the soil, and the novel cognition that natural products also could be released into the environment from vital and living plants, necessitate a reconsideration of our understanding of plant-plant-interactions. Up to now, only “typical allelochemicals”, i.e., those compounds that reveal certain significance in plant-plant interactions, e.g., by inhibiting the growth or the germination of potential competitors, have in this context been taken into consideration.

The outlined transfer of natural products from donor to acceptor plants, which seems to be a general feature in plant-plant interactions, presents new explanations of several hitherto unexplained processes related to beneficial effects of crop rotations or the co-cultivation of certain vegetables.

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Strategies for Enhancing Artemisinin Production in *Artemisia annua* Under Changing Environment

M. Naeem, Tariq Aftab and M. Masroor A. Khan

Abstract *Artemisia annua* L. (family Asteraceae), is the only source of a potent anti-malarial drug, artemisinin. Artemisinin, a sesquiterpene lactone containing an endoperoxide bridge, has been recognized as an effective and safe remedy against malaria parasite *Plasmodium falciparum*. However, the concentration of artemisinin in the plant is very low, ranging from 0.01 to 0.8%. Exposure of *A. annua* plants to abiotic stresses, such as of light, temperature, salinity, heavy metals and UV light, have been reported to enhance ROS generation, which augment artemisinin yield by facilitating rapid conversion of artemisinic acid to artemisinin. In fact, generation rate of H_2O_2 increased consistently under abiotic stress conditions, suggesting a direct relationship between generation of H_2O_2 and artemisinin biosynthesis in *A. annua*. Various scientific approaches have been attempted to increase artemisinin production including chemical synthesis and genetic engineering of the pathway genes involved in artemisinin biosynthesis in *A. annua*; but, not much success has been accomplished till now due to high cost of procedure or complex nature of regulation and expression of the genes responsible for artemisinin biosynthesis. Plant growth regulators (PGRs) exhibit a broad spectrum of physiological effects in plants which may influence the inter-relationships between primary and secondary metabolism leading to increased biosynthesis of secondary metabolites. As per our study, employing pot and field experiments, some PGRs (gibberellic acid, salicylic acid, methyl jasmonate etc.) and radiation-processed polysaccharides (sodium alginate and carrageenan) proved as non-conventional elicitors that enhanced the overall yield of artemisinin under normal and abiotic stress conditions. In order to increase the desired production of artemisinin and utilize the land unsuitable for food crops, cultivation of *A. annua* might be employed with new scientific approaches and convenient strategies.

Keywords Artemisinin · Abiotic stresses · Irradiated polysaccharides · PGRs · Mineral nutrients

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Introduction

Artemisinin, isolated from *Artemisia annua*, is a potent drug that could be effective against multidrug-resistant strains of the malaria parasite, *Plasmodium*, responsible for a variety of various diseases including antimalarial and anti-cancer activities, contains an endoperoxide bridge, which is rarely found in other secondary metabolites. Artemisinin and its derivatives are administered to restrain cancer and tumor activity (Crespo-Ortiz and Wei 2012) besides their effective use in the treatment of malaria (Haynes 2006). However, artemisinin-derived drugs are not available to millions of the world's poorest people because of the low yield (0.1–0.5% of dry weight) of artemisinin in naturally-grown artemisia plants (Wallaart et al. 1999). Genetic improvement of natural artemisinin varieties has been attempted, but the maximum artemisinin-yield achieved so far is 2% of leaf dry-weight (Graham et al. 2010). Although artemisinin can be obtained by chemical synthesis, the method is complicated and is not feasible economically because of the poor yield of the drug. Since the plant is the only viable source of artemisinin production, the enhanced biosynthesis of artemisinin content in the plant is extremely advantageous (Weathers et al. 2005; Aftab et al. 2010a, b, c, 2011a, b, 2014; Kiani et al. 2016; Lv et al. 2016).

As per the World Health Organization (WHO), the toxin-free, artemisinin-based combination therapy (ACT) is most effective against the drug-resistant malaria parasite (Davis et al. 2005; WHO 2010). Various scientific approaches have been used to enhance artemisinin production including chemical synthesis (Xu et al. 1986; Avery et al. 1992) and genetic engineering of the pathway genes involved in artemisinin biosynthesis (Chen et al. 2000; Xie et al. 2001; Ro et al. 2006; Nguyen et al. 2011); but, not much success has been accomplished due to high cost of procedure or complex nature of regulation and expression of the genes responsible for artemisinin biosynthesis.

The biomass and artemisinin production in *A. annua* are mostly regulated by genetic factors (Ferreira et al. 1995b). Apart from this, a variety of plant growth regulators (PGRs) including gamma-irradiated polysaccharides are reported to influence diverse plant developmental processes including terpenoid biosynthesis in this aromatic plant (Aftab et al. 2010a, b). These polysaccharides have been used as a wonderful growth-promoting substance in its depolymerized form for a number of medicinal plants including *Artemisia annua* (Aftab et al. 2013, 2014, 2016). On the other hand, exposure of *A. annua* to several abiotic factors such as, light, temperature, salinity, heavy metals and UV light, have been reported to stimulate ROS generation, which enhance artemisinin yield by facilitating rapid conversion of dihydroartemisinic acid to artemisinin (Pu et al. 2009; Wallaart et al. 2000; Qureshi et al. 2005; Aftab et al. 2010a, b, c) during biosynthetic pathway.

Therefore, there are several factors (e.g. PGRs, elicitors, mineral nutrients, abiotic stresses) that affect plant biomass and artemisinin yield. Apparently, the artemisinin precursor dihydroartemisinic acid (DHAA) is conjectured to act as a scavenger of reactive oxygen species (ROS) before getting transformed into artemisinin in the plant. However, artemisinin is only one the of 16 molecules that are formed from DHAA (Brown 2010), indicating that scavenging for reactive oxygen species is not the main function of DHAA. In the following chapter, we provide detailed description of various factors that affect the morphology, physiology and artemisinin production of this enormous plant with an emphasis on PGRs, elicitors, mineral nutrients, as well as abiotic stresses on plant performance and artemisinin production.

Effect of Plant Growth Regulators and Elicitors

Shukla et al. (1992) reported that artemisinin and herbage yield of *A. annua* plants increased owing to application of triacontanol (TRIA) and 2-chloroethyl trimethyl ammonium chloride (chlormequat). TRIA produced a significant increase on artemisinin concentration as well as on plant height, leaf and herbage yield. Chlormequat also increased artemisinin level, decreased the plant height at higher concentrations and increased the leaf and herbage yield at lower concentrations. They suggested that the effect of TRIA on artemisinin yield seems to be mediated through GA_3 and ABA-like activities on plant growth.

Smith et al. (1997) explored the effect of gibberellic acid (GA_3) on the growth and artemisinin production of hairy roots of *A. annua*. They applied six different concentrations of GA_3 to find out the optimum concentration. GA_3 levels from 0.01 to 0.001 mg L^{-1} (28.9–2.89 lM) provided the most significant increase in biomass and 0.01 mg L^{-1} (28.9 lM) produced the highest amount of artemisinin. They also studied growth kinetics and found that the use of GA_3 at 0.01 mg L^{-1} (28.9 lM) increased the growth rate of hairy roots of *A. annua* by 24.9%.

Wang et al. (2002) focused their research on artemisinin accumulation in hairy root cultures of *A. annua* using (22S, 23S)-homobrassinolide (SSHB). They observed that when 1 µg L^{-1} of SSHB was added to the hairy root cultures, the production of artemisinin reached 14 mg L^{-1}, an increment of 57% over the control. Furthermore, artemisinin accumulation in hairy roots was found to be dose dependent as well in the treatment with SSHB. They also found that the SSHB treatments at 0.1–10 µg L^{-1} increased the root biomass up to 12–15 g L^{-1} from that of the control. However, if SSHB concentrations were higher than 100 µg L^{-1}, a decline in growth was detected with some root browning, an indication of the cell necrosis.

Weathers et al. (2005) examined the effect of various phytohormones on the growth and secondary metabolism of *A. annua*. They measured growth, development and production of artemisinin in *A. annua* hairy roots in response to the five main hormones: auxins, cytokinins, ethylene, gibberellins (GA_3) and abscisic acid (ABA). Single roots grown in six-well plates in medium B5 with 0.01 mg L^{-1} GA_3 produced the highest values overall in terms of the number of lateral roots, length of the primary root, lateral root tip density, total lateral root length and total root length. When the total root length was compared, the best conditions for stimulating root elongation were with 0.01 mg L^{-1} GA_3. Bulk yields of biomass were inversely proportional to the concentration of each hormone tested in their study. All root cultures provided with ABA yielded the highest amount of biomass. Both 6-benzylaminopurine and 2-isopentenyladenine inhibited root growth, however, only 2-isopentenyladenine stimulated artemisinin production, more than twice to that of the B5 controls, and more than any other hormone studied.

Ferreira and Janick (1996a) used the growth regulators, benzyladenine (BA), kinetin, chlormequat (CCC) and daminozide, to induce shoot development. BA increased shoot proliferation at 0.5 and 5.0 lM but decreased root production at all concentrations. A highly significant correlation was observed between shoot artemisinin concentration and the number of roots (r = 0.775**), while shoot number and artemisinin were unrelated (r = −0.198). The authors reported that the highest levels of shoot artemisinin (0.29%) in shoot cultures were obtained with hormone-free medium (control) when root production was maximized. In the same study, removal of roots from shoot cultures grown in hormone-free medium reduced shoot artemisinin in 53% and arteannuin B by 60%, confirming the pivotal role of roots in artemisinin biosynthesis.

The research of Pu et al. (2009) provided evidence that salicylic acid (SA) can activate artemisinin biosynthesis in *A. annua*. They observed that exogenous application of SA to *A. annua* leaves was followed by a burst of reactive oxygen species (ROS) and the conversion of dihydroartemisinic acid into artemisinin. Within 24 h from application, SA led to a gradual increase in the expression of the 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) gene and a temporary peak in the expression of the amorpha-4, 11-diene synthase (ADS) gene. However, the expression of the farnesyl diphosphate synthase (FDS) gene and that of the cytochrome P450 monooxygenase (CYP71AV1) gene changed little. At 96 h after SA (1.0 mM) treatment, the concentration of artemisinin, artemisinic acid and dihydroartemisinic acid were 54, 127 and 72% higher than that of the control, respectively. On the basis of their results, they suggested that SA induces artemisinin biosynthesis in at least two ways: by increasing the conversion of dihydroartemisinic acid into artemisinin caused by a burst in ROS and by upregulating the expression of genes involved in artemisinin biosynthesis.

Jing et al. (2009) evaluated the effect of different concentrations of abscisic acid (ABA) on artemisinin concentration in *A. annua*, under tissue culture conditions. Artemisinin content in plants treated with 10 lM ABA was 65% higher than that in control plants (1.1% on a dry weight basis) and ranged from 1.5 to 1.84%. They also studied gene expression analysis and showed that in both ABA-treated plants and cell suspension cultures, the important genes in the artemisinin biosynthetic pathway, such as HMGR, FPS, CYP71AV1 and CPR (cytochrome P450 reductase), were significantly induced. While only a slight increase in ADS expression was observed in ABA-treated plants, no expression of ADS was detected in cell suspension cultures. They suggested that there is probably a crosstalk between the ABA signalling pathway and artemisinin biosynthetic pathway and that CYP71AV1, which was induced most significantly, may play a key regulatory role in the artemisinin biosynthetic pathway.

Aftab et al. (2010a) investigated the effects of foliar sprays of triacontanol (TRIA) alone and in combination with gibberellic acid (GA_3) on growth attributes, photosynthesis, enzymatic activities, essential oil and artemisinin content and yield of *A. annua*. The results indicated that combination of TRIA and GA_3 significantly increased activities of nitrate reductase and carbonic anhydrase by 25.9 and 21.5%, and net photosynthetic rate, stomatal conductance and internal CO_2 by 25.4, 14.1 and 15.4%, respectively, when compared to unsprayed plants. Combination of Tria and GA_3 also significantly enhanced artemisinin content and yield.

Wang et al. (2010) reported the effects of exogenous foliar methyl jasmonate (MJ) on artemisinin biosynthesis and secondary metabolites in *A. annua* under greenhouse conditions. They found a 49% increase in artemisinin concentration on day 8 after treatment with MJ, associated with an 80% increase in artemisinic acid and 28% in dihydroartemisinic acid, the latter currently accepted as being the main precursor of artemisinin. In addition, they also worked out some other secondary metabolites using metabolite profiling after exogenous methyl jasmonate treatment. Their content also changed significantly after MJ treatment, including a 50% increase in methyl artemisinic acid, a 67% increase in squalene and a 60% increase in an unidentified sesquiterpenoid. They argued that these compounds might act as promising targets for further studies on artemisinin biosynthesis.

In a different study conducted by Banyai et al. (2011), cloned plants grown in mixed soil potting medium in a growth chamber, the production of artemisinin and leaf biomass in *A. annua* (varieties 007 and 253-2) was significantly increased by exogenous GA_3 applied to the soil. They also worked out the effects of GA_3 application on expression of key enzymes involved in artemisinin biosynthesis. They postulated that the increased artemisinin content (close to 1% DW) from exogenous GA_3 treatment was associated with increased expression of key enzymes in the artemisinin biosynthesis pathway. Interestingly, exogenous GA_3 continuously enhanced artemisinin content from the vegetative stage to flower initiation in both plant lines involved and gave significantly higher leaf biomass than in control plants. Consequently, the artemisinin yield in GA_3-treated plants was much higher than in control plants. In their work, although the maximum artemisinin content was found at the full blooming stage, the highest artemisinin yield in GA3-treated plants was obtained during the flower initiation stage. This was 26.3 and 27.8% higher, respectively, than in non-treated plants.

Aftab et al. (2010b) illustrated that salicylic acid (SA) acts as a potential plant growth promoter and plays an important role in regulating a number of plant physiological and biochemical processes under field conditions. Four levels of SA (0, 0.25, 0.50 and 1.00 mM SA) were applied in the form of diluted aqueous sprays on the aboveground plant parts in their study. Plant height, dry weight, chlorophyll and carotenoid contents were improved significantly as the level of SA increased. Furthermore, significant enhancement in net photosynthetic rate (31.7%), the activity of nitrate reductase (17.2%) and carbonic anhydrase (10.9%) was noticed as the level of SA application was increased from 0 to 1.00 mM SA. Most importantly, the content and yield of artemisinin were increased by 25.8 and 50.0%, respectively, after treatment with SA.

In another study, Aftab et al. (2013) concluded that sodium alginate, irradiated by Co-60 gamma rays together with various nitrogen doses, was used to study its effect on growth attributes, physiological and biochemical processes and production of artemisinin in *A. annua*. Of the various applied doses of nitrogen, N80 (80 kg N ha^{-1}) together with ISA80 (80 mg L^{-1} Irradiated Sodium Alginate) proved superior in positively regulating almost all the parameters studied. An increase of 38.1 and 80.5% in artemisinin content and yield respectively, was also noticed at this treatment.

Aftab et al. (2014), also conducted experiments on *A. annua* with an aim to evaluate artemisinin production and overall plant development though depolymerized sodium alginate application and nutrient supply. In their study, sodium alginate, irradiated by Co-60 gamma rays together with various phosphorus doses (10, 20, 30, 40 and 50 kg ha^{-1}), was used to study their effect on growth, physiological and biochemical processes and production of artemisinin in *A. annua*. Among various applied doses of phosphorus fertilizer, P40 (40 kg P ha^{-1}) together with ISA80 significantly improved all the parameters studied. Increase in plant height as well as weight was noted at this treatment. Dry leaf yield, artemisinin concentration in leaves and artemisinin yield was also significantly enhanced by the applied treatment.

In order to increase the plant growth and artemisinin content, a field experiment was conducted for two consecutive years, using foliar application of an aqueous solution of gamma-ray irradiated sodium alginate along with split application of nitrogen and phosphorus fertilizers (Aftab et al. 2016). Crop performance was assessed at pre-flowering and flowering stage in terms of growth, physiological and biochemical parameters and production of artemisinin in *A. annua*. Foliar application of 80 mg L^{-1} of irradiated sodium alginate along with split application of nitrogen (40 + 40 kg N ha^{-1}) and phosphorus (20 + 20 kg P ha^{-1}) (i.e. ISA80 + N40 + 40 + P20 + 20) proved the best integrated treatment that gave maximal values for the attributes studied at flowering stage. As compared with the control, effect of this treatment (ISA80 + N40 + 40 + P20 + 20) was positive on dry leaf yield, leaf artemisinin concentration and artemisinin yield. They concluded that the combined application enhanced the content and yield of artemisinin by 43.3 and 87.9%, respectively.

Patra et al. (2013) used different precursors and elicitors for enhancement of artemisinin. The maximum artemisinin content obtained was 3.45 mg/g on 15d by using the elicitor methyl jasmonate (40 μg/L) and the precursors casein acid hydrolysate (50 μg/L), sodium acetate (500 μg/L). Effect of addition time of elicitors and precursors on overall optimum biomass growth and artemisinin yield and productivity was also recorded.

Ahlawat et al. (2014) studied the effect of different concentrations of methyl jasmonate (MeJA), fungal elicitors (*Alternaria alternate*, *Curvularia limata*, *Fusarium solani*, and *Piriformospora indica*), farnesyl pyrophosphate, and miconazole on artemisinin production in hairy root cultures of *A. annua* were evaluated. Among all the factors used individually for their effect on artemisinin production in hairy root culture system, the maximum enhancement was achieved with *P. indica* (1.97 times). Increase of 2.44 times in artemisinin concentration by this system was, however, obtained by combined addition of MeJA and cell homogenate of *P. indica* in the culture medium. They suggested that the effects of these factors on artemisinin production were positively correlated with regulatory genes of MVA, MEP, and artemisinin biosynthetic pathways, viz. hmgr, ads, cyp71av1, aldh1, dxs, dxr, and dbr2 in hairy root cultures of *A. annua* L.

Tahir et al. (2016) studied the effect of some plant growth regulators on the in vitro initiation of callus using the leaves of Chiyong variety of *A. annua*. The explants were sterilized and incubated on Murashige and Skoog basal media augmented with varying concentrations ranging from 0.5 to 3.0 µm/L of GA_3, Benzyle amino purine (BAP), Napthaleneacetic acid (NAA), 2,4-Dichlrophynoxyacetic acid (2,4-D), Indole butyric acid (IBA) and Kinetin (Kin). Low concentration of BAP (0.5 µm/l) and a high concentration of NAA (3.0 µm/L) as well as equal concentration of BAP of 1.0 µm/l and IBA of 1.0 µm/L had the least days (7 days) to regeneration. For regeneration percent, equal low concentrations of BAP of 0.5 µm/L and NAA of 0.5 µm/L had the highest performance of 94%. However, no significant difference was observed between treatments with respect to vigor. Equal low concentrations of BAP (0.5 µm/L) and (0.5 µm/L) NAA and BAP (1.0 µm/L) combined with IBA (1.5 µm/L) had the highest height. They concluded that combined BAP and NAA treatment is an efficient protocol for callus initiation and proliferation. A combination of GA_3 and BAP is suitable for shoot initiation from callus using cultures of *A. annua*.

Effect of Mineral Nutrients

Singh (2000) conducted a field experiment to study the effect of levels of nitrogen (N), phosphorus (P) and potassium (K) on herb, oil and artemisinin yield of *A. annua*. Herbage, essential oil and artemisinin yields were increased significantly with application of 50 kg N ha^{-1} compared to control (0 kg N ha^{-1}), but were found statistically similar at 100 kg N ha^{-1}. Application of 50 and 100 kg N ha^{-1} increased herbage, yield by 26.2 and 40.1% respectively compared with control.

Ayanoglu et al. (2002) studied the effects of different N doses (0, 6, 12 and 18 kg ha^{-1}) on the growth parameters, yields and quality of *A. annua* ecotype from Adana, Turkey. A two-year experiment was conducted under Adana ecological conditions. During their study, plant height, number of branches per plant, fresh and dry herb yield, dry leaf yield, content and yield of essential oil and artemisinin were investigated. Their results indicated that application of different N doses significantly affected the essential oil content and oil yield only the first year of the study, however, content and yield of artemisinin were not significantly affected.

Khudsar et al. (2004) investigated the response of *A. annua* to different concentrations of zinc [50, 100, 200, 300 and 400 µg g^{-1}(soil dry mass)]. Total leaf area, dry mass of leaves, length and dry mass of shoots and roots were increased with the age of the plant but the magnitude of increase declined significantly under the influence of Zn treatment. Net photosynthetic rate, intercellular carbon dioxide concentration and stomatal conductance were highest at flowering stage in control and treated plants and decreased at post flowering stage. According to their study, contents of chlorophyll *a*, chlorophyll *b*, carotenoids, proteins and nitrate reductase activity in leaves increased from pre-flowering to maximum level at flowering stage and decreased thereafter in both control and treated plants. Presence of Zn in the soil drastically decreased/inhibited all the parameters, and the magnitude of decline increased with increasing Zn concentration.

Kapoor et al. (2007) studied the effects of two arbuscular mycorrhizal (AM) fungi, *Glomus macrocarpum* and *Glomus fasciculatum*, either alone or supplemented with P-fertilizer, on artemisinin concentration in *A. annua*. In their study, there was significant increase in concentration of artemisinin in non-mycorrhizal P-fertilized plants as compared to control, the increase was less compared to mycorrhizal plants grown with or without P-fertilization. They suggested that the increase in artemisinin concentration may not be entirely attributed to enhanced P-nutrition and improved growth. The plants supplied with AM fungi and P-fertilizer produced up to ten fold more shoot biomass than control plant. Also, AM fungi and P-fertilizer combined resulted in significant increases in concentrations of chlorophyll *a*, chlorophyll *b* and carotenoids. Maximum artemisinin content was found in the plants fertilized with P, and inoculated with AM fungi.

Özgüven et al. (2008) evaluated yield, yield components and artemisinin content of *A. annua* grown under N application (0, 40, 80 and 120 kg ha^{-1}) for two successive years. In their study, N doses had no significant effect on plant height, number of branches, fresh herbage yield, dry herbage yield, fresh leaf yield, dry leaf yield or essential oil content. However, artemisinin concentration were significantly increased by N application and ranged from 6.3 to 27.5 mg 100 g^{-1} among the treatments.

Peyvandi et al. (2009) investigated the effects of different N and P application on the plant growth parameters, yield and essential oil composition of *A. annua*. They observed that differences between the average height, number of branches and dry weight were significantly increased by the treatments. The maximum number of branches and plant height increased in $N_{80}P_{40}$ treatment. Increasing P-fertilizer more than 40 kg ha^{-1} decreased the growth parameters significantly. However, they did not observe the changes in artemisinin content due to the applied treatments.

Davies et al. (2009) examined the effect of various concentrations of N and K on artemisinin concentration and yield of *A. annua*. The nutrients were supplied in irrigation water to plants in pots. Nitrogen enhanced plant N concentration and biomass production successively up to 106 mg N L^{-1} for biomass and 206 mg N L^{-1} for leaf nitrogen; further increases in nitrogen had no influence on biomass. Artemisinin concentration in dried leaf material was maximum at a N application of 106 mg L^{-1}, but declined at higher concentrations. Increasing K application from 51 to 153 mg L^{-1} increased total plant biomass. Potassium application enhanced leaf K concentration, but there was no effect on leaf artemisinin concentration or leaf artemisinin yield.

Malik et al. (2009) studied the effects of nutrients on the essential oil content of *A. annua*. Plants were treated with basal N, P, K and S application (in the form of urea P_2O_5, K_2O and gypsum at a rate of 120, 50, 100 and 50 kg ha^{-1}, respectively) amounted to 0.32 ± 0.03% of fresh weight and consisted mainly of thujyl alcohol (33.3%), -caryophyllene (15.5%), cis-undec-5-ene (14.4%), artemisia ketone (6.0%), trans-nerolidol (5.8%) and undec-4-ene (4.6%). The hydrodistillation of *A. annua* treated with *Glomus* (Mycorrhizal soil 50 g/plant) produced 0.50 ± 0.02% essential oil on fresh weight basis.

Aftab et al. (2011a) evaluated varying levels of soil-applied N with foliar GA_3. Application of GA_3 proved effective in increasing growth, photosynthesis and enzyme activities of *A. annua*. However, N levels combined with GA_3 led to further improvement in shoot lengths and dry weights, as well as photosynthetic rate. Furthermore, N combined (80 mg kg^{-1} soil) with GA_3 augmented the content and yield of artemisinin in the treated plants over the control.

Jha et al. (2011) assessed the effect of organic manure and chemical fertilizers on the accumulation of artemisinin and biomass in various plant parts during the developmental stages of *A. annua*. They found that content and yield of artemisinin in dried leaves increased significantly (27.3 and 53.6%, respectively) at pre-flowering stage in the plants treated with NPKS and NPK (18.2 and 33.5%, respectively) when compared with control. Maximum dry leaf yield ranged from 2596 to 3141 kg ha^{-1} at pre-flowering stage with various treatments.

Davies et al. (2011) examined the response of *A. annua* to different levels of phosphorus (P) and boron (B) on the basis of dry matter and artemisinin concentration. Increases in P application enhanced plant growth and total dry matter production up to 30 mg P L^{-1}. Although application of P had no influence on leaf artemisinin concentration, optimal yields of artemisinin per plant were achieved at P from 30 to 60 mg L^{-1}, reflecting the increase in biomass caused by P application. Increasing B application rate had no significant effect on dry matter production. Leaf artemisinin concentration was significantly increased by 20% (0.65–0.78%) as B concentration was increased from 0.1 to 0.6 mg L^{-1}. Further, increasing B concentration to 0.9 mg L^{-1} had no further effect on artemisinin concentration or yield.

Rostkowska et al. (2016) reported that accumulation of silicon (Si) and artemisinin stored in glandular trichomes of *A. annua*. Their study aimed to look into Si effects on *A. annua* trichome, artemisinin concentration, and whether leaf infusion from Si-treated *A. annua* plants is able to control *Toxoplasma gondii* growth. The experimental design consisted of *A. annua* seedlings randomly planted in soil treated with different doses of calcium/magnesium silicate (0, 200, 400, 800, and 1600 kg ha^{-1}). Analysis of foliar macronutrients showed significant increases of N content only at the highest dose of silicate. Foliar micronutrients, Si concentrations, and plant height were not affected by any of the silicate doses. However, the dose of 400 kg ha^{-1} of silicate increased the trichome size, which in turn raised artemisinin concentration in leaves and the infusion. In contrast, the 800 and 1600 kg ha^{-1} doses dramatically decreased artemisinin concentration. Further, HeLa cell treatment with the infusion of *A. annua* grown in soil treated with 400 kg ha^{-1} of silicate decreased parasite proliferation in a dose-dependent manner when the treatment was carried out after or along with *T. gondii* infection. However, this effect was similar to *A. annua* grown in soil without silicate treatment. Thus, it was concluded that, Si applied to the soil at 400 kg ha^{-1} had a positive effect on the size of glandular trichome and the artemisinin concentration of *A. annua*.

Effect of Environmental Stresses

Prasad et al. (1998) investigated the effect of soil salinity (mixture of cations and anions) on the growth, yield, mineral composition and artemisinin concentration of *A. annua* cultivar. They noticed that plant height decreased as salinity stress increased, and the leaf-to-stem ratio was generally increased in salinized plants as compared with control plants not subjected to salinity stress. Artemisinin content in vegetative tissue was present 0.01% (g/100 g dry weight) and was not influenced by salinity levels from 0.9 to 10.4 dS/m, but decreased to 0.006% at 14 dS/m. The concentration of nitrogen was significantly higher, and the concentrations of phosphorus and calcium were lower in plants subjected to salinity stress. Potassium concentration and the potassium-to-sodium ratio in shoots decreased, while the sodium and magnesium concentrations increased with salinity stress to 6.0 dS/m. The potassium-to-sodium ratio was significantly and negatively correlated with the dry weight of shoot.

Qureshi et al. (2005) reported the effects of NaCl (0–160 mM) and lead acetate (0–500 lM) on 90 (S1 treatment)- and 120-day-old (S2 treatment) *A. annua* plants. Plants were assessed for lipid peroxidation rate, photosynthetic rate, chlorophyll content, content and yield of artemisinin, and for total biomass accumulation, at 100, 130 and 160 DAS in S1, and at 130 and 160 DAS in S2 treatments. Treatments enhanced lipid peroxidation at all stages of plant growth and increased the concentration and yield of artemisinin at 100 and 130 DAS in S1 and S2, respectively, while other parameters declined at all growth stages. The magnitude of changes was greater in lead-treated than in salt-treated plants. Both treatments induced oxidative stress, which might have damaged the photosynthetic apparatus resulting in a loss of chlorophyll content and a reduction in photosynthetic rate, biomass accumulation and artemisinin production.

Liu et al. (2002) studied the effects of light irradiation on growth and production of artermisinin in hairy root cultures of *A. annua* L. When the hairy roots were cultured under illumination at 3000 Lux for 16 h using several cool-white fluorescent lamps, and then darkness for 8 h, the dry weight and artemisinin concentration reached 13.8 g/L and 244.5 mg/L, respectively. The results obtained showed that the growth and artemisinin accumulation increased on improving the conditions of light irradiation, which affected metabolite formation conspicuously. Under optimal conditions, kinetics of growth, artemisinin accumulation and nutrient uptake were studied.

Ferreira (2007) first time reported that a cloned, greenhouse-grown *A. annua* subjected to an acidic soil and macronutrient deficit was evaluated for artemisinin production. Lack of lime (L) and macronutrients (N, P and K) reduced leaf biomass accumulation. When L was provided, the highest average leaf biomass was achieved with the "complete" (+N, +P, +K and +L) treatment, and the least biomass was achieved with the untreated (−N, −P, −K and −L) treatment. The macronutrient least required for biomass accumulation per plant was K (49.0 g), followed by P (36.5 g) and N (14.3 g). The artemisinin concentration (g/100 g) was significantly higher (75.5%) in −K plants when compared to plants under the complete treatment. Although the artemisinin total yield was 21% higher in −K plants, it was not significantly different from plants under the complete treatment, due to the lower biomass accumulation caused by deficiency of K. There was no marked treatment effects for concentration or yield of both dihydroartemisinic acid and artemisinic acid, although higher levels were achieved in plants under the complete or −K treatments. There was a positive and significant correlation between artemisinin and both artemisinic acid and dihydroartemisinic acid, in g/100 g and g/plant.

Marchese et al. (2010) grown *A. annua* plants in growth chambers and exposed to five water deficit treatments. Water deficits of 38 and 62 h increased leaf artemisinin content, but only 38 h led to a significant increase in both leaf and plant artemisinin, with no damage to plant biomass production. The other treatments had no effect on, or decreased artemisinin accumulation. They concluded that moderate water deficit prior to harvesting the crop may not only reduce time and costs in drying the crop, but can also induce artemisinin accumulation, both of which increase crop profit margins.

Aftab et al. (2010c) studied the effect of increasing levels of boron (B) on oxidative stress, antioxidant defence response and changes in artemisinin content in *A. annua*. Toxicity caused by B reduced growth parameters such as stem height, fresh weight and dry weight. Treatments induced oxidative stress resulting in lower net photosynthetic rate, stomatal conductance, internal CO_2 and total chlorophyll content. The increased activities of antioxidant enzymes like CAT (catalase), POX (peroxidase) and SOD (superoxide dismutase) were also noted in response to increasing levels of B stress. However, H_2O_2 and artemisinin content were found to be high up to 1.00 mM concentration of boron compared to control, and on applying higher doses, further reduced contents were obtained. Their results suggest that a mild stress of B can be utilized to enhance artemisinin production.

The role of salicylic acid (SA) in inducing salinity tolerance was studied in *A. annua* L., by Aftab et al. (2011b). When applied to leaves at 1.0 mM, SA provided considerable protection against salt stress imposed by 50, 100 or 200 mM NaCl to soil. Salt stress negatively affected plant growth as assessed by length and dry weight of shoots and roots. Salinity also reduced the values of photosynthetic attributes and total chlorophyll content and inhibited the activities of nitrate reductase and carbonic anhydrase. Furthermore, salt stress significantly increased electrolyte leakage and proline content. Salt stress also induced oxidative stress as indicated by the elevated levels of lipid peroxidation compared to the control.

A foliar spray of SA at 1.0 mM promoted the growth of plants, independent of salinity level. The activity of antioxidant enzymes, namely CAT, POX and SOD, was upregulated by salt stress and was further enhanced by SA treatment. Artemisinin concentration increased at 50 and 100 mM NaCl but decreased at 200 mM NaCl. The application of SA further enhanced artemisinin concentration when applied with 50 and 100 mM NaCl by 18.3 and 52.4%, respectively. Their results indicated that moderate saline conditions can be exploited to obtain higher artemisinin content in *A. annua* plants, whereas the application of SA can be used to maintain plant growth and induce its antioxidant defence system under salt stress.

Aftab et al. (2011b) conducted a study to determine whether the exogenous application of methyl jasmonate (MJ) on *A. annua* could counteract the ill effects of excessive B present in the soil. According to the results obtained, B toxicity induced oxidative stress and reduced stem height, as well as fresh and dry masses of the plant significantly. The excessive amounts of soil B also lowered the net photosynthetic rate, stomatal conductance, internal CO_2 concentration and total chlorophyll content in the leaves. In contrast, the foliar application of MJ enhanced the growth and photosynthetic efficiency in both the stressed and non-stressed plants. The excessive B levels also increased the activities of antioxidant enzymes, such as CAT, POX and SOD. Endogenous H_2O_2 and O_2^- levels were also high in stressed plants. However, MJ application on stressed plants reduced the amount of lipid peroxidation and stimulated the synthesis of antioxidant enzymes, enhancing the concentration and yield of artemisinin as well. They concluded that MJ might be utilized in mitigating B toxicity and improving both concentration and yield of artemisinin in *A. annua*.

Further, Aftab et al. (2012) reported the effects of boron (B) and aluminium (Al) contamination in soil, carried out with or without application of exogenous sodium nitroprusside or SNP (a nitric oxide donor), on various plant processes in *A. annua*, including changes in artemisinin content. The addition of B or Al to soil significantly reduced the yield and growth of plants and lowered the values of net photosynthetic rate, stomatal conductance, internal CO_2 concentration and total chlorophyll content. The follow-up treatment of NO donor favoured growth and improved the photosynthetic efficiency in stressed and non-stressed plants. Artemisinin content was enhanced by 24.6 and 43.8% at 1.0 mol of soil-applied B or Al. When SNP was applied at 2.0 mol concentration with either 1.0 mol of B and/or Al, it further stimulated artemisinin biosynthesis compared to the control. Application of B, Al and SNP proved to be the best treatment combination to increase artemisinin concentration in *A. annua* leaves.

Zanjani et al. (2012) assessed the effect of salt stress on physiological traits of *A. annua* L. under salicylic acid (SA) application and non application conditions. They applied four salinity levels (0 (control), 4, 8, and 12 ds. m^{-1} NaCl) and two SA levels (SA non application (control) and SA application (0.5 mM). Chlorophyll *a* and *b* content, leaf electrical conductivity, stomatal resistance, leaf relative water content, and canopy temperature difference were determined. Their results revealed that application of SA in both stress and non stress conditions increased the chlorophyll *a* and *b* content and also leaf relative water content, although leaf electrical conductivity, stomatal resistance and canopy temperature difference decreased by SA application in both stress and non-stress conditions.

Rai et al. (2011) studied physiological, biochemical and molecular changes in *Artemisia annua* under arsenic (As) stress. Plants accumulated As in a dose dependent manner with bioconcentration factor 13.4 and translocation factor 0.97. While a similar trend of As accumulation was observed under soil culture experiments, the transfer factor went up to 2.1, depicting high efficiency of As translocation from roots to shoots by *A. annua*. Plants raised in 0–3000 µg L^{-1} As containing nutrient solution registered increase in root length, biomass, and carotenoid contents without any visual toxicity symptoms. A dose dependent increase in the activities of enzymes such as superoxide dismutase, ascorbate peroxidase, glutathione reductase and guaiacol peroxidase followed by a gradual decline at higher concentrations suggested their role in alleviating oxidative stress. Significant increase in the levels of thiols, GSH, and pcs gene transcript up to 3000 µg L^{-1} As attested their roles in As detoxification. Enhanced artemisinin production under As stress and upregulation of the transcripts of the genes HMGR, FDS, ADS, and CYP71AV1 involved in artemisinin biosynthesis.

Further, study of Rai et al. (2014) provided the first proteomic evidence of As tolerance and interactive regulatory network between primary and secondary metabolism in *A. annua*. While chlorophyll fluorescence and photosynthetic rate depicted mild inhibition, there was a significant enhancement in PSI activity, whole chain, ATP, and NADPH contents in 100 µM As treatments compared to the control plants. The most interesting observation was an increased accumulation of LEAFY like novel protein conceivably responsible for an early onset of flowering in *A. annua* under As stress. Their study not only affirmed the role of energy metabolism proteins but also identified potential candidates responsible for As tolerance in plants.

Paul and Shakya (2013) investigated the effect of As(III), Cr(VI) and NaCl on plant growth, antioxidant enzymes, SOD, TBRAS, protein, cDNA amplification of key genes of artemisinin pathway and artemisinin biosynthesis to explore the actual changes in total herb and artemisinin yield in a crop cycle of *A. annua*. Enhanced TBARS and SOD activity (4 U mg^{-1}), decreased catalase activity and total cholorophyll content were observed under metal(loid) and NaCl stress. Accumulation of As(III; µg mg^{-1} DW) was higher in roots (10.75 ± 0.00) than shoot (0.43 ± 0.00) at 10 µg ml^{-1}. While Cr(VI; µg ml^{-1} DW) accumulated more in shoots (37 ± 9.6, 41.1 ± 7.2 and 52.71 ± 19.6). cDNA template of these treated plants along with control were amplified with HMGR, ADS and CYP71AV1 genes (artemisinin biosynthetic pathway genes); showed very low expression with Cr(VI) while As(III) (5 and 7.5 µg ml^{-1}) showed higher expression than control. Their study suggested that *A. annua* can grow well with favoring artemisinin biosynthesis with treatment of As(III) 5, 7.5 µg ml^{-1} and NaCl, while 10 µg ml^{-1} As(III) and all doses of Cr(VI) affect artemisinin synthesis and total herb yield of plant.

Li et al. (2014) investigated the physiological response of varying level of salt stresses (50, 100, 200, 300, or 400 mM NaCl) on plant growth and artemisinin content of *A. annua* L. and salt damage mitigation by exogenous salicylic acid (0.05 or 0.1 mM). Plant growth, antioxidant enzyme activity, proline, and mineral element level were determined. In general, increasing salt concentration significantly reduced plant growth. Superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were stimulated by salt treatment to a higher enzyme activity in treated plants than those in untreated plants. Content of proline had a visible range of increment in the salt-treated plants. Distribution of mineral elements was in inconformity: Na^+ and Ca^{2+} were mainly accumulated in the roots; K^+ and Mg^{2+} were concentrated in leaves and stems, respectively. Alleviation of growth arrest was observed with exogenous applications of salicylic acid (SA) under salt stress conditions. The activity of SOD and POD was notably enhanced by SA, but the CAT action was suppressed. While SA had no discernible effect on proline content, it effectively inhibited excessive Na^+ absorption and promoted Mg^{2+} absorption. Ca^{2+} and K^+ contents showed a slight reduction when supplemented with SA.

Chaturvedi et al. (2014) conducted experiment to study the effect of various extrinsic factors viz., temperature, moisture and salt concentrations affecting ex situ seed germination of different populations of *A. annua*. All the populations were susceptible to changes in abiotic conditions viz., moisture and salinity levels in dose dependent manner. According to Agrawal (2015), In vitro enhancement in artemisinin content has been achieved employing biotic (*Piriformospora indica* and *Agrobacterium rhizogenes* strain A4) and abiotic (such as heavy metals and salicylic acid) elicitors with different explants in various MS media compositions. A significant enhancement in artemisinin content of 60% was achieved in shoot cultures co-cultivated with *P. indica*. Hairy root cultures raised through leaf explants of *A. annua* when exposed to different concentration of Pb, Hg, Co and SA have shown a tremendous enhancement in artemisinin, the maximum elevation reaching to 1450% in lead nitrate (100 mg/L) supplemented medium over control. For abiotic stress, nodal explants if exposed to various heavy metal (Ag, Cu, Hg, Co and Zn) salts too revealed significant increase in artemisinin production, the optimum being 50% at 100 mg/L of Cu and Zn. The crude extract of *A. annua* has been fractionated, isolated and characterized through CC, TLC, FT-IR and NMR. Bioassays conducted with crude extract of leaves against larvae of malaria (*Anopheles stephensi*) and dengue (*Aedes aegypti*) vectors have shown a strong larvicidal activity and on human oral cancer cell line causing 98.5% mortality. This is our first report of elicitation of artemisinin in *A. annua* employing *P. indica* and hairy roots coupled with abiotic stresses.

Yadav et al. (2014) find out the modulation of artemisinin and essential oil constituents in plants grown under prolonged water stress conditions. *A. annua* CIM-Arogya plants grown in pots were subjected to mild (60% ± 5) and moderate (40% ± 5) water stress treatment and continued during entire developmental period. Their study revealed that artemisinin, arteannuin-B, artemisinic acid, dihydroartemisinic acid and essential oil content were positively controlled by the growth and development however negatively modulated by water deficit stress. Interestingly, some of minor monoterpenes, all sesquiterpenes and other low molecular weight volatiles of essential oil components were induced by water deficit treatment. Camphor which is the major essential oil constituents did not alter much while 1, 8 cineole was modulated during development of plant as well as under water stress conditions. Water deficit stress induces a decrease in glandular trichome density and size as well.

Malik et al. (2013) conducted an experiment to assess the impact of organic residues from urban areas and industries on *A. annua* L. Application of industrial wastes enhanced content and yield of artemisinin, herb dry yield and plant characteristics. Highest artemisinin content in leaf (0.53%) was found at flower initiation stage and highest artemisinin yield (29.97 kg ha^{-1}) was obtained from sewage sludge biosolid treatment. Their study revealed that organic waste amendments enhance herbage yield and artemisinin content so these, can be utilized for the commercial production of *A. annua* with additional benefits of mitigation of environmental pollution caused by the accretion of these large quantities.

A pot experiment was conducted by Naeem (2015) according to randomized block design using five replicates to screen the *A. annua* L. varieties, namely ‘CIM-Arogya’ and ‘Jeevan Raksha’ under arsenic stress. Four concentrations of arsenic [0 (control), 15, 30 and 45 mg kg^{-1} soil] were applied to the soil. Measurements for growth characteristics, physiological attributes, biochemical parameters, yield and quality attributes were carried out at pre-flowering [90 days after planting (DAP)] and flowering (120 DAP) stages. The variety ‘Jeevan Raksha’ was more adversely affected by arsenic stress than the ‘CIM-Arogya’, suggesting that the ‘CIM-Arogya’ was more arsenic tolerant than the ‘Jeevan Raksha’. The highest arsenic concentration (45 mg kg^{-1} soil) proved most toxic dose as compared to other arsenic doses applied. It reduced the rate of photosynthesis and leaf-chlorophyll content significantly both at 90 and 120 DAP. The activities of CAT, POX and APX were also rapidly stimulated due to the highest concentration of arsenic at both the growth stages. Noticeably, all applied doses of arsenic significantly increased the production of artemisinin in the leaves. As compared to control, ‘CIM-Arogya’ contained the maximum artemisinin content under the highest arsenic stress, increasing the artemisinin content by 38.0 and 42.6% at 90 and 120 DAP, respectively; while, ‘Jeevan Raksha’ showed an increase in artemisinin content by 32.6 and 35.7% at the respective dates of sampling. At the highest arsenic stress, the yield of artemisinin was increased by 42.9 and 45.7% in ‘CIM-Arogya’ and by 37.5 and 40.5% in ‘Jeevan Raksha’. Generation rate of H_2O_2 increased consistently when arsenic was applied irrespective of the varieties used, indicating the role of H_2O_2 in artemisinin biosynthesis in *A. annua* L.

Kumari et al. (2016) suggested that *A. annua* is a good As-accumulator. They examined differential responses of roots and leaves of *A. annua* to different arsenic concentrations (50, 100, and 150 μM) and treatment durations (1, 3, 5, or 7 days). Above and below ground plant biomass was enhanced at 100 μM As but 150 μM As significantly reduced them. As-treatment caused membrane damage more in the roots than in the leaves as reflected by higher degree of lipid peroxidation in the roots than in the leaves. In response to As stress, plant activated antioxidative defense for detoxification of induced reactive oxygen species, As sequestration via phytochelatins (PCS) as well as wide range of secondary metabolites. All of them were activated differently in roots and leaves. Among enzymatic antioxidants, leaves significantly elevated superoxide dismutase (SOD), ascorbate peroxidase, and glutathione reductase, whereas in roots SOD, catalase, and peroxidase played significant role in ROS detoxification.

Soni and Abdin (2016) examined the effects of long-term water shortage-induced oxidative stress on morphophysiological parameters, proline metabolic genes, and artemisinin content in *A. annua* L. under greenhouse conditions. Plant growth, biomass accumulation, relative water content, and chlorophyll content were reduced under drought. Leaf water potential ranged from −0.3248 MPa to −1.22 MPa in stress conditions. Increased levels of proline accumulation, protein concentration, and lipid peroxidation were detected in water-stressed plants. They postulated that higher expression of biosynthetic genes and lower expression of catabolic genes at the preflowering stage confirmed the important role of proline in flower development. Artemisinin content decreased owing to water stress, but the slightly higher amounts were detected in leaves of severely stressed plants compared with moderately stressed plants.

Conclusion

Various scientific strategies have been applied to enhance the production of artemisinin, which include agronomical practices, genetic engineering, selective breeding and bio-transformation approach. Agronomical practices have always been a successful tool for the improved production of commercial crops with prominent effects on the secondary metabolite production. However, it is the need of the hour to explore newer strategies to enhance productivity of this antimalarial plant and also to minimize the expenses of this drug. Therefore, in order to increase the desired production of artemisinin, exploitation of scientific approaches with cheaper and more convenient strategies should be considered.

Also, the agricultural aspects of artemisinin production such as soil fertility and pH, plant density, water availability, latitude and altitude, mineral nutrients, elicitors, hormones, harvesting and drying strategies must be fine-tuned for each geographic area where *Artemisia* is to be cultivated for artemisinin production. Furthermore, efforts should be made to enhance the production of artemisinin both in vivo and in vitro with good quality and authentic seeds generated from high-artemisinin yielding parents. The study also concluded that abiotic stresses play an important role in amplifying the production of artemisinin by scavenging ROS through dihydroartemisinic acid. It is, therefore, fundamental to look for non-conventional, alternate strategies, which are economically viable for commercial production of artemisinin.

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Plant Nanobionics a Novel Approach to Overcome the Environmental Challenges

Mansour Ghorbanpour and Shohreh Fahimirad

Abstract Plant nanobionics is a new field of bioengineering that inserts nanoparticles into the cells and chloroplasts of living plants, which then alter or amplify the functioning of the plant tissue or organelle. The broader vision is to create a wide array of wild-type plants capable of imaging objects in their environment, self-powering themselves as light sources, infrared communication devices, and also function as self-powered ground water sensors. Plants are uniquely suited to perform such roles due to their ability to generate energy from sunlight and photosynthesis. In the field of nanobiotechnology, researchers want to develop bionic plants that could have better photosynthesis efficiency and biochemical sensing.

Keywords Plant nanobionics · Nanoparticles · Nanobiotechnology · Self-powering plants

Introduction

Nanoscale science and nanotechnology is the study and application of small sized objects range from 1 to 100 nanometers (nm), where novel characteristics make new and wide uses possible. Nanomaterials are therefore characterized as natural or engineered substances with at least one dimension in the size less than 100 nm. With quite diverse appearance, engineered nanomaterials can be spherical or near-spherical, tubular, or irregularly (non-spherical) shaped, which have been found in single, fused, and agglomerated forms with compositionally homogenous

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or heterogeneous (Hatami et al. 2016; Service 2003). There are many specific reasons that show why nanoscale has become so prominent; some of which are as follows (Mansoori 2017):

(1) Quantum mechanical (wavelike) characteristics of electrons inside matter are influenced by variations on the nanoscale. By nanoscale design of materials it is possible to vary their micro and macroscopic attributes including charge capacity, magnetization and melting temperature, without changing their chemical composition.

(2) A key attribute of biological entities is the systematic organization of matter on the nanoscale. Development in nanotechnology and nanoscience has allowed us to place man-made nanoscale things inside living cells (Ebrahimi and Mansoori 2014). It has also made it possible to study micro and macro structure of materials using molecular self-assembly (Xue and Mansoori 2010). This certainly is a powerful tool in materials science.

(3) Nanoscale components have unique properties such as very high surface to volume ratio, making them ideal for use in composite materials, reacting systems, drug delivery, and energy storage, etc.

(4) Macroscopic systems made up of nanostructures can have much higher density than those made up of microstructures. They can also be better conductors of electricity, resulting in new electronic device concepts, smaller and faster circuits, more sophisticated functions, and greatly reduced power consumption simultaneously by controlling nanostructure interactions and complexity.

Results of research and developments in the field of nanotechnology and nanoscience are entering into all aspects of our lives including, but not limited to, aerospace, defense, energy, environment, materials, manufacturing, medicine, agriculture and plant sciences (Ghorbanpour and Hadian 2015; Hatami et al. 2017; Ghorbanpour 2015; Baiazidi-Aghdam et al. 2016; Ghorbanpour and Hatami 2014; Hatami et al. 2014), etc. It is truly an atomic and molecular approach for building biologically, chemically and physically stable structures one atom or one molecule at a time. Presently some of the active nanoscience and nanotechnology research areas include nanolithography, nanodevices, nanorobotics, nanocomputers, nanopowders, nanostructured catalysts and nanoporous materials, molecular manufacturing, nanolayers, molecular nanotechnology, medicine such as Alzheimer's disease (Nazem and Mansoori 2008, 2014) and cancer (Ebrahimi and Mansoori 2014; Mansoori et al. 2007, 2010) prediction, prevention and treatment through nanotechnology), nanobiology, organic nanostructures to name a few.

Also, nanotechnology has the potential to enable new and enhanced functional properties in photosynthetic organelles and organisms for the enhancement of solar energy harnessing and biochemical sensing. Nanobionics engineering of plant function may contribute to the development of biomimetic materials for light-harvesting and biochemical detection with regenerative properties and enhanced efficiency (Giraldo et al. 2014). Thus, nanobionics aims to give plants superpowers.

Plant Nanobionics with Improved Photosynthesis Efficiency

Plant Nanobionic with Broaden Solar Light Absorption

In most kinds of plants, thylakoid membranes within chloroplasts are main location of the photosynthetic machinery. Chloroplasts are able to absorb visible range of the light spectrum which comprise of 50% of the incident solar energy radiation. Furthermore, Plants typically make use of only about 10% of the sunlight available to them (Zhu et al. 2010).

Thus, researchers have tried to improve photosynthetic efficiency by extending the range of solar light absorption (Blankenship et al. 2011).

Nanomaterials with perfect chemical and physical traits in chloroplast-based photocatalytic complexes form cause enhanced and new functional properties (Giraldo et al. 2014).

SWNTs are able to capture visible and near-infrared spectra of light wavelengths while chloroplast antenna pigments absorption rates are limited in this case.

Giraldo et al. (2014) successfully designed highly charged single-walled carbon nanotubes (SWNTs) coated with DNA and chitosan (a biomolecule derived from shrimp and other crustacean shells) which were able to spontaneously penetrate into chloroplasts. This new lipid exchange envelope penetration (LEEP) process for incorporating the nanostructures involves wrapping CNTs or nanoparticles with highly charged DNA or polymer molecules, enabling them to penetrate into the fatty, hydrophobic membranes that surround chloroplasts (Fig. 1).

Single walled carbon nanotubes (SWNTs) embedded within chloroplasts has the potential to enhance the light reactions of photosynthesis with their distinctive optical properties. SWNTs are able to capture visible and near-infrared spectra of light wavelengths while chloroplast antenna pigments absorption rates are limited in this case (Fig. 2). SWNTs convert this absorbed solar energy into excitons which transfer electrons to the photosynthetic machinery (Han et al. 2010).

Incorporation of CNTs into chloroplasts extracted from plants enhanced choloroplast's photosynthetic activity by 49% compared to the control. When these nanocomposites were incorporated into leaf chloroplasts of living plants, the electron flow associated with photosynthesis was enhanced by 30%. SWNT real-time sensing of NO in extracted chloroplasts and leaves could also be extended to detect a wide range of plant signalling molecules and exogenous compounds such as pesticides, herbicides and environmental pollutants.

Plant Nanobionic with Higher ROS Savaging Ability

Interestingly, SWNT-based nanosensors were able to monitor single-molecule dynamics of free radicals within chloroplasts for optimizing photosynthetic environmental conditions (light and CO_2) (Zhang et al. 2010).

Fig. 1 SWNT transport through chloroplast double membrane envelope via kinetic trapping by lipid exchange (Giraldo et al. 2014)

The major limitation in the use of extracted chloroplasts for solar energy applications is that they easily break down due to light- and oxygen-induced damage to the photosynthetic proteins. Giraldo et al. (2014) illustrated that cerium oxide nanoparticles (nanoceria) were combined with a highly charged polymer

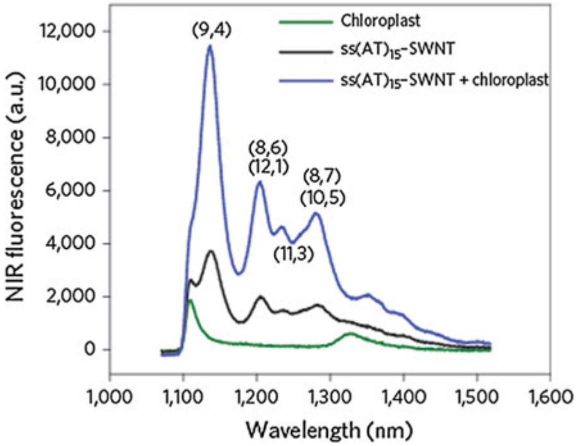

Fig. 2 Chloroplast autofluorescence was masked from near-infrared images by along-pass 1100 nm filter

(polyacrylic acid) pass through the outer membranes of the chloroplast and locate in the stroma, and remarkably prevent damage to the photosystems by quenching reactive oxygen species that are widely dispersed throughout the chloroplast and enable real-time monitoring of free-radical species and environmental pollutants using in vivo and ex vivo embedded nanosensors (Siddiqui et al. 2015) (Fig. 3).

In addition, solar energy is captured by chlorophylls in the two types of pigment-protein complexes (photosystems I and II, designated PSI and PSII,

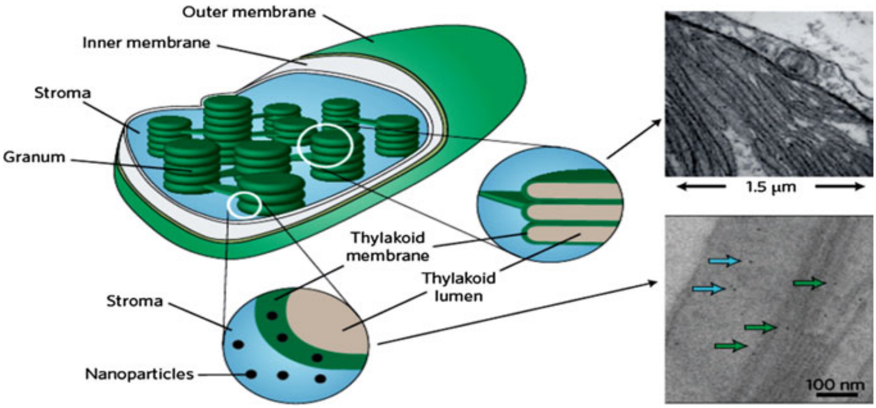

Fig. 3 Natural and nanobionic chloroplasts. The photosynthetic apparatus is mostly embedded in the thylakoid membranes of chloroplasts. Flattened thylakoids are stacked into grana, as shown by a micrograph of the cryptophyte alga *Proteomona ssulcata* (top right). The nanoparticles localize in both the thylakoid membrane and the stroma (bottom left schematic; green and blue arrows, respectively, in the bottom right micrograph) (Scholes and Sargent 2014)

respectively) and are converted into electrochemical energy to produce ATP and NADPH that are used for CO_2 fixation. PSII performs the light-induced oxygen evolution reaction and transfers electrons from water to plastoquinone in the membrane and PSI produces strong reducing power using electrons supplied by PSII and reduces ferredoxin and NADPH. Noji et al. (2011) illustrated that nanomesoporous silica compound (SBA) conjugated with photosystem II (PSII) maintained the high and stable oxygen-evolving ability of *T. vulcanus* PSII even inside silica nanopores. The activity lasted more than 3 h under the moderate illumination/dark cycles. Combination of PSII-SBA conjugates with the mediator recycling systems can remove the harmful effects of electron acceptors and light-induced radicals and have properties to develop for photosensors and artificial photosynthetic system.

Plant Nanobionic Designed as Detector for Various Chemicals Presented in Environment

Because the water evaporates, chemicals drawn up along with the fluid that don't easily vaporize get concentrated in the leaves. This means plants can detect very low concentrations of chemicals. Plant nanobionics has also enabled us to use plants as detectors for the presence of different chemicals in the soil and water, and even in the air. When one of these chemical compounds are present in the groundwater being absorbed and sampled naturally by the plant, the embedded carbon nanotubes will emit a fluorescent signal that can be read with an infrared camera that can be attached to a small computer similar to smart phone. The computer will then send an e-mail to the user.

Nanobionic Plant as Nitroaromatics Detector

It may seem exquisite but imitate from simple plant transpiration process. Plants draw up water and other analytes from the ground, and can accumulate even trace levels of analytes within tissues. Knowing this rule, Wong et al. (2016) made a nanobionic plant that can both detect explosives in groundwater and alert a user to their presence in the area. IR-fluorescent carbon nanotubes (CNTs) based sensors that selectively respond to nitro aromatics were injected into a spinach plant's leaves. These nanotubes quench in fluorescence intensity in the presence of nitroaromatics. Then a reference sensor that is invariant in signal intensity was designed. The plant draws the nitroaromatics or the common explosives component picric acid (2,4,6-trinitrophenol) up through the roots into its leaves, where the suppressed IR signal is imaged with a night-vision camera and sent to a smart phone via a WiFi signal. With the reference sensor embedded in the leaf as well, the technique produces high contrast images (Fig. 4).

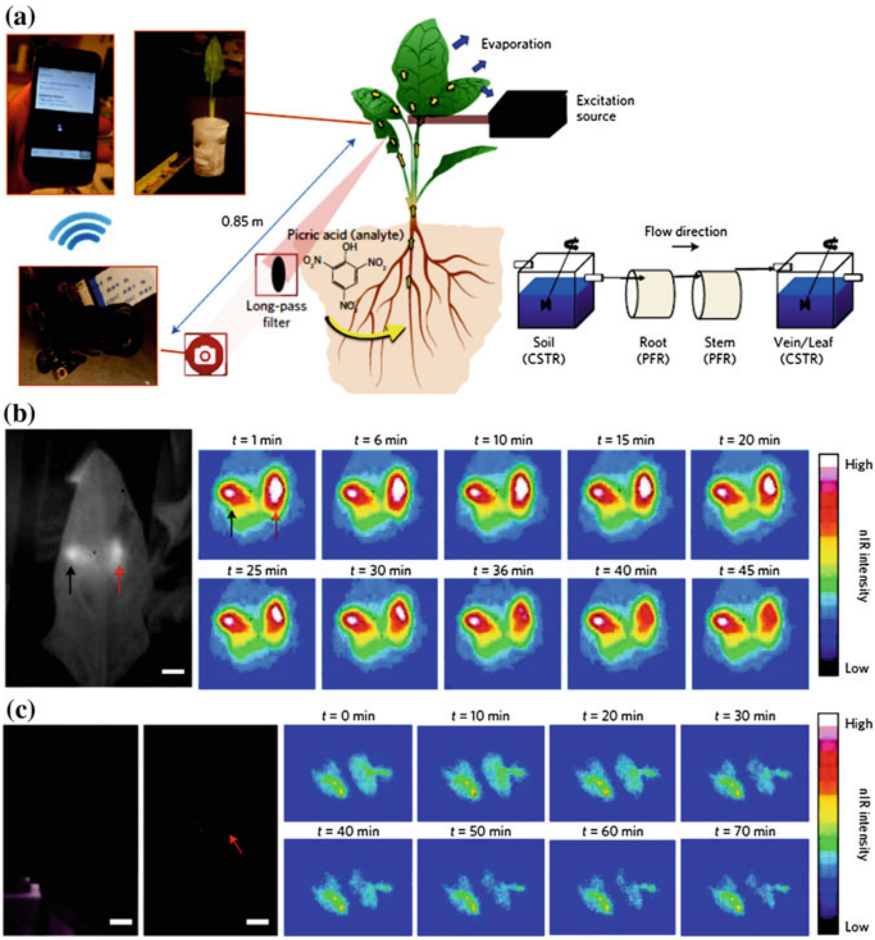

Fig. 4 Detection of picric acid using a nanobionic spinach plant. **a** Diagrammatic depiction of detection set-up with the nanobionic sensing plant shown here using a Raspberry Pi CCD detector (no infrared filters). **b** (Left) Bright-field image of spinach plant leaf infiltrated with SWCNTs and under 785 nm excitation. P-SWCNTs and B-SWCNTs indicated by black and red arrows, respectively. (Right) False-coloured time-lapse pictures show temporal changes in nIR fluorescence of a plant infiltrated with B-SWCNTs and P-SWCNTs as picric acid is transported from the roots to the leaves via the plant vascular system. While P-SWCNT nIR fluorescence remains stable, the B-SWCNT intensity drops as the leaf transpire a solution of picric acid (400-M) in 10 mM KCl. nIR images were taken with a Princeton Instruments OMA V InGaAs detector equipped with a 900 nm long-pass filter. SWCNTs inside leaves were excited with 785 nm laser incident at 15mW. Scale bar, 0.5 cm. **c** SWCNT nIR emission can be similarly detected by a Raspberry Pi CCD detector (infrared filters removed), which then transmit pictures wirelessly and in real time via an email interface to a smart phone. (Left) Bright-field imageof spinach plant infiltrated with SWCNT sensors. (Centre) nIR emission from embedded P-SWCNTs (black arrow) and B-SWCNTs (red arrow) is visualized with 785 nm laser excitation (15 mW). (Right) False-coloured time-lapse pictures similarly show temporal changes in nIR fluorescence of a plant infiltrated with B-SWCNTs and P-SWCNTs as picric acid is transported from the roots to the leaves via the plant vascular system. Scale bar, 0.5 cm (Wong et al. 2016)

Nanobionic Plant as Temperature Detector

Cyberwood was designed by employing a new synthetic carbon nanotubes which mechanical and structural properties resembling wood embedded and exquisitely sensitive to temperature changes into a matrix of plant cells from the tobacco. Preserving plant cells' natural ability to sense temperature variations even after their death cause electricity conductivity of this kind of carbon nanotubes change with temperature. The presence ofmulti-walled carbon nanotubes (MWCNTs) confers structural stability and a high electrical conductivity,which can be exploited to connect the samples to anexternal circuit (Di Giacomo et al. 2015). In fact, pectins and charged atoms (ions) play a key role in the temperature sensitivity of both living plant cells and the dry cyberwood. Pectins are sugar molecules found in plant cell walls that can be cross-linked, depending on temperature, to form a gel. Calcium and magnesium ions are both present in this gel. As the temperature rises, the links of the pectin break apart, the gel becomes softer, and the ions can move about more freely. As a result, the material conducts electricity better when temperature increases.

To synthesize cyberwood, several conventional nano-synthesis approaches were combined. At First step, undifferentiated tobacco BY-2 cells derived from the callus of seedlings of *Nicotiana tabacum* were cultured in a growth medium that contained MWCNTs. Spontaneous aggregation of cells was observed with tobacco cells combined with MWCNTs. After 24 h a gel-like material formed, was collected and dried at 47 °C for 15 d. The material formed had a complex, hierarchical structure, similar to wood (Fig. 5).

This construct it may find applications in thermal sensors, for example, for thermal cameras, or in distance sensors for consumer products and security systems Because of its exquisite temperature sensitivity. The cyberwood sensor can identify warm bodies even at distance; for example, a hand approaching the sensor from a distance of a few dozen centimeters. The sensor's conductivity depends directly on the hand's distance from the sensor (Fig. 6). The very high responsively to temperature changes of cyberwood suggests that it can be used as a temperature

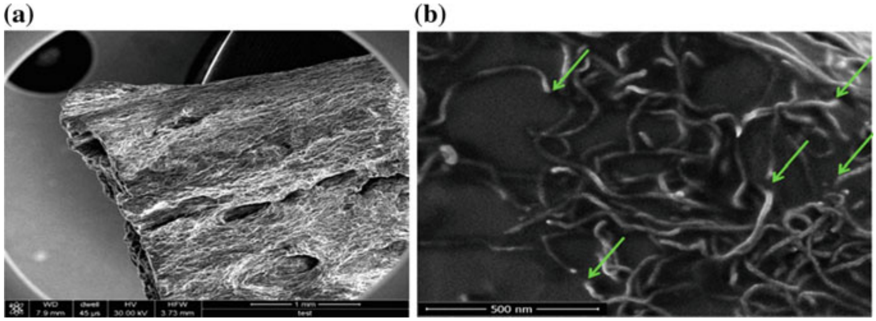

Fig. 5 **a** Low-magnification SEM picture of the cyberwood. **b** Top view of a cell wall of BY-2 with MWCNTs on top. Arrows emphasize some penetration points (Di Giacomo et al. 2015)

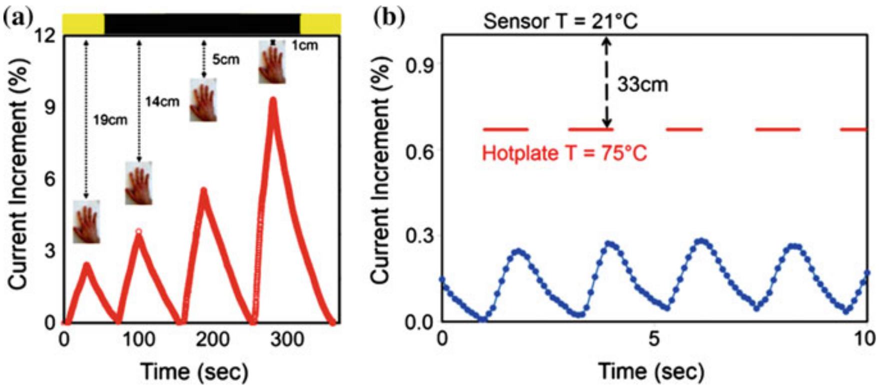

Fig. 6 Cyberwood as a thermal distance sensor. Plots show variations of the current in different cyberwood samples, as a function of the position, in- andoff-axis, of heat-emitting bodies in time. **a** Larger sample detecting the position of a hand. **b** Microsample detecting the position of a hotplate (Di Giacomo et al. 2015)

distance sensor. The distance of a warm body from the sensor can be inferred from temperature measurements performed at constant environmental conditions (Di Giacomo et al. 2015).

Conclusion and Future Prospects

Utilization of nanoparticles to create nanobionic-plant-enabled sensors for environment monitoring is a novel complex strategy. The researchers' interest to design creative nanosensors give real-time information from a plant is growing sharply. The product of this kinds of researches will Make human dreams of carrying a plant speak about their surroundings to a reality. Researchers are trying to increase the number of sensors that can be applied to plants and enhance chemicals detections in both the air and groundwater by plants. Monitoring plant signaling pathways of pest infestations, damage, and drought while being capable to real-time analysis will be a new revolution in agriculture industry. It is not far to have commercial sensing plants in home send messages directly on smart phone data about temperature, humidity and pollutants.

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Medicinal Plants: Influence of Environmental Factors on the Content of Secondary Metabolites

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Abstract Current research in secondary metabolites from medicinal plants should take into account the prevalence and the healing properties of which plant. These compounds have been provided opportunities to development of new drugs leads against several diseases. However, to obtain compounds from edible medicinal plants as well as that used to prepare infusions, numerous challenges are encountered including the environment and stressing factors to which the plants are submitted. Extensive phytochemicals analysis has lead to the identification of biotic and abiotic stress factors that directly influence in the metabolism of plant. This chapter provide an overview of some environmental and stressing factors that may direct the secondary metabolism in medicinal plants.

Keywords Bioactive compounds · Biotic stress · Abiotic stress · Nutrients · Saline stress · Drought Stress · Radiation

Introduction

The search for substances that decrease or avoid diseases, and the concern with the human health, has extended the studies aiming to understand the synthesis mechanism, as well as the environmental factors, that increase chemical compounds with medicinal/therapeutic potential. Plants are natural sources of these chemical compounds, which are originated from its secondary metabolism and have been used by humans, mainly due to its pharmacological properties. The use of plants in a therapeutic way is quite old and a large portion of world's population still rely on a traditional medicine system based on herbal drugs (WHO 2013). The secondary metabolites (SMs) and/or phytochemicals have biological activity responsible for the plants medicinal properties, besides taking part in the production of drug used

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by the pharmaceutical industry for the healing of various diseases. These SMs, as well as the primary metabolites, perform a vital role in the plants survival, and its synthesis can be induced by the plant-environment interaction (Kliebenstein 2013).

The vegetal kingdom produces thousands of SMs that are limited to certain taxonomic groups. As a survival strategy and to create diversity at organism level, the capacity of synthesizing some classes of SMs is variable between plants groups. Even in the same species there are variations in the chemical compounds content. These characteristics are related to the genetic variability and, mainly, to the differences of the growth conditions (Radušienė et al. 2012). Additionally, the synthesis of these metabolites are, many times, strictly regulated and, generally, restricted to specific vegetal tissues or stages of development, under response to environmental stimulation (Gargallo-Garriga et al. 2014; Osbourn et al. 2003; Sampaio et al. 2016).

The chemical interaction between plants and the environment is mediated, mainly, by the biosynthesis of SMs, which perform biological roles as a plastic adaptive response to the environment. Plants interact with the environment in order to survive, thus they are influenced by environmental factors, including biotic and abiotic stimulations that regulates the SMs biosynthesis (Verma and Shukla 2015; Zhi-lin et al. 2007).

Environmental Influence on the Contents of Bioactive Compounds

Abiotic stresses, as the ones caused by light incidence, temperature, humidity, water and nutrients availability, heavy metals and salinity in the soil, have been demonstrated to significantly affect the content of SMs in plants (Sampaio et al. 2016; Khan et al. 2016; Bernstein et al. 2010). Biotic stresses are consequences of damages induced by living organisms as bacteria, virus, fungi, parasites, insects, etc. Mechanisms for acclimatization and adaptation to environmental conditions based in the activation of specific physiological and molecular responses, lead to modifications in the plants metabolism in order to minimize the damages induced by the stress, as a form of defense and survival of the species against specific growth conditions.

The mechanisms involved in the synthesis, degradation and accumulation of SMs in plants becomes of major importance to the development of strategies that increases these phytochemicals in medicinal plants. Under stress, the plants tend to generate reactive oxygen species (ROS) such as superoxide anion (O_2^-), H_2O_2 and hydroxyl radical ($OH^\bullet$), which can promote cellular damage by triggering off a oxidative chain reaction (Imlay 2003). For example, the superoxide anion can destroy the photosynthetic apparatus (Jakob and Heber 1996), causing damages in the production of primary metabolites, leading to a decrease in the plant growth and development, and possibly to cell death. In order to eliminate the ROS, the plants produce defense compounds, besides the SMs, through enzymatic mechanisms (peroxidases, superoxide dismutase, catalase, among others) and non enzymatic, as some secondary

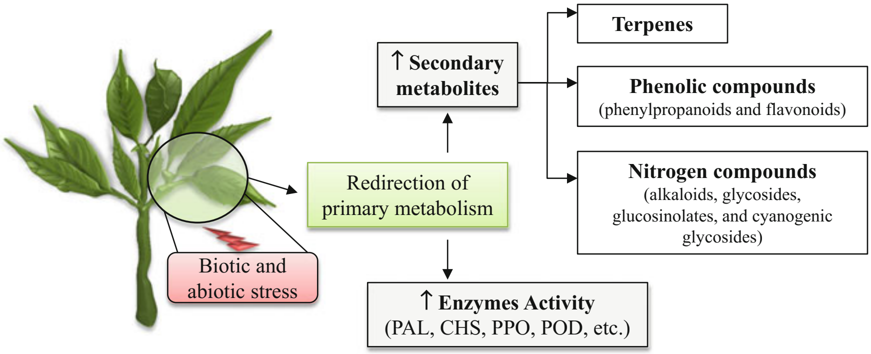

Fig. 1 Biotic and/or abiotic stress redirect the primary metabolism of plants, and results in increased enzymatic activity and synthesis of secondary metabolites. Enzymes as phenylalanine ammonia lyase (PAL), chalcona synthase (CHS), polyphenoloxidase (PPO), and peroxidase (POD) are essential components in this plant response. The secondary metabolites are metabolically classified in three main groups, terpenes, phenolic compounds, and nitrogen compounds

metabolism compounds. For an adequate ROS balance, not harmful to cells, the combination of enzymatic and non enzymatic mechanisms is fundamental.

Based on the biosynthetic pathway, the SMs are classified in three main groups (Fig. 1), terpenes, phenolic compounds (phenylpropanoids and flavonoids) and nitrogen compounds (alkaloids, glycosides, glucosinolates, and cyanogenic glycosides) (Fang et al. 2011). Even though the SMs are structurally different, they derive from the synthesis of products from the primary metabolism (carbohydrates, lipids, and amino acids) (Crozier et al. 2006). For example, both the pentose phosphate pathway as the Calvin cycle provide carbonic skeletons for the erythrose-4-phosphate, which form shikimate through some reactions, in order to produce phenylalanine, which is the precursor for the phenylpropanoid metabolism (Caretto et al. 2015; Lattanzio et al. 2009). The shikimic acid pathway, non-mevalonate (MEP) pathway and mevalonate (MVA) pathway form many chemical compounds (Wilson and Roberts 2014), divided in classes, such as terpenoids, monoterpene indole alkaloids, isoquinoline alkaloids, flavonoids, and anthocyanins.

Generally, under stress, plants tend to increase their enzymatic activity that synthesize secondary metabolism compounds. This accumulation is due to the raise of the activity of enzymes as phenylalanine ammonia lyase (PAL) (EC 4.1.3.5) and chalcone synthase (CHS) (EC 2.3.1.74). Both the PAL and the CHS are key enzymes in the flavonoids synthesis and can have it activity affected in function of the environmental stress. The PAL is the main enzyme related to plant defense by producing phenols and lignins. It is also the first enzyme in the phenylpropanoids pathway and one of the responsible for the pholyphenols synthesis, catalyzing the deamination of L-phenylalanine to produce *trans*-cinnamic acid, intermediary in the biosynthesis of phenolics (Dixon et al. 1992), compounds related to scavenge of ROS in plants under stress. The phenylpropanoids have a fundamental role in the plants responses against abiotic and biotic stresses that includes herbivory and infection by pathogens.

The phenolic compounds provide many physiological functions to the plants survival and have fundamental importance in plants adaptation to imposed environmental alterations (Verma and Shukla 2015). Some phenolic compounds can be used in ROS scavenging reactions as well as in the formation of o-quinones, by the action of enzymes as polyphenoloxidase (PPO) (EC 1.14.18.1) and peroxidase (POD) (EC 1.11.1.7), forming compounds related to defense system against some pathogens. The phosphoenolpyruvate (PEP)-carboxylase (EC 4.1.1.31) is an important enzyme in the plant carbohydrate metabolism that promotes β-carboxylation of phosphoenolpyruvate to yield oxaloacetate. It activity can also increase in response to the stress (e.g. saline), suggesting a modification in its synthesis pathway to support the plant defense (Landolt et al. 1997), e.g. production of metabolites related to plants defense/resistance, as the phenolic compounds.

Plants, as the medicinal/aromatic plants, under certain types of stress, salinity for example, can also accumulate terpenes, in the form of essential oils. The main constituents of the essential oils are the terpenoids, however, we can also find phenylpropanoids. The terpenoids synthesis occurs from five carbon units of isopentenyl pyrophosphate (IPP) and its isomer, dimethylallyl pyrophosphate (DMAPP). Prenyl pyrophosphate synthase bind IPP unities with DMAPP, forming monoterpenoids (C10) and sesquiterpenoids (C15) of prenyl pyrophosphates (Sangwan et al. 2001).

The glucosinolates can also have their content affected in function of the stress. Many studies have shown that the synthesis of these compounds in Brassicaceae can be influenced by environmental factors, such as salinity, drought, temperature, deficiency of nutrients and soil acidity (low pH), beyond the biotic factors (Steinbrenner et al. 2012). The glucosinolates are a class of SMs that gained a particular interest due to its biological activity, mainly in the prevention of cancer (Johnson 2002; Finley 2005). These compounds are very studied in vegetables and its consumption has been recommended for the treatment of some diseases.

In plants, amino acids are precursors of a wide range of metabolites, in especial the secondary metabolites produced in response to environmental stress or as defense mechanisms. The aliphatic SMs are from alanine, leucine, isoleucine, valine, and methionine, while the indole glucosinates and the aromatics derive from tryptophan, phenylalanine, and/or tyrosine (Fig. 2). Aromatic amino acids probably are the major precursors of SMs related to plant defense. Tryptophan is the precursor of alkaloids, phytoalexins, and indole glucosinolates. Phenylalanine is a basic precursor of the most phenolic compounds, such as flavonoids, condensed tannins, and phenylpropanoids, whereas tyrosine is the precursor of isoquinoline alkaloids and quinones (Maeda and Dudareva 2012). The metabolic pathway of synthesis for these compounds is performed in three phases, where the chain elongation is affected by the stress (Sønderby et al. 2010; Ishida et al. 2014; Martínez-Ballesta et al. 2013; Khan et al. 2011).

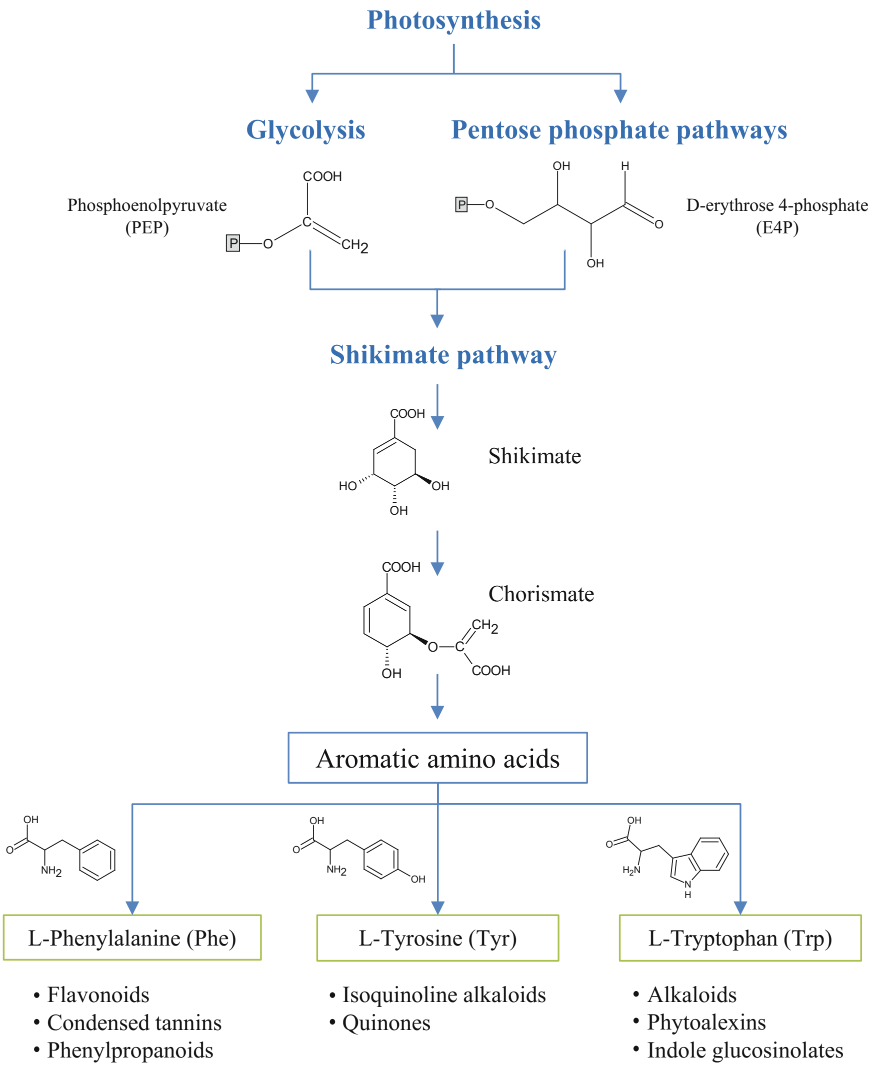

Fig. 2 Resumed scheme of aromatic amino acids derived secondary metabolites production in plants

Thus, the investigation of the mechanisms involved in the synthesis, degradation, and accumulation of the SMs in plants becomes of great importance for the development of strategies in the production of medicinal plants, aiming the increase of these phytochemicals. Even though there is an infinity of traditional plants with great phytotherapy potential, most of these plants are not used in large scale, that is, in industrial scale. The main reason for this is that many of these SMs produced by plants and with pharmacologic properties are results of a response to the imposed stress and when the plant is taken from the nature and cultivated in large-scale, the stress factors are removed and, consequently, the content of these compounds with medicinal properties are largely reduced. Due to this problem, the studies with elicitors, both biotic and abiotic, appear as a possible help to maximize the production of these biologically active substances. The elicitation is already been used in studies of resistance to plants diseases as well as in metabolic studies (Gorelick and Bernstein 2014).

Biotic and Abiotic Stress Effects on Plant Metabolism

Due to the great scientific and industrial interest for the increase of the SMs production, studies have been performed with many types of stress (biotic or abiotic) as substances elicitors, such as essential oils, polyphenols, among others with therapeutic potential.

Abiotic Factors

Various climatic factors, such as water availability (Sampaio et al. 2016; Zhou et al. 2017), temperature and solar radiation (Sampaio et al. 2016; Nascimento et al. 2015; Ferreira et al. 2016) are described as capable of influencing the production of SMs. Thus, the plants under stress conditions induced by the environment (e.g., water, drought, high temperatures, freezing, high thermal amplitude, and high levels of solar radiation) can show changes in the production of different classes of SMs. The plants modify specifically the genetic expression in a very different way, under a stress condition. These alterations in the genetic expression lead to a specific regulation of the metabolome, depending on the vegetal species and on the specific stress conditions (Rizhsky et al. 2002). When a plant is exposed to stress agents, many times denominated elicitors, enzymatic pathways are induced, affecting the SMs contents (Gorelick and Bernstein 2014). This is already established for compounds well known for their pharmacological properties, such as terpenoids (Lake et al. 2009), alkaloids (Nascimento et al. 2015), and phenolic compounds (Verma and Shukla 2015).

Environmental factors as temperature, rainfall, humidity and solar radiation, as well as the quantity of macro and micronutrients in the soil show a strong influence on the variation of SMs in many species. In *Tithonia diversifolia*, there were found compounds as sesquiterpene, lactone, flavonoids and t-cinnamic acid in, which were directly correlated to the amount of rainfall and changes in temperature (Sampaio et al. 2016).

Nutrients

It has been described in the literature an influence of many nutrients in the content of SMs. Their production is higher in plants cultivated soils that are poor in nutrients, mainly some phenolic derivates (Gobbo-Neto and Lopes 2007). The availability of certain nutrients in the soil, especially the macronutrients and the copper affect the metabolic profile in inflorescences in *T. diversifolia* (Sampaio et al. 2016), plant with many biological activities that can be used as anti-inflammatory, anti-malaric, cytotoxic, gastric protector, antimicrobial, chemopreventive, and anti-hyperglycemic. The inflorescences and roots were grouped according to the availability of certain nutrients in the soil, especially macro (Ca, Mg, P e K) and micronutrients (Cu). Cu is an essential nutrient that generates ROS for being a redox-active metal. Low levels of macronutrients cause accumulation of esters derived from the trans-cinnamic acid in the roots (Sampaio et al. 2016). The accumulation of these esters can be a response to the lower nutrient availability, once the polyphenols bonded to the sesquioxides have the capacity of inhibiting the phosphate adsorption. Additionally, the phenolic acids also have the capacity of adsorbing the bonded phosphate, increasing the availability of inorganic P from the soil, beyond the fact that the phenolic compounds can also retain inorganic exchangeable cations (Ca, Mg and K) providing absorption sites on acid soils (Hättenschwiler and Vitousek 2000).

The deficiency of N can promote alterations in the secondary metabolism of medicinal plants because it induces the formation of ROS. In leaves and roots of *Matricaria chamomilla*, the lack of N during the cultivation promoted an increase in the total phenolic content (Kováčik and Bačkor 2007). In *Achillea collina* cultivated under N starvation were observed a decreasing in the plant growth, as well as a reduced content of substances related to the primary metabolism as carotenoids and chlorophyll, and increased total phenols content and antioxidant capacity of leaves and roots (Giorgi et al. 2009). Higher PAL activity, and increased synthesis of anthocyanin and phenylethanoid glycosides were described in *Castilleja tenuiflora*, a plant used to treat cancer, when cultivated under the deficiency of N (Medina-Pérez et al. 2015).

Compounds containing N as cyanogenic glycosides and alkaloids are generally reduced by low nitrogen concentrations, due to the decrease in its synthesis, because in these substances the nitrogen atom is part of the heterocyclic ring. In environmental with limited C and saturation conditions of N, the accumulation of alkaloids becomes favored in *Cyrtanthus guthrieae*, plant used for medicinal purposes in South Africa, while the polyphenols content increases proportionally to C levels in the ambient, being less influenced by the quantities of N (Ncube et al. 2014).

The foliar application of gamma irradiated sodium alginate (ISA) (0, 40, 80 and 120 mg L^{-1}) alone and in combination with phosphor applied in the soil (soil-applied phosphorus) (40 kg p ha^{-1}) were used as trigonelline elicitors, an alkaloid found in *Trigonella foenum-graecum* L. (fenugreek), which controls the insulin secretion, cell regeneration and stimulation of the enzymatic activities related to the glucose metabolism. The treatments employed with 80 mg L^{-1} of ISA and applied at 40 kg P ha^{-1} proved to be the best in raising these nitrogenated compounds content (increased the seed yield by 131%, trigonelline content by 17.84%, trigonelline yield by 174%, seed alkaloid content by 32.98%, and seed alkaloid yield by 208.64% over the control) (Dar et al. 2016).

Metals

Soils contaminated with heavy metals can also influence the SMs content in response to the ROS production. *Matricaria recutita* cultivated in nutritive solution for 7 days under levels of cadmium (Cd) or copper (Cu), increased the SM content. Doses of 60 and 120 μM of Cu strongly elevated PAL activity, and consequently increased the phenolic compounds and lignin content one day after the begin of the treatment. This effect could indicate the formation of a barrier against metal entrance (Kováčik and Klejdus 2008).

In *Robinia pseudoacacia* L. high content of saponins, phenolic compounds, and flavonoids, were observed in plants cultivated in soils containing Cd and Pb, and under effect of high temperatures (Zhao et al. 2016). This metabolic pattern was attributed to a mechanism of prevention against damages caused by Cd and Pb. In opposition, the presence of low Cd and Al concentration induced a decrease in the hypoxoside synthesis in *Hypoxis hemerocallidea* Fisch. and C.A. Mey corn, a medicinal plant traditionally used in Africa. However, when the exposition occurred in low Cd and Al concentrations, there was an increase in the phenols and flavonoids content (Okem et al. 2015). Even though plants grown in contaminated soils are a source of compounds with high biological activity, there are still not enough studies that grant that these metals are not present as contaminants in the plants used for medicinal purpose.

Saline and Drought Stress

Stressing situations as the lack of water in the environment causes a raise in the synthesis of many bioactive compounds, which are produced by the plants in order to combat the hydric deficit (Khan et al. 2011). Both, saline and drought/water stress affect the plant productivity and many times can have a contradictory effect in medicinal plants. Naturally, these stresses occur more commonly in arid or semiarid environments. However, nowadays, with the modifications in the rainfall regime and the lack of water, these types of stresses are becoming more common. Drought stress as consequence of the lack of rain or soil humidity can induce many biochemical and physiological factors and diverse genetic responses, depending on the specie or cultivar (Zhou et al. 2017). Plants cultivated in water stress can present accumulation of carbon in the form of carbohydrates, and allowing the carbon to be allocated to the secondary metabolism (Herms and Mattson 1992). In water stress as well as in other types of stress, the plants can present a raise in the phosphoenolpyruvate carboxylase activity, suggesting a formation of SM from sucrose (primary metabolism) (VanderPlas et al. 1995).

Drought stress can cause a reduction in the photosynthetic rates with a consequent increase in the production of reactive oxygen species (ROS), resulting in high production of phenolic compounds as a plant defense mechanism (Sampaio et al. 2016). This metabolic mechanism was observed in *Labisia pumila*, where an increase in the total phenolics, total flavonoids and total anthocyanins after the imposition of high water stress (50% evapotranspiration replacement) compared to severe water stress (25% evapotranspiration replacement) (Jaafar et al. 2012).

In saline stress, high sodium levels can occur, leading to potassium unbalance and, when accumulated in the cytoplasm, it causes an inhibition of many enzymes. Simultaneously, the stressor environment triggers an oxidative stress response, associated with the ROS production (Iqbal et al. 2006). Most of the plants show hypersensitivity when cultivated in saline soils. The excess of intercellular sodium (Na^+) is toxic to the plant metabolism, leading to a decrease in the growth in most of the cultures (Jaleel et al. 2008). Saline environments can affect the plant physiological responses, inducing readjusts in its metabolism, resulting from the harmful effects caused by the decrease of osmotic potential, specific ion toxicity, and nutrient ion deficiency (Luo et al. 2005).

The increase of polyphenols in response to salinity or drought stress can be related to the activity of enzymes such as the PAL and CHS. It is well known that under some types of stress, as osmotic, saline, droughts/water, there is a raise in levels of the aminoacid L-proline, for the osmotic readjust in many vegetal species. Although this aminoacid belongs to the primary metabolism, it can be related to the formation of SMs, through PAL action. With the increase of the proline levels, by the action of Δ1-pyrroline-5-carboxylate-synthetase (P5CS) and Δ1-pyrroline-5-carboxylate-reductase (P5CR), there is simultaneously a raise of $NADP^+$/NADPH ratio, inducing the formation of polyphenols precursors, by the pentose phosphate pathway (PPP) (Lattanzio et al. 2009). In the PPP there is the formation of precursors for many polyphenols, including flavonoids. It was suggested that the flavonoids are a secondary system of ROS elimination in plants under great salinity stress, showing a severe excess of excitation energy being channeled to the photosynthetic apparatus (Agati et al. 2011).

An interesting factor that can be observed is that the environmental factors do not seem to have constant effect on the general metabolism and each part of the plant responds differently to the environmental changes that they are exposed to. This difference in the metabolic response, according to the parts of the plant, was described in a comparative study of the *Holcus lanatus* L. and *Alopecurus pratensis* L. (Poaceae) metabolisms (Gargallo-Garriga et al. 2014). Differences in the accumulation of primary and SMs between the shoots and roots of both species, in different seasons and drought conditions were observed by means of spectral data of LC-MS and RMN (Gargallo-Garriga et al. 2014).

In *Limonium bicolor* were found evidences that salt stress can induce the accumulation of specific phenolic compounds, both in leaves [myricetin-3-*O*-glucoside/galactoside, quercetin-3-*O*-rhamnoside, myricetin-3-*O*-(*O*-galloyl)-rhamnoside, gallic acid, and vanillic acid–hexose] and in roots (epigallocatechin gallate and myricetin-3-*O*-rhamnoside), suggesting that some compounds can be part of the resistance system against the salt stress (Wang et al. 2016). Similarly, *Salvia mirzayanii* cultivated under salinity stress shown an increase in the leaf total phenolic compounds under mild (6.8 dS m^{-1}) salinity. Furthermore, an increased production of α-terpinyl acetate (at 2.3 dS m^{-1}), 1,8-cineole (4.5 dS m^{-1}), and linalyl acetate (at 9.1 dS m^{-1}) were described (Valifard et al. 2014). The authors affirm that plants exhibiting tolerance to salt stress are useful systems for production of SMs.

Studies inducing hydric and salt stress have been performed in various medicinal plants with the aim of observe the content, as well as the composition, of some SMs as the essential oils. Japanese mint plants shown a reduction in essential oil contens when cultivated in hydric stress (Misra and Srivastava 2000). In *Satureja hortensis* L., used for treating stomach and intestinal disorders, water stress, besides having promoted the decrease of the oil production, also induced a raise of bicyclogermacrene and caracrol, and the decrease of β-caryophyllene and ϒ-terpinene (Baher et al. 2002). In *Matricaria camomila*, both the salt and the hydric stress promoted a reduction in number of branches and flowers per plant, as well as the essential oil content (Razmjoo et al. 2008). Other medicinal plants cultivated in environments with high saline concentration also showed a reduction in the essential oil content, as described in *Mentha piperita* (Tabatabaie and Nazari 2007). In contrast, in *Coriandrum sativum* leaves, levels from 25 to 50 mM of NaCl induced raise of 0.15 and 0.18%, respectively, in the essential oils production. However when 75 mM NaCl was used, there was a decrease of 38% (Neffati and Marzouk 2008). Additionally, there was a raise of (E)-2-decenal, (E)-2-dodecenal and dodecanal in *C. sativum* cultivated in low salinity level, while the levels of 75 mM promoted a decrease in the contents of these compounds. The essential oil content of *C. sativum* fruits in saline environment, reached 0.53 and 0.55% under 50 and 75 mM NaCl, respectively (Neffati et al. 2011). The same metabolic behavior was induced *Salvia officinalis*, in which the essential oil content increased in a salinity dose-dependent manner (Ben et al. 2010).

The raise of the oil production can be attributed to an indirect effect of salinity in the liquid assimilation (photosynthesis) (Charles et al. 1990) or in the reduction of growing and differentiation processes, induced by the osmotic stress, resulting in a partitioning of compounds and increasing the supply of carbon skeletons for the terpenes synthesis and accumulation (Ben et al. 2010).

The salinity can also affect the alkaloids content, as observed in *Catharanthus roseus* (L.) G. Don (Apocynaceae), a medicinal plant rich in alkaloids. There was an increase in the alkaloids content in response to the different soil salinity regimes that were imposed in the soil (50 and 100 mM NaCl), when compared to the control (Jaleel et al. 2008). In *Datura inoxia* grown under salt stress, a similar behavior in alkaloids content was observed, more specifically in tropane alkaloids (Selmar

2008). The effect of inorganic salt stress was also evaluated in the *Achnatherum inebrians*, and modifications in secondary metabolism and high alkaloids levels were reported in plants cultivated under salt stress (Zhang et al. 2011).

Beyond the medicinal plants, many fruits and vegetables can be sources of important bioactives, as the glucosinolates. In radishes cultivated under 100 mM NaCl, higher glucoraphasatin and total glycosinolates contents were found (Yuan et al. 2010). Similarly, an increase of glucosinolates in *Brassica oleracea* L. var. italica, when cultivated under salt stress (80 mM NaCl) was showed (López-Berenguer et al. 2008). These results indicate that, in order to obtain plants with higher levels of these compounds, which are studied by many researchers due to the medicinal effect, the use of elicitors such as the salinity can be beneficial.

Temperature

The temperature stress has been described for inducing or increasing antioxidant enzymes, as superoxide dismutase, catalase, peroxidase and many compounds with antioxidant activity. This stress can cause alterations in proteins, promoting the denaturation as well as alterations of lipids and in the membrane integrity. All these changes induce modifications in the SMs content (Levitt 1980). Generally, because temperature is a consequence of various factors, few studies are performed under its direct influence in the SM production and composition (Gobbo-Neto and Lopes 2007).

The temperature can cause variation in the medicinal plants metabolism, inducing reductions in the vigor and growth, by affecting the photosynthesis, one of the most sensible processes to the temperature differences (Zobayed et al. 2005; Yamori et al. 2014). Oscillations in the photosynthetic rates induced by stresses can promote the SMs formation through restoring defense mechanisms (Zobayed et al. 2005). High temperatures modify the opening and closing mechanism of the stomata, affecting the carbon assimilation, causing an imbalance and influencing directly in the ribulose 1,5 biphosphate carboxylase/oxygenase activity (Weis and Berry 1988). In opposition, plants can also show a decrease in the photosynthesis when submitted to low temperatures, in function of the metabolism type, e.g. plants C3, C4 and CAM (Crassulacean acid metabolism).

Radiation

The environmental levels of the UV solar radiation may represent an environmental stress to the plants, inducing the production of reactive oxygen species (ROS), instigating protective effects that promotes the biosynthesis of SMs that can absorb UV-B (e.g., alkaloids, anthocyanins, carotenoids, flavonoids, lignin, phytosterols, saponins, and tannins) (Nascimento et al. 2015; Takshak and Agrawal 2014), as well as alterations in the antioxidant enzymatic activity (e.g. enzymes from the phenylpropanoids pathway) (Takshak and Agrawal 2014). Luminous intensity can increase the terpenoids and phenolic compounds levels in many plants species (Lake et al. 2009; Park et al. 2007). Even though there is a slight reduction of the vegetative and reproductive growth in plants, due to a dislocation of carbons to the SMs production (Chalker-Scott 1999), the accumulation of these compounds is essential to protect the plants tissues from the excess of solar radiation.

The UV-B radiation induce synthesis of compounds responsible for the UV-B light radiation absorbance in plants and is an important factor of SMs induction. However, the production of these compounds is variable among plants. The increase of UV-B radiation (produced by UV-B fluorescent lamps—40 W, 305 nm) increased significantly the contents of anthocyanins and compounds capable of absorbing UV-B in medicinal chrysanthemum flowers (*Chrysanthemum morifolium Ramat*) (Ma et al. 2016). The anthocyanins belong to a class of flavonoids that are important pigments in flowers, fruits and leaves, distributed mainly in the epidermal cells of flowers and other parts, regulating the UV-B radiation absorbance. The phenolic compounds are widely distributed in the plants and many works relate the increase of these chemical compounds with several enzymatic activities. The coordinated enzymatic activity in the phenylpropanoids pathway is responsible for the production of flavonoids under stress caused by UV-B. The UV-B radiation effect is also known for stimulating the expression of genes encoding enzymes that participate directly in the anthocyanins biosynthesis. The increase in the anthocyanins content is related to high activity of various enzymes as CHS (chalcone synthase), DFR (dihydroflavonol reductase) and F3H (flavanone 3-hydroxylase) (Park et al. 2007).

Increases in the activities of PAL (phenylalanine ammonia lyase), CAD (cinnamoyl alcohol dehydrogenase), 4CL (4-coumarate CoA ligase), CHI (chalcone-flavanone isomerase) and DFR (dihydroflavonol reductase), and consequently high concentrations of flavonoids, anthocyanins and tannins contents were described in *Withania somnifera* submitted to UV-B radiation (Takshak and Agrawal 2014). In studies performed with *Curcuma longa* leaves and rhizomes, the total phenol content, POD and PAL activity increased with the exposition to light and decreased after UV exclusion (Ferreira et al. 2016). Curiously, the curcuminoids content was not affected by solar radiation. However, the UV exclusion provided by the anti-UV film led to improvements in the total curcuminoids efficiency. These results showed a correlation between different processes, as the curcumin biosynthesis, photosynthetic development, plants growth and rhizome biomass production, regarding that the biomass production and the CO_2 assimilation during the growth were higher in plants grew under the UV exclusion. In chrysanthemum, the UV-B radiation increased significantly the flavonoids and chlorogenic acid contents in the four stages of floral development, which are the main phytochemicals responsible for the medicinal properties in flowers belonging to this species (Ma et al. 2016). Additionally, other SMs can be affected by radiation stresses. Compounds as withferin A and alkaloids also increased with the exposition to UV-B radiation, in *W. somnifera* (Takshak and Agrawal 2014).

Biotic Factors

The plants developed a wide range of defense mechanisms in their habitats against insects, herbivores and several microorganisms (e.g. bacteria, filamentous fungi and protozoa), which establish many interactions with the host plant. These continuous interactions of parasitism and/or mutualism are the basis for the synthesis of specific metabolites. Natural products resulting from this interaction are classified in three large groups: phytoalexins, phytoanticipins and signaling molecules, for example the salicylic acid. The phytoalexins are molecules with low molecular weight, synthesized and accumulated after the plants were exposed to the microorganisms. The phytoanticipins are synthesized in the plants before attacks by pathogens (Cheynier et al. 2013).

The SMs, such as phenolic compounds, among others, are important elements in the plant chemical defense against insects and pathogens (Kim et al. 2008), besides the beneficial aspects for some organisms as pollinators or symbionts (Wink and Schimmer 1999). The phenolic compounds contribute to the reduction of reactive oxygen species and therefore impact cellular processes sensitive to redox effects. However, the plants phenolics have been implied in more direct interactions, such as transduction pathways and signals transport. In this context, the salicylic acid (SA) performs a regulator role in multiple physiological processes, including the immune response. Thus, when the plant is attacked by a pathogen, sophisticated innate immune systems recognize signals from injured cells and respond by activating effective immune responses through SA signaling cascade and interactions with other phytohormones. Nevertheless, the complexity of the interaction plant-microbe can represent different adaptive mechanisms in the plants (Zhi-lin et al. 2007).

During the fungi attack, the plant epidermal cells tend to resist to the penetration, by forming papillae that strengthen the cell wall, attempting to stop the microorganism penetration and also, accumulating fungitoxic substances as the phenolic compounds and ROS. In the attempted penetration site, the hydrogen peroxide (H_2O_2) can be accumulated, presumably providing the oxidative power for cross linking of materials and hardening of the papillae (Zeyen et al. 2002).

Another way of avoiding the organisms attack is by the increase of specific enzymes as PPO. The PPO activity increases in wounded plants, infected by pathogens or attacked by other types of pests (Vanitha et al. 2009). In order to avoid the attack of fungi and other microorganisms, the plants oxide phenolic compounds, producing o-quinones, which are antimicrobial compounds. These o-quinones decrease the dietetic proteins nutritional value by reacting with amino groups and free tiols, decreasing the palatability (Constabel et al. 2000). These enzymes are also involved with the cells lignification during the pathogens invasion (Mohammadi and Kazemi 2002).

Beyond the quinones, compounds as glucosides of hydroxynitrile, including the cyanogenic glucosides, are known for providing defense against herbivores and some pathogenic fungi, improving the plant response to environmental challenges.

Many other substances can be affected in function of the biotic stress. Several alkaloids are synthesized in response to the plant defense against microorganism attacks. The alkaloids are important secondary compounds involved in the chemical defense in many plants. For example, compounds as trigonelline, castanospermine and camptothecin are increased due to reactions with arbuscular mycorrhizal fungus inoculum (Jia et al. 2016).

In response to the herbivory, certain plants tend to change the essential oils content and composition. The terpenes are SMs present in essential oils and the monoterpenes are considered the main components of many species. Studies show that these substances are related to a possible plant defense system against biotic stress, when acting as an antifungal, antimicrobial, antiherbivore as well as in allelopathic functions (Isman 2000; Bekele and Hassanali 2001). In *Minthostachys mollis* leaves attacked by insects (feedong), there was the decrease of menthone compared to the undamaged plants. In contrast, in these same conditions, the pulegone concentration increased in all of the tested treatments (Banchio et al. 2005). Pulegone is an inhibitor of the acethylcholinesterase and is related to destroying symbionts of herbivores, creating a biochemical barrier against the utilization of plants with high pulegone contents as food (Harrewijn et al. 2000).

The conjugation of compounds to minimize the stressor effect of the environment caused by the microorganisms attack is also described in many researches. For example, we can cite compounds as phenolamides (also named hydroxycinnamic acid amides) that are combinations of phenolic portions, as coumaric and ferulic acid, with polyamines or aromatic deaminated aminoacids. These compounds have specific functions in the plants development and defense responses, either as an intermediate product or, as final product (Bassard et al. 2010). When there is a biotic stress, e.g. caused by a fungus, there is the synthesis of these substances in plant cell wall, increasing the plant resistance to degradation by the hydrolytic enzymes produced by the pathogen. Additionally, these compounds show anti-microbial activity acting directly on the pathogen and/or disease (Bassard et al. 2010; Kristensen et al. 2004; Mikkelsen et al. 2015).

Conclusion

The plant defense system against several types of stress is important to increasing the SMs, which can be a source of medicinal substances for the population and possible auxiliaries in the cure of many diseases. Additionally, for presenting this type of metabolism (secondary), the plants can survive in inhospitable environments. However, the plant growth and development will be affected, because there is a relocation of primary metabolites for the formation of SMs. The production of SMs, induced by stressing factors, is not similar for all species and represents an important study object. Nevertheless, the plants can adapt to the environment through some biochemical and genetic mechanisms, which can be beneficial for the plants survival as well for obtaining medicines.

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The Most Important Fungal Diseases Associated with Some Useful Medicinal Plants

Faezehossadat Abtahi and Seydeh Lavin Nourani

Abstract When the ability of the cells of a plant to carry out one or more of these essential functions is interfered with by either a pathogenic organism or an adverse environmental factor, the activities of the cells are disrupted, the cells malfunction or die, and the plant becomes diseased. Tens of thousands of diseases affect cultivated and wild plants. Plant diseases are grouped according to the type of pathogen that causes the disease. All plants are attacked by some kinds of fungi, and each of the parasitic fungi can attack one or many kinds of plants. Fungi are small, generally microscopic, eukaryotic, usually filamentous, branched, spore-bearing organisms that lack chlorophyll. Fungi cause local or general symptoms on their hosts and such symptoms may occur separately or concurrently or may follow one another. Medicinal plants like other plants have specific fungal diseases. In this chapter, considering the importance of fungal diseases on this group of plants, we review some of the most important fungal diseases of the number of medicinal plants.

Keywords Medicinal plants · Plant diseases · Fungi · Symptoms

Introduction

Plants have been used for thousands of years to flavor and conserve food, to treat health disorders and to prevent diseases including epidemics. The knowledge of their healing properties has been transmitted over the centuries within and among human communities. Active compounds produced during secondary vegetal

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metabolism are usually responsible for the biological properties of some plant species used throughout the globe for various purposes, including treatment of infectious diseases. Currently, data on the antimicrobial activity of numerous plants, so far considered empirical, have been scientifically confirmed, concomitantly with the increasing number of reports on pathogenic microorganisms resistant to antimicrobials. Products derived from plants may potentially control microbial growth in diverse situations and in the specific case of disease (Silva and Fernandes Junior 2010). Plant that possess therapeutic properties or exert beneficial pharmacological effects on the human-body are generally designated as medicinal plants (Motaleb 2011). Traditional medicine is used globally and has a rapidly growing economic importance. Complementary-alternative and traditional medicine are gaining more and more respect by national governments and health providers in developing countries (Grigoryan et al. 2015).

A plant disease may therefore be defined as any harmful deviation or alteration from the normal functioning of physiological processes. When the ability of the cells of a plant or plant part to carry out one or more of these essential functions is interfered with by either a pathogenic organism or an adverse environmental factor, the activities of the cells are disrupted, altered, or inhibited, the cells malfunction or die, and the plant becomes diseased. On average, each kind of crop plant can be affected by a hundred or more plant diseases. Some pathogens affect only one variety of a plant. Other pathogens affect several dozen or even hundreds of species of plants. Plant diseases are grouped according to the cause of the disease, on this basis, plant diseases are classified as infectious plant diseases (caused by fungi, prokaryotes, parasitic higher plants and green algae, viruses and viroids, nematodes and protozoa) and noninfectious plant diseases too low or too high a temperature, lack or excess of soil moisture, lack or excess of light, lack of oxygen, air pollution, nutrient deficiencies, soil acidity or alkalinity (pH) and etc.

Fungi are small, generally microscopic, eukaryotic, usually filamentous, branched, spore-bearing organisms that lack chlorophyll. They have cell walls that contain chitin and glucans (but no cellulose) as the skeletal components. These are embedded in a matrix of polysaccharides and glycoproteins. More than 10,000 species of fungi, however, can cause disease in plants. All plants are attacked by some kinds of fungi, and each of the parasitic fungi can attack one or many kinds of plants. In many diseases, the fungal pathogen grows, or produces various structures such as mycelium, sclerotia, sporophores, fruiting bodies, and spores, all called signs, on the surface of the host. Signs are distinct from symptoms, which refer only to the appearance of the infected plants or plant tissues. Thus, in the mildews, for example, one sees mostly the signs consisting of a whitish, downy or powdery growth of fungus mycelium and spores on the plant leaves, fruit, or stem, whereas the symptoms consist of chlorotic or necrotic lesions on leaves, fruit, and stem, reduced growth of the plant, and so on.

Fungi cause local or general necrosis of plant tissues, and they often cause reduced growth (stunting) of plant organs or entire plants. A few fungi cause excessive growth of infected plants or plant parts. The most common necrotic symptoms are: leaf spots, blight, canker, dieback, root rot, damping-off, soft rots and dry rots, anthracnose, scab, decline, clubroot, galls, warts, leaf curls. Other diseases, such as rusts, mildews, wilts may cause stunting of the plant as a whole.

Medicinal plants like other plants have specific fungal diseases. In this chapter, considering the importance of fungal diseases on this group of plants, we review some of the most important fungal diseases of the number of medicinal plants.

Introduction to Species

Common Fungal Diseases of Species

1. *Bunium persicum* (Boiss.) B. Fedtsch.

Common Name: Black Caraway, Black Cumin

Kingdom	Plantae
Order	Apiales
Family	Apiaceae
Genus	*Bunium*
Species	*B. persicum.*

1.1 Description

Black caraway (*Bunium persicum* Boiss.) is a perennial aromatic and medicinal herb, distributed in temperate areas of the world and mostly restricted to the sub-alpine mountain slopes. Its species grow wild in North Himalayan regions, Iran, Pakistan and generally it is native to central and Southern parts of Asia, with a wide geographical distribution in arid regions in Iran. The fruit of *B. persicum* is widely used in traditional Iranian medicine to control colic pain and dysmen-orrhoea (Dar et al. 2011; Zendehdel et al. 2015; Faravani et al. 2015).

1.2 Some of the important fungal diseases

Studies conducted by researchers showed that incidence of disease like leaf blight and umbel blight (*Alternaria alternata*), tuber rot (*Fusarium solani*) and powdery mildew significantly influence its yield potential. It has been reported that disease incidence causes up to 38% reduction in yield in Kala zeera. Blight disease was found to attack the crop first immediately after the emergence of sprout whereas powdery mildew appeared at flower bud and developing grain stages. Tuber rot shows the highest incidence (35.2%) followed by blight (19.87%) whereas powdery mildew was mild (5.4%) (Parvaze et al. 2009). The disease incidence of leaf blight and tuber rot were recorded at vegetative, flowering and harvesting stages of the crop growth, whereas umbel blight was recorded at flowering and harvesting stages at all the locations. Gupta et al. (2012) gave first report of tuber rot disease of Kalazira in 2008 caused by a member of the *Fusarium solani* species complex in India (Dar et al. 2014).

2. *Taxus baccata* L.

Common name: Common yew, English yew

Kingdom	Plantae
Order	Pinales
Family	Taxaceae
Genus	*Taxus*
Species	*T. baccata.*

2.1 Description

Taxus baccata L. is a native evergreen non-resinous gymnosperm tree up to 20(−28) m, often with multiple trunks and spreading, rounded or pyramidal canopy (Thomas and Polwart 2003). The yew is native to most of Europe, the Atlas Mountains and Asia Minor. In Europe, yew woodland can be found over most areas, but it grows best in oceanic climates with moderate temperatures (Thomas and Polwart 2003). Its distribution is limited in northern Europe beyond Britain, Ireland and southern Scandinavia by low temperatures and waterlogging and in the south by drought and high temperature (Linares 2013; Farjon and Filer 2013). The yew tree is a highly toxic plant that has occasionally been used medicinally, mainly in the treatment of chest complaints. Modern research has shown that the plants contain the substance 'taxol' in their shoots. Taxol has shown exciting potential as an anti-cancer drug, particularly in the treatment of ovarian cancers. Unfortunately, the concentrations of taxol in this species are too low to be of much value commercially, though it is being used for research purposes (Bown 1995).

2.2 Some of the important fungal diseases

Seedlings of *T. baccata* can be killed by pathogenic fungi (Lewandowski et al. 1995). Stem canker has been seen in Switzerland (Hassler et al. 2004) and has been identified as the cause of death of some yews in Great Britain (Strouts and Winter 1994; Benham et al. 2016). The fungus *Cryptocline taxicola* is known to occur on needles of *T. baccata.* It has been shown that this fungi obviously possesses a large degree of parasitic ability on *T. baccata* (Wulf and Pehl 2002; Bukvayova 2007). Based on research conducted on the reasons of dying down of needles and branches of common yew (*Taxus baccata* L.), 34 fungal species on the diseased tissues of *T. baccata* were showed. The following fungi occurred most frequently in the community of the fungi isolated from the investigated material: *Phomopsis juniperivora*, *Alternaria alternata*, *Colletotrichum gloeosporioides* and *Zythiostroma pinastri* (Mirski 2008).

3. *Elaegnus angustifolia* L.

Common name: Russian olive, Oleaster

Kingdom	Plantae
Order	Rosales

Family	Elaeagnaceae
Genus	*Elaegnus*
Species	*E. angustifolia*.

3.1 Description

There are more than 90 species of the *Elaeagnus* found around the world which are mainly distributed in subtropical regions of Asia, Europe and some parts of North America. *Elaeagnus angustifolia* L. is a deciduous tree or large shrub which is commonly called Russian olive since its appearance resembles an olive tree with small reddish-brown, elliptic shaped fruits. Russian olive is a long lived tree (80–100 years) which grows rapidly up to 10 m in height and 30 cm in diameter and starts to fruit after 5–6 years. This tree can tolerate a wide range of harsh environmental conditions such as flood, severe drought, stony, sandy and high salinity or alkalinity of the soils. Different parts of *E. angustifolia* plant, especially the fruits and flowers, have been used traditionally in treating a variety of common illnesses such as nausea, cough, asthma, fever, jaundice, and diarrhea. The use of fruit powder and extract of *E. angustifolia* have shown to be effective in alleviating pain in patients with rheumatoid arthritis and also in reducing the healing time of wounds in injured person. In addition, some recent reports have indicated the anti-oxidant, anti-inflammatory, antimicrobial, anticancer and some other properties of oleaster plant. The other important property of this plant would be its role in bio-monitoring the environment for some toxic elements and also its action as a bio-fertilizer agent in distressed lands. It seems that with more advanced studies on *E. angustifolia* and its bioactive components, this plant might be potentially effective and can be used as a natural alternative resource in pharmaceutical industries for treating chronic and serious problems (Fonia et al. 2009; Taheri et al. 2010; Kiseleva and chindyaeva 2011; Asadiar et al. 2013; Sahan et al. 2013; Saboonchian et al. 2014; Hamidpour et al. 2017).

3.2 Some of the important fungal diseases

Several canker diseases attack the branches and trunk of *E. angustifolia*, particularly in the humid east. The leaves on infected branches turn brown and persist on the tree. Usually, only the branch tip is affected. One of the most serious diseases of Russian olive is a canker and dieback caused by the fungus *Phomopsis elaeagni* (formerly called *Fusicoccum elaeagni*). Unlike most canker-producing fungi, *P. elaeagni* is an aggressive pathogen that can attack vigorous trees. Phomopsis-infected trees often appear ragged, with several dying or dead twigs and branches. Current-season twigs and small branches often wilt and die. The dead, withered leaves remain attached for some time. An examination of the large branches and trunks may eveal oval-to-elongate, depressed cankers of varying size, mostly 1–6 inches long. The diseased bark on cankers varies from orange-brown to dark reddish-brown. Ridges often form around the margins. Cankers on branches under an inch in diameter usually are not noticeably sunken or as obvious as the larger ones. The bark remains smooth and turns reddish-brown with dark brown margins. Branches girdled by the enlarging cankers wilt and die. The white sapwood underneath the cankers turns brown or black. The discoloration often extends beyond the margins of the canker (https://ipm.illinois.edu/diseases/rpds/606.pdf). *Cytospora* species are important phytopathogens causing severe canker disease with a worldwide distribution and broad host range. Canker productive species including *Cytospora elaeagni*, *C. hippophaes* and *C. nivea* have been recorded from *E. angustifolia* (Leifan et al. 2015). The most common disease is verticillium wilt, caused by fungi that live in the soil (*Verticillium albo-atrum, Verticillium dahliae*). They invade the tree through wounds in the roots and are then carried by the sap through the entire vascular system. They gradually block the conductive vessels and cause the leaves to wilt suddenly; the twigs and branches then dry out (http://espacepourlavie.ca/en/diseased-russian-olive-trees).

4. *Hippophae rhamnoides* L.

Common name: Sea buckthorn, Seaberry

Kingdom	Plantae
Order	Rosids
Family	Elaeagnaceae
Genus	*Hippophae*
Species	*H. rhamnoides*.

4.1 Description

Hippophae rhamnoides L. a unique and valuable plant has recently gained worldwide attention, mainly for its medicinal and nutritional potential. Sea buckthorn (SBT) is a thorny nitrogen-fixing deciduous shrub of cold arid region native to Europe and Asia. It is currently domesticated in several parts of the world due to its nutritional and medicinal properties (Rousi 1971; Li 2003; Suryakumar and Gupta 2011). Currently, SBT has gained the status of one of the most sought after plant in the pharmaceutical and cosmetic based industries, besides health food processing industries the world over (Suryakumar and Gupta 2011).

4.2 Some of the important fungal diseases

Among the diseases that can occur in sea buckthorn plantation we mention: verticillium wilt (*Verticillium albo—atrum*, *V. dahliae*), fusariosis (*Fusarium* sp.) and decay caused by *Phytium*, *Alternaria* and *Botrytis*. Both in Asia and in Europe, wilt disease are the most important causes of damage of SBT (Ruan et al. 2013; Coutuna et al. 2014). *Verticillium* wilt can occur quite often in sea buckthorn plantations, the pathogen *Verticillium* sp. being dangerous and able to kill the shrubs during two years. SBT is one of its favourite hosts. Often *Verticillium* sp. can be confounded with the damages produced by other diseases or by the unfavourable environmental conditions that can have identical symptoms (Ash-Cynthia 2013). After the entering in the plant tissues the fungus produces toxins and invades the xylem and then continuates the advancing in the plant through the produced spores. The toxins produced by *Verticillium* can close the plant cells at some distance from those directly invaded. From this reason the fungus cannot be isolated many times from the apex of the diseased shoots or from wilted branches, even the deterioration is very evident (Coutuna et al. 2014). Also *Fusarium* and *Pythium* diseases can be important diseases at the seedling stage of (www.Worldagroforestry.org/treedb/AFTPDFS/Hippophae_rhamnoides.PDF).

5. *Colchicum luteum* Baker.

Common name: Autumn crocus, meadow saffron

Kingdom	Plantae
Order	Liliales
Family	Colchicaceae
Genus	*Colchicum*
Species	*C. luteum.*

5.1 Description

Colchicum Luteum Baker. is known by the name of colchicicum in English, Suranjan in Sanskrit and Hirantutiya in Hindi. The corms of the plant are usually used to make natural medicines. *C. luteum* is known to be Kapha and Vata suppressant. These are the principle stabilizing energies that govern the body as well as the mind. It is connected with the structure, lubrication, fluid balance and stability of the entire human body. It is native to Europe, West Asia, parts of the Mediterranean coast, East Africa, South Africa and the region of Western Cape. This plant contains an alkaloid called colchicines. It also contains a significant amount of starch (http://www.alwaysayurveda.com/colchicum-luteum/).

5.2 Some of the important fungal diseases

The crop, in general, is not attacked by any insect. There is no disease infestation as well (NMPB 2008).

6. *Rhamnus frangula* L.

Common name: Glossy buckthorn, Fen Buckthorn, Alder Buckthorn

Kingdom	Plantae
Order	Rhamnales
Family	Rhamnaceae
Genus	*Rhamnus*
Species	*R. frangula.*

6.1 Description

Rhamnus frangula L. (*Frangula alnus* Mill.) is native to Europe, northernmost Africa, and western Asia, from Ireland and Great Britain north to the 68th parallel in Scandinavia, east to central Siberia and Xinjiang in western China, and south to northern Morocco, Turkey, and the Alborz and Caucasus Mountains, in the northwest of its range (Ireland, Scotland), it is rare and scattered. It is also introduced and naturalised in eastern North America. It is a deciduous shrub or small tree of wet or dry sites. In full sun plants may bear fruit in as little as 3 years from seed (Rushforth 1999; Possessky and Moriarity 2000; Zecchin et al. 2016). Buckthorn is used in many herbal medications, especially as a laxative, as a tonic and as an ingredient in the Hoxsey cancer formula. Alder buckthorn is high in anthraquinone glycosides. Resins, tannins, and lipids make up the bulk of the bark's other ingredients. Anthraquinone glycosides have a cathartic action, inducing the large intestine to increase its muscular contraction (peristalsis) and increasing water movement from the cells of the colon into the feces, resulting in strong, soft bowel movements (www.tipdisease.com/2014/11/benefits-of-alder-buckthorn-rhamnus.html).

6.2 Some of the important fungal diseases

The alder buckthorn is generally free from any significant pests and disease (Hemery and Simblet 2014). It can be a host in the life cycle of the crown rust fungus (*Puccinia coronata*), which affects oat and barley (Murphy 1935; Liu and Hambleton 2013). In southern Spain relict populations of *F. alnus* subsp. *baetica*, which occur at the limits of geographic range for this species, are in decline and threatened by increasing summer droughts (Hampe 2005). On the contrary, in North America this species is ranked as “highly invasive”, due to its tendency to replace native plants, and several prevention and control programmes have been started where dominant alder buckthorn can negatively affect native species richness, simplify vegetation structure, disrupt food webs and delay succession (Gucker 2008; Zecchin et al. 2016).

7. *Trigonella foenum-graceum*

Common name: Fenugreek; fenugrec (France), fieno greco (Italy) alholva, feno-greco (Spain), helba (Arabic), methi (India, Pakistan)

Kingdom	Plantae
Order	Fabales
Family	Fabaceae
Genus	*Trigonella*
Species	*T. foenum-graceum.*

7.1 Description

Fenugreek (*Trigonella foenum-graecum*) is a legume and it has been used as a spice throughout the world to enhance the sensory quality of foods. It is known for its medicinal qualities such as antidiabetic, anticarcinogenic, hypocholesterolemic, antioxidant, and immunological activities (Wani and Kumar 2016). This plant is cultivated in India, the Mediterranean region, China, parts of Africa, Europe and Australia and, in recent years, in North America (Acharya et al. 2010). The leaves and seeds of the Fenugreek plant are widely used in Indian cuisine. The herb has been traditionally used in Ayurvedic compositions as a moisturizer, specially formulated to hydrate dry skin. Applied topically, the herb is also helpful in treating boils, abscesses and ulcers. Fenugreek is a natural source of protein, which rebuilds and strengthens the hair shaft. It reduces hair fall and promotes hair growth. Fenugreek seeds function as an appetite suppressant and give a feeling of satiety more quickly. The German Commission E documented its mild antiseptic property. The British Herbal Pharmacopoeia reported that the herb's actions as demulcent and hypoglycemic (lowering blood sugar levels). ESCOP and the WHO monographs indicate the use of the seeds as an adjuvant in therapies for diabetes mellitus, anorexia, and high cholesterol (Khare 2007).

7.2 Some of the important fungal diseases

Diseases caused by fungus are one of the major constraints to the production of fenugreek in field condition. The yield of the plant is seriously affected by the fungal diseases mostly the seed borne ones. The two major fungal diseases that affect fenugreek are powdery mildew caused by *Erysiphe polygoni, Oidipsis* sp. and *Leveillula taurica* and Cercospora leaf spot caused by *Cercospora traversiana*. This despite the fact that Cercospora leaf spot is a destructive and widespread disease of fenugreek. The disease is devastating in nature spreading quite uniformly in the growing areas in absence of specific control measures. In case of severely infected plants only a few terminal leaves survive. The size of the lesions on mature leaves increases resulting in rapid sporulation resembling a whitish dorsal surface. The necrotic areas are characterized by the presence of an encircling yellowish halo. Most severe infections are observed in the stem and the pods. Infected areas of the pod are characterized by loss of color with most seriously infected areas are found to under go structural deformation. In case of severe infection even the youngest leaves have been reported to be wilted and dead (Zimmer 1984; Acharya et al. 2010, 2014; Prasad et al. 2014). Being among one of the most common and serious diseases of fenugreek, the powdery mildew, affects both biomass and yield (Basu et al. 2006; Petropoulos 2002) of the plant under moist agro-climatic conditions in north America, such as Creston in British Columbia (Canada) and Vermont (USA). It is most commonly found in hot and humid tropical and sub-tropical areas, as well as in temperate to subtemperate regions (Basu et al. 2006; Palti 1959; Rouk and Mangesha 1963). Fenugreek plants infected by *Oidiopsis* sp. showed slightly blister like areas on the young leaves which soon becomes covered with white to grey powdery mass. Powdery mildew caused by *L. taurica* is identified by mycelial patches on leaves and is favored by comparatively dry weather conditions (Petropoulos 1973). The collar rot or the root rot or the foot rot or the damping off of fenugreek by *Rhizoctonia solani* is another important disease of the plant which reduces the yield of the plant significantly. It has been reported from all parts of India (Hiremath and Prasad 1985). Wilt of fenugreek by *Fusarium oxysporum* is one of the most important disease causing moderate to extensive damage to the crop. The pathogen remains present both in soil as well as in seeds of fenugreek (Bansal and Gupta 2000; Hashmi and Thrane 1990; Komaraiah and Reddy 1986; Pierre and Francis 2000; Khare et al. 2014). *Ascochyta* leaf spot is a serious afflicting fenugreek (Petropoulos 1973). It is another important seed borne disease of fenugreek (Petropoulos 2002; Walker 1952).

8. *Conium maculatum* L.

Common name: Poison Hemlock, Hemlock, Devil's porridge, Beaver Poison, Herb Bennet.

Kingdom	Plantae
Order	Apiales
Family	Apiaceae
Genus	*Conium*
Species	*Conium maculatum.*

8.1 Description

Conium maculatum is one of the most toxic members of the plant kingdom. There are numerous reports of deaths for a wide range of animal species including humans. *C. maculatum* has a worldwide distribution (Vetter 2004). The plant is reported as a very common weed in Europe, North and South America, North Africa, Australia and New Zealand, and there are data about its occurrence in Ethiopia (Mekkonen 1994), and in Pakistan (Ahmed et al. 1989). Poison hemlock contains five alkaloids that are toxic to humans and livestock if ingested and can be lethal. All parts of the plants contain the toxic alkaloids with levels being variable throughout the year. Symptoms of toxicity include nervousness, trembling, and loss of coordination followed by depression, coma, and/or death. Initial symptoms will occur within a few hours of ingestion. Cases of poisoning due to poison hemlock ingestion are rare as the plants emit a mousy odor that makes it undesirable and unpalatable to livestock and humans. Consumption and toxicity in animals usually occurs in poorly managed or overgrazed pastures where animals are forced to graze poison hemlock (www.btny.purdue.edu/weedscience).

8.2 Some of the important fungal diseases

In 2008, a powdery mildew was observed on poison-hemlock growing in coastal (Monterey and Santa Clara counties) California. The causal agent was determined to be *Erysiphe heraclei* DC., previously unreported on this host in North America. Signs of the pathogen included effuse patches of white mycelia on adaxial leaf surfaces of older foliage, leaf petioles, flower pedicels, and immature seed capsules of wayside plants. Disease was widespread on poison-hemlock plants that were surveyed in both counties, with disease incidence averaging 33%. The hemlock is infected often by one or more virus strains such as ringspot virus, carrot thin leaf virus (CTLV), alfalfa mosaic virus (AMV) or celery mosaic virus (CeMV) and powdery mildew did not appear to cause obvious damage to this host (Howell and Mink 1981; Koike and Glawe 2009).

9. *Anethum graveolens* L.

Common name: Dill

Kingdom	Plantae
Order	Apiales
Family	Apiaceae
Genus	*Anethum*
Species	*A. graveolens*.

9.1 Description

Anethum graveolens L. contained essential oils, fatty oil, proteins, carbohydrates, furanocoumarin, polyphenols, mineral and many other biologically active constituents. It is widely used traditionally. The pharmacological studies showed that *A. graveolens* induced antimicrobial, antiinflammatory, analgesic, gastric mucosal protective and antisecretory effects, smooth muscle relaxant effect, hyperlipidaemic, increased progesterone concentration, and many other effects. This plant is a plant with wide range of chemical constituents which exerted many pharmacological effects. There is a great promise for development of novel drugs from *A. graveolens* to treat human diseases as a result of its effectiveness and safety (Al-Snafi 2014).

9.2 Some of the important fungal diseases

From 2010 to 2014, typical symptoms of powdery mildew were observed on dill crops (*A. graveolens*, Apiaceae) in several greenhouses in Apulia (southern Italy). The disease appeared at all stages of growth, from transplanted seedlings to harvest. If not controlled promptly, a white fungal efflorescence spread progressively from the basal leaves and petioles to the whole plant with disease incidence and severity often reaching 100% that Based on the morphology of the conidia and sequence homology, the isolated fungus was identified as the anamorph of *E. heraclei* (Bubici 2015). Cercospora leaf blight by *Cercosporidium punctum* is another fungal diseases of dill. Small, necrotic flecks on leaves which develop a chlorotic halo and expand into tan brown necrotic spots; lesions coalesce and cause leaves to wither, curl and die is the symptoms of the fungus. Other plant diseases of dill include: damping-off (*Pythium* spp. and *Rhizoctonia solani*) and *Peronospora umbellifarum* (http://www.cabi.org/cpc/datasheet/3472).

10. *Capsicum annuum* L.

Common name: There are more than 200 common names in use for this species. The most common include chilli pepper, paprika (sweet varieties); bell pepper, cayenne, halapenos, chitlepin (hot varieties); and Christmas peppers (ornamental).

Kingdom	Plantae
Order	Solanales

Family	Solanaceae
Genus	*Capsicum*
Species	*C. annuum*.

10.1 Description

Capsicum annuum L. is a species of the plant genus *Capsicum* native to southern North America and northern South America (GRIN 2010; Latham 2009). This species is the most common and extensively cultivated of the five domesticated capsicums. The species encompasses a wide variety of shapes and sizes of peppers, both mild and hot, ranging from bell peppers to chili peppers (Francis 2003).

10.2 Some of the important fungal diseases

Diseases caused by *Colletotrichum gloeosporioides*, *C. capsici*, *C. coccodes*, *Cercospora capsici*, *Macrophomina phaseolina*, *Choanephora cucurbitarum*, *Rhizoctonia solani*, *Phytophthora* spp., *Fusarium* spp., *Pythium* spp., *Peronospora tabacina*, *Fusarium solani*, *F. oxysporum* f.sp. *capsici*, *Stemphylium solani*, *S. lycopersici*, *Botrytis cinerea*, *Phytophthora capsici*, *Leveillula taurica*, *Sclerotium rolfsii*, *Verticillium albo-atrum*, *V. dahliae* and *Sclerotinia sclerotiorum* are the most important fungal diseases of *Capsicum* [(Common Names of Diseases, The American Phytopathological Society) (Pepper Diseases (Fact Sheets and Information Bulletins), The Cornell Plant Pathology Vegetable Disease Web Page)].

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Weed and Weeding Effects on Medicinal Herbs

Alessandra Carrubba

Abstract Competition with weeds exerts significant depressive effects on yield and quality features of Medicinal Plants (MPs). According to the crop, the part of plant to be harvested, the environmental features (including cropping technique) and the severity of infestation, yield losses due to the presence of weeds may vary within wide intervals. Furthermore, unlike the majority of other crops, MPs are cultivated with the goal to obtain relevant quantities of specific secondary metabolites, whose final quantity determines the quality level (and, consequently, the market value) of the harvested drug. Almost all papers addressed to this topic agree on the statement that unrestricted weed growth may alter MP production also from the qualitative point of view, that is, determining an overall decrease in the yield of active substances for unit area. In part, this outcome can be attributed to the general decrease of harvestable biomass, but in some cases also modifications of crop metabolic pathways have been observed, resulting in a general unpredictability of the chemical characteristics of the product obtained in weedy fields. Competition with weeds may assume a different severity according to the time and duration of competition period. In the starting phases of cultivation, the outcome of an early weed infestation is expected to be severe, since very often weeds grow much faster than crops. The maximum tolerance period, i.e. the period when weeding operations must be started, varies according to the tolerated loss values, and in annual crops the time span when fields must be kept totally weed-free may cover more than 60% of the entire crop cycle. The tools that are used for weeds removal may affect MPs production in many ways. Chemical treatments have been studied with contrasting results, but an interference of herbicides with the metabolism of secondary products was found in some cases. Furthermore, the interest in growing MPs with organic or environmentally friendly methods is increasing. Hence, besides the traditional (and highly expensive) method of hand-weeding, other non-chemical methods are studied, including mechanical treatments, mulching, flaming, and even grazing by goats or lambs. There is scope for further research, embracing a larger number of

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MPs and different environments, also including the effects of weeds on MPs metabolic pathways.

Keywords Competition · Yields · Essential oil (EO) · Weeding management · Critical period for weed control (CPWD) · Relative losses (RL)

Introduction

Although a role in maintaining sustainability of agro-ecosystems (providing for example habitat for beneficial insects, or enhancing the overall biodiversity level of farm) is today acknowledged to weeds (Kristiansen et al. 2001), they are mostly considered a major issue for all crops. Their presence in cultivated fields is not only an obstacle to mechanization and proper field management, but is also responsible for yield shortage and decreased quality. It is generally assumed that injuries caused by weeds are tightly dependant upon many factors, including the severity of weed infestation (i.e. the total amount of weeds retrievable in cultivated fields) and its duration (i.e. the time span of crop-weed interference). In many "major" crops, such as wheat, maize or cotton, much research has been done to evaluate the effects exerted by competing weeds, and many aspects of the crop-weed relationships have been examined. Both quantitative yield losses and effects on quality have been largely studied, and much information is available about weed effects within a range of different conditions, i.e. with variable duration of competition, contrasting nutrient availability, and so on. Otherwise, in Medicinal Plants (MPs), research about this topic is sparse, also due to some very specific features of these crops. A first important issue is linked to what exactly is termed "yield": whereas in some MPs the whole plant is harvested, in others only a special plant part (very commonly the reproductive fraction) has a commercial value. In either cases, the outcome of weed interference will be dramatically different, as well as the tactics and tools to minimize yield shortage.

The aspects concerning quality issues are even less argumented; weeds are generally reputed to be responsible for a reduction in harvested crop quality, but apart from this very general statement, scarce attention has been devoted to evaluate in detail the effects of competing weeds on the various plant traits that concur in forming crop quality. In MPs this issue may be crucial, because they owe their economical importance to the presence of special plant metabolites, whose production and storage may be affected by weed competition in an unpredictable way. In this paper, an attempt is made to collect information available from the worldwide literature on the effects of weeds on MPs.

Medicinal Plants and Weeds

There is general agreement about the necessity to remove weeds from cultivated stands of MPs, and almost all technical papers providing indications for cultivation clearly state that MPs fields must be kept weed free as much, and as long, as possible. De la Fuente et al. (2003) demonstrated that on biomass and seeds yield of coriander, especially under poor soil conditions, weeding had a greater effect than did N fertilization. Furthermore, the absence of weeds from herbal products is mandatory in order to declare their high quality, irrespective whether they come from cultivation or wild collecting (FAH 2003). Yet, a surprisingly reduced number of works have been expressely addressed to the evaluation of the effects that weeds may exert on MPs. Studies about this topic are scattered in the world literature, and very rarely the argument is treated in detail.

MPs have the distinguishing property to be graded by the market according to their content in active components, i.e., the special metabolites that confer to them their medicinal properties. In many cases, these metabolites are synthesized by plants under environmental stress conditions; as competition with weeds is a special and often severe cause of stress, it should be argued that the best conditions for producing medicinal metabolites would be under weedy conditions. Actually, experiments all over the world have demonstrated that this is seldom true, and MPs response to weeds is widely variable according to crop species, metabolites of interest, but also environmental conditions, and characteristics (severity, duration, and composition) of weed population. It is widely recognised that a proper cultivation technique, by inducing modifications in MPs' growth environment, may lead to significant variations of plant secondary metabolism, and therefore may be a useful tool to tailor the content of some specially welcomed active compounds (Palevitch 1991; Canter et al. 2005). Of course, this approach needs a in-depth knowledge of mechanisms underlying secondary metabolites production, and it requires as well an exact view of the scope of the cultivation. Technical management, in fact, will be different whether the cultivation is addressed to obtain plant biomass, or some special part of it, and it should be clear as well which compound, or mixture of compounds, will be of marketable interest. For these reasons, the topic of weed and weeding effects on medicinal plants will be treated taking into consideration first, the overall effects of competition on the productive behaviour of medicinal plants, and then, the various outcomes of weeds in such crops, discussing separately (whenever possible) the effects on the whole plant, those on yield components, and the effects on the secondary metabolites.

Effects of Intra- and Interspecific Competition in Medicinal Plants

As known, intra-specific competition is due to the simultaneous presence of many individuals of the same species, whereas inter-specific competition occurs when two or more species grow together. The first is the case of usual monoculture, where the yield per area unit is obtained by the simple combination between the average yield of one plant and the value of plant population. In most cases, the average yield per plant is inversely correlated to plant density: the higher the plant population, the lower the unitary plant mass, and vice versa (Weiner and Freckleton 2010). Many studies about the effect of plant population on biomass yield of MPs have confirmed this general trend, and this effect was shown to be achieved by means of a number of morphological adjustments. Plants of *Salvia officinalis* were smaller at high densities, because of a lower number of leaves per plant and a reduced leaf size (Bezzi et al. 1992; Karamanos 2000); in *Artemisia absinthium* a lower density was accompanied by a higher unitary plant mass, as plants were taller and more branched (Maw et al. 1985); in *Asparagus racemosus*, Tewari and Misra (1996) measured a higher fresh weight and number of roots per plant under wider spacings; fennel (*Foeniculum vulgare* Mill) exhibited a higher number of ramification and umbels per plant when sown at wider spacings (Al-Dalain et al. 2012). Hence, provided there is a satisfactory level of water and nutrients in the soil, a high number of plants per unit area seems to be compensated for by the reduction of unitary plant mass, and the maximum areic yield is obtained when any increase in plants density is exactly counterbalanced by a decrease in unitary plant mass. In some cases, however, other mechanisms take some relevance. One of these is self-thinning, that is a self-arrangement mechanism that has been recorded in several plants, including some MPs. In this case plant populations, irrespective of their starting values, tend spontaneously to converge to a common value, whose level is mostly depending upon resource availability. For example in *A. absinthium*, after the first year of cultivation, different plant stands were shown to converge to a density of 20 plants per 625 cm^2 (Maw et al. 1985).

When the harvestable yield is not the entire plant biomass, but only a part of it (flowers, or seeds, or roots), the outcome of the relationship between yield and density starts to be influenced by the intra-plant competition for the allocation of resources, i.e. the competition between the different organs of the same plant. In this case, the optimum plant population level will be different (and often lower) than the one indicated for maximizing the whole plant biomass. A number of works have been carried out which put in relation seeding rate or plant population, by one side, and marketable yields, by the other. In same examples, such as *Nigella sativa* (Moghaddam and Motlagh 2007) or *Cuminum cyminum* (Shakeri et al. 2015), similarly to single-plant biomass, also seeds yield per plant was found to decrease with increasing plant density. When the total seeds yield for unit area is taken into account, however, very commonly a linear increase is observed until a specific density level, and once it is reached, seeds yield starts decreasing (Falzari et al. 2006; Shakeri et al. 2015).

An additional feature that is typical of MPs, is that in such plants, cultivation is addressed to obtain relevant quantities of specific plant compounds, or mixtures of compounds. Being affected by all those environmental factors that variously influence plant growth, also the production of plant secondary metabolites may be modified by competition effects. These effects do not necessarily have the same trend of the raw matter that they are extracted from, and farmers may face the necessity to choose if the desired product is represented by essential oil or by herbal product. Several researches have taken into consideration the effects of intra- and inter- specific competition on the yield and composition of secondary metabolites from MPs. In plants where the entire biomass is harvested, such as oregano (*Origanum vulgare* L.), a decrease in essential oil yield was detected as plant density was increasing, although such a decrease had probably to be attributed to the higher percentage of stems and woody parts (having a lower essential oil content) in the harvested material (Scarpa et al. 2004). Otherwise in basil (*Ocimum basilicum L.*), the increased intraspecific competition due to a higher plant population induced a higher essential oil production; hence, a lower density was considered more suitable to obtain fresh herb than essential oil (Putievsky and Galambosi 1999). In *Thymus vulgaris* L., the wider the spacing, the greater were the yields of essential oil per plant; however, closer spacing significantly increased the yield of oil per unit area (Shalby and Razin 1992).

In species where the essential oil is obtained from harvested seeds, contrasting results may be found in the literature about the effect of plant population on essential oil content. EO percentage resulted unaffected in *Carum copticum* (Ghilavizadeh et al. 2013), whereas in fennel (*Foeniculum vulgare* Mill), the maximum essential oil percentage was obtained with the minimum plant density, and vice versa (Khorshidi et al. 2009). However, things may change when essential oil areic yields are considered, due to the modifications of plant harvestable biomass (i.e. the reproductive fractions), that may be detected with varying plants density. In fennel, some variations of essential oil areic yields were attributed to the different incidence of primary, secondary and tertiary umbels (dealing with different percentages of essential oil) in the harvested biomass (Falzari et al. 2006). The same occurrence was thought to be responsible for the differences in some essential oil components that were found by the same Author: since trans-anethole content increases with umbel maturity, a higher number of high-order, immature umbels (as obtained with wider spacings) would reasonably have been the cause for a decrease in total trans-anethole yield. This could probably explain the variability also found by other Authors (Khorshidi et al. 2009) in some components of essential oil of fennel grown with diverse plant densities. As concerns other species, different spacings did not influence chemical components of silymarin (taxifolin, silychristin etc.) in *Silybum marianum* Gaertn (Belitz and Sams 2007).

Inter-specific competition is due to the contemporary presence of two species (or more). Within a certain limit, and with a number of exceptions, the effects of inter-specific competition do not differ from those of intra-specific one, and yield of plants grown in mixture may be different from yield of plants grown alone. This phenomenon is exploited when MPs are grown in intercropping mixtures, including either other MPs or different crops, as experienced for fennel and dill (Carrubba et al. 2008), liquorice and cereals (De Mastro et al. 1993), fenugreek and anise (Mardani et al. 2015), coriander and fenugreek (Pouryousef et al. 2015), chickpea and black cumin (Gholinezhad and Rezaei Chiyaneh 2015). However, the mechanisms underlying, and the outcome of cultivation, are probably more complex than in the intra-specific case. Hence to choose the best-yielding intercropping mixture is not a simple task, since the ratio between intercropped plants must be finely tuned according to the species and the environmental conditions, and even when sowing rates and crop management are carefully planned, the outcome of cultivation may be extremely variable.

Weed Population in Medicinal Plants

Since the works about weeds and medicinal plants are not many, also few are the available lists concerning weeds retrieved under the different cropping conditions. Table 1 summarizes the information (name and family, growth habitus-annual, biennial or perennial- and Raunkiaer's life form) collected by means of a search in the literature about weeds retrieved on MPs cultivations. One hundred and six weed species, belonging to 29 botanical family are listed. As expected, weed communities vary with the geographical site of cultivation and with the farming practices in use in each region. No species seems to be "typical" of a certain MP, but some of them are ubiquitous, such as *Amaranthus retroflexus*, *Convolvulus arvensis* and *Chenopodium album*, that have been detected in different environments and cropping conditions. As shown, the majority of retrieved weeds are annual species, whereas fewer are biennials and 26 (about 25%) are perennials. The analysis of the life form spectrum shows the sharp predominance of Therophytes: 66 out of 106 species, i.e. more than 62%, belong to this group, and 9 more may occasionally behave also as Hemicryptophytes or Chamaephytes. Therophytes are followed by Hemicryptophytes (14, i.e. 13% of all retrieved species), and Geophytes (12, i.e. about 11%). Although an excessive generalization would be misleading, due to the huge differences among environments and cropping situations involved, all data converge towards the definition of a general frame, where the level of disturbance is relatively low, compared to that of more intensive and productive cropping systems. As a matter of fact, cropping techniques are a crucial factor in the establishment of weed population (Martínez-Ghersa et al. 2000). In horticultural systems, where crop management is intensive and rotational schemes are narrow, annuals are usually the predominant weeds and Therophytes may reach an incidence close to 100% (Zanin et al. 2001). Interestingly, data from Table 1 reveal instead an overall tendency

Table 1 Botanical names, growth habitus and Raunkiaer's life form of some weeds retrieved in several surveys about MPs. Denominations of taxa were checked and updated according to Euro + Med PlantBase (www.bgbm.org), USDA-NRCS (plants.usda.gov), US-NPGS (npgsweb.ars-grin.gov), TROPICOS (tropicos.org). Life forms were assigned after Ellenberg and Mueller-Dombois (1965–1966) and Pignatti (1982)

		Hab[a]	LF[b]	Anethum graveolens L. (1)	Apiaceae (all) (2)	Cymbopogon winterianus Jowitt.; C. flexuosus (Nees ex Steud.) Wats.; C. martinii (Roxb.) Wats. var. motia Burk.; Ocimum basilicum L. (3)	Curcuma longa L. (4)	Foeniculum vulgare Mill (5, 6, 7)	Hypericum perforatum L. (8)	Mentha arvensis (9)	Muscari comosum (L.) Mill (10)
Amaranthaceae Juss.	Achyranthes aspera L.	P	Ch suffr			X					
	Aerva lanata (L.) Schult.	A	Th			X					
	Amaranthus blitoides S. Wats.	A	Th					X			
	Amaranthus retroflexus L.	A	Th					XX			
	Amaranthus spinosus L.	A	Th				X				
	Amaranthus viridis L.	A	Th				X				
	Celosia argentea L.	A	Th			X					
Apiaceae Lindl.	Apium leptophyllum (Pers.) F.Mueller	A	Th								
	Bowlesia incana Ruiz & Pov.	A	Th								
Apocynaceae Juss.	Catharanthus roseus (L.) G. Don.	P	Ch suffr			X					

(continued)

Table 1 (continued)

		Hab[a]	LF[b]	Anethum graveolens L. (1)	Apiaceae (all) (2)	Cymbopogon winterianus Jowitt.; C. flexuosus (Nees ex Steud.) Wats.; C. martinii (Roxb.) Wats. var. motia Burk.; Ocimum basilicum L. (3)	Curcuma longa L. (4)	Foeniculum vulgare Mill (5, 6, 7)	Hypericum perforatum L. (8)	Mentha arvensis (9)	Muscari comosum (L.) Mill (10)
Brassicaceae Burnett	Capsella bursa-pastoris (L.) Medik.	B	H								
	Coronopus didymus (L.) Sm.	A	Th								
	Descurainia sophia (L.) Prantl.	A	Th								
	Diplotaxis tenuifolia (L.) DC	P	H scap	X							
	Eruca vesicaria (L.) Cav.	A	Th								
	Goldbachia laevigata (M. Bieb.) DC.	A	Th					X			
	Lepidium virginicum L.	A	Th						X		
	Raphanus sativus L.	A	Th								
	Sinapis alba (L.) Spach	A	Th								
	Sisymbrium irio L.	A	Th					X			
Caryophyllaceae Juss	Arenaria serpyllifolia L	A	Th							X	
	Polycarpaea corymbosa (L.) Lam.	A	Th								
	Stellaria graminea L.	P	H scap								
	Stellaria media (L.) Villars.	A	Th								X
Chenopodiaceae Vent.	Chenopodium album L.	A	Th				X	XXX			
	Salsola kali L.	A	Th					X			

(continued)

Table 1 (continued)

		Hab[a]	LF[b]	Anethum graveolens L. (1)	Apiaceae (all) (2)	Cymbopogon winterianus Jowitt.; C. flexuosus (Nees ex Steud.) Wats.; C. martinii (Roxb.) Wats. var. motia Burk.; Ocimum basilicum L. (3)	Curcuma longa L. (4)	Foeniculum vulgare Mill (5, 6, 7)	Hypericum perforatum L. (8)	Mentha arvensis (9)	Muscari comosum (L.) Mill (10)
Compositae Giseke	Bidens pilosa L.	A	Th				X				
	Carduus nutans L.	A or B	Th/H bienn								
	Cirsium arvense (L.) Scop.	P	G rad								X
	Glebionis segeta (L.) Fourr. = Chrysanthemum segetum L	A	Th	X							
	Helminthotheca echioides (L.) Holub	A	Th								
	Launaea nudicaulis (L.) Hook. f.	P	H								
	Matricaria spp.	A	Th		X						
	Parthenium hysterophorus L.	A	Th			X					
	Sonchus arvensis L.	P	H								
	Sonchus asper (L.) Hill	A, somet. B	Th/H bienn		X		X				
	Sonchus oleraceus L.	A	Th				X	X			
	Tragopogon spp.	A or P	Th/H					X			
	Tridax procumbens L.	P	Ch			X					
	Tussilago farfara L.	P	G rhiz		X						
	Xanthium strumarium L.	A	Th					X			

(continued)

Table 1 (continued)

		Hab[a]	LF[b]	Anethum graveolens L. (1)	Apiaceae (all) (2)	Cymbopogon winterianus Jowitt.; C. flexuosus (Nees ex Steud.) Wats.; C. martinii (Roxb.) Wats. var. motia Burk.; Ocimum basilicum L. (3)	Curcuma longa L. (4)	Foeniculum vulgare Mill (5, 6, 7)	Hypericum perforatum L. (8)	Mentha arvensis (9)	Muscari comosum (L.) Mill (10)
Convolvulaceae	Convolvulus arvensis L.	P	G rhiz	X				XX			
	Evolvulus alsinoides L.	P	H			X					
Cyperaceae Juss.	Cyperus rotundus L.	P	G rhiz				X			X	
Equisetaceae DC	Equisetum palustre L.	P	G rhiz								
Euphorbiaceae Juss.	Acalypha australis L.	A	Th				X				
	Euphorbia hirta L.	A, somet. P	Th			X					
Fabaceae Lindl.	Acacia farnesiana (L.) Willd. = Mimosa indica L.	P	P caesp				X				
	Glycyrrhiza aspera Pall.	P	G rhiz					X			
	Melilotus indicus (L.) All.	A	Th							X	
	Melilotus albus Medik.	A or B	Th								
	Tephrosia purpurea (L.) Pers.	A or P	Ch			X					
Geraniaceae Juss.	Erodium cicutarium (L.) L'Hér.	A, somet. B or P	Th/H ros					X			
	Erodium ciconium (L.) L'Hér.	A or B	Th/H bienn								

(continued)

Table 1 (continued)

		Hab[a]	LF[b]	Anethum graveolens L. (1)	Apiaceae (all) (2)	Cymbopogon winterianus Jowitt.; C. flexuosus (Nees ex Steud.) Wats.; C. martinii (Roxb.) Wats. var. motia Burk.; Ocimum basilicum L. (3)	Curcuma longa L. (4)	Foeniculum vulgare Mill (5, 6, 7)	Hypericum perforatum L. (8)	Mentha arvensis (9)	Muscari comosum (L.) Mill (10)
Lamiaceae Lindl.	Hyptis suaveolens (L.) Poit. = Mesosphaerum suaveolens (L.) Kuntze	A or P	Th			X					
	Lamium amplexicaule L.	A	Th								
	Lamium purpureum L.	A	Th								X
	Leonurus cardiaca L.	P	H scap								
Malvaceae Juss.	Abutilon theophrasti Medik.	A	Th								
	Malva neglecta Wallr.	A	Th					X			
	Malva sylvestris L	P, rar A	H scap/Th								
Nyctaginaceae Juss.	Boerhavia diffusa L.	A, rar P	Th/Ch rept			X					
Oxalidaceae	Oxalis corymbosa DC	P	G bulb				X				
Papaveraceae Juss.	Argemone mexicana L.	A	Ch								
	Fumaria officinalis L.	A	Th								X
	Papaver rhoeas L.	A	Th								X
Phyllanthaceae	Phyllanthus amarus Schum. et Thonn.	A	Th/Ch			X					
	Phyllanthus niruri L.	A	Th								

(continued)

Table 1 (continued)

		Hab[a]	LF[b]	Anethum graveolens L. (1)	Apiaceae (all) (2)	Cymbopogon winterianus Jowitt.; C. flexuosus (Nees ex Steud.) Wats.; C. martinii (Roxb.) Wats. var. motia Burk.; Ocimum basilicum L. (3)	Curcuma longa L. (4)	Foeniculum vulgare Mill (5, 6, 7)	Hypericum perforatum L. (8)	Mentha arvensis (9)	Muscari comosum (L.) Mill (10)
Poaceae Barnhart	Aristida adscensionis L.	P	H caesp			X					
	Avena fatua L.	A	Th	X	X						
	Chloris barbata Sw	A or P	H caesp			X					
	Cynodon dactylon (L.) Pers.	P	G rhiz/H rept			X					
	Digitaria ciliaris (Retz.) Koeler	A	Th				X				
	Digitaria ischaemum (Schreb.) Muhl.	A	Th							X	
	Digitaria radicosa (J.Presl.)Miq. = D. timorensis (Kunth) Balansa	A	Th				X				
	Digitaria sanguinalis (L.) Scop.	A	Th						X		
	Echinochloa crus-galli L. (P.Beauv.)	A	Th					XX	X		
	Eleusine indica (L.) Gaertn.	A	Th				X			X	

(continued)

Table 1 (continued)

		Hab[a]	LF[b]	Anethum graveolens L. (1)	Apiaceae (all) (2)	Cymbopogon winterianus Jowitt.; C. flexuosus (Nees ex Steud.) Wats.; C. martinii (Roxb.) Wats. var. motia Burk.; Ocimum basilicum L. (3)	Curcuma longa L. (4)	Foeniculum vulgare Mill (5, 6, 7)	Hypericum perforatum L. (8)	Mentha arvensis (9)	Muscari comosum (L.) Mill (10)
	Elytrigia repens (L.) Nevski = Elymus repens (L.) Gould	P	G rhiz/H caesp		X						
	Lolium multiflorum Lam.	A or P	Th/H scap	X							X
	Ochlopoa annua (L.) H. Scholz = Poa annua L.	A	Th							X	
	Panicum capillare L.	A	Th						X		
	Panicum repens L.	P	G rhiz				X				
	Paspalum distichum L.	P	G rhiz				X				
	Phalaris spp.	A	Th					X			
	Rottboellia exaltata L. f. = R. cochinchinensis (Lour.) W.D. Clayton	A	Th				X				
	Setaria viridis (L.) P. Beauv.	A	Th					XX	X		
	Setaria pumila (Poir.) Roem.& Schult. = Setaria glauca L.	A	Th						X		
	Setaria faberi Herrm.	A	Th								
	Sorghum halepense (L.) Pers.	P	G rhiz								

(continued)

Table 1 (continued)

		Hab[a]	LF[b]	Anethum graveolens L. (1)	Apiaceae (all) (2)	Cymbopogon winterianus Jowitt.; C. flexuosus (Nees ex Steud.) Wats.; C. martinii (Roxb.) Wats. var. motia Burk.; Ocimum basilicum L. (3)	Curcuma longa L. (4)	Foeniculum vulgare Mill (5, 6, 7)	Hypericum perforatum L. (8)	Mentha arvensis (9)	Muscari comosum (L.) Mill (10)
Polygonaceae	Polygonum aviculare L.	A	Th		X						
	Polygonum convolvulus L.	A	Th								
	Rumex crispus L.	P	H scap					X			
Portulacaceae Juss.	Portulaca oleracea L.	A	Th						X		
Primulaceae Vent.	Anagallis arvensis L.	A	Th					X			
Resedaceae Gray	Reseda luteola L.	B, rar P or A	H scap/Th		X						
Rubiaceae	Galium aparine L.	A	Th								
Scrophulariaceae Juss.	Veronica hederifolia L.	A	Th								X
	Veronica persica Poir.	A	Th								
Solanaceae	Datura metel L.	A	Th			X					
	Datura stramonium L.	A	Th								
	Physalis subglabrata Mack. and Bush.	P	Th						X		
	Solanum nigrum L.	A	Th				X	X			
	Solanum carolinense L.	P	H scap						X		
Xanthorrhoeaceae	Asphodelus tenuifolius L.	B	H								
Zygophyllaceae R.Br.	Tribulus terrestris L.	A	Th			X					

(continued)

Table 1 (continued)

		Hab[a]	LF[b]	Nepeta cataria L. (8)	Nigella sativa L. (11, 12)	Origanum vulgare L. (13)	Plantago ovata Forsk (14)	Rosmarinus officinalis L. (15, 16)	Silybum marianum Gaertn. (17, 18)	Thymus vulgaris L. (19)
Amaranthaceae Juss.	Achyranthes aspera L.	P	Ch suffr							
	Aerva lanata (L.) Schult.	A	Th							
	Amaranthus blitoides S. Wats.	A	Th						X	
	Amaranthus retroflexus L.	A	Th		X				XX	
	Amaranthus spinosus L.	A	Th							
	Amaranthus viridis L.	A	Th				X			
	Celosia argentea L.	A	Th							
Apiaceae Lindl.	Apium leptophyllum (Pers.) F. Mueller	A	Th			X				
	Bowlesia incana Ruiz & Pov.	A	Th			X				
Apocynaceae Juss.	Catharanthus roseus (L.) G. Don.	P	Ch suffr							
Brassicaceae Burnett	Capsella bursa-pastoris (L.) Medik.	B	H		X			X		
	Coronopus didymus (L.) Sm.	A	Th			X				
	Descurainia sophia (L.) Prantl.	A	Th		X					
	Diplotaxis tenuifolia (L.) DC	P	H scap							
	Eruca vesicaria (L.) Cav.	A	Th					X		
	Goldbachia laevigata (M. Bieb.) DC.	A	Th							
	Lepidium virginicum L.	A	Th							
	Raphanus sativus L.	A	Th			X				
	Sinapis alba (L.) Spach	A	Th					X		
	Sisymbrium irio L.	A	Th			X				
Caryophyllaceae Juss	Arenaria serpyllifolia L	A	Th							
	Polycarpaea corymbosa (L.) Lam.	A	Th				X			
	Stellaria graminea L.	P	H scap		X					
	Stellaria media (L.) Villars.	A	Th			X				

(continued)

Table 1 (continued)

		Hab[a]	LF[b]	Nepeta cataria L. (8)	Nigella sativa L. (11, 12)	Origanum vulgare L. (13)	Plantago ovata Forsk (14)	Rosmarinus officinalis L. (15, 16)	Silybum marianum Gaertn. (17, 18)	Thymus vulgaris L. (19)
Chenopodiaceae Vent.	Chenopodium album L.	A	Th		X	X	X		XX	
	Salsola kali L.	A	Th							
Compositae Giseke	Bidens pilosa L.	A	Th							
	Carduus nutans L.	A or B	Th/H bienn			X				
	Cirsium arvense (L.) Scop.	P	G rad						XX	
	Glebionis segeta (L.) Fourr. = Chrysanthemum segetum L	A	Th					X		X
	Helminthotheca echioides (L.) Holub	A	Th					X		X
	Launaea nudicaulis (L.) Hook. f.	P	H				X			
	Matricaria spp.	A	Th							
	Parthenium hysterophorus L.	A	Th							
	Sonchus arvensis L.	P	H		X			X		
	Sonchus asper (L.) Hill	A, somet. B	Th/H bienn							
	Sonchus oleraceus L.	A	Th							
	Tragopogon spp.	A or P	Th/H							
	Tridax procumbens L.	P	Ch							
	Tussilago farfara L.	P	G rhiz						X	
	Xanthium strumarium L.	A	Th						X	
Convolvulaceae	Convolvulus arvensis L.	P	G rhiz		X			X	XX	X
	Evolvulus alsinoides L.	P	H							
Cyperaceae Juss.	Cyperus rotundus L.	P	G rhiz				X			
Equisetaceae DC	Equisetum palustre L.	P	G rhiz					X		

(continued)

Table 1 (continued)

		Hab[a]	LF[b]	Nepeta cataria L. (8)	Nigella sativa L. (11, 12)	Origanum vulgare L. (13)	Plantago ovata Forsk (14)	Rosmarinus officinalis L. (15, 16)	Silybum marianum Gaertn. (17, 18)	Thymus vulgaris L. (19)
Euphorbiaceae Juss.	Acalypha australis L.	A	Th							
	Euphorbia hirta L.	A, somet. P	Th							
Fabaceae Lindl.	Acacia farnesiana (L.) Willd. = Mimosa indica L.	P	P caesp							
	Glycyrrhiza aspera Pall.	P	G rhiz							
	Melilotus indicus (L.) All.	A	Th							
	Melilotus albus Medik.	A or B	Th				X			
	Tephrosia purpurea (L.) Pers.	A or P	Ch							
Geraniaceae Juss.	Erodium cicutarium (L.) L'Hér.	A, somet. B or P	Th/H ros							
	Erodium ciconium (L.) L'Hér.	A or B	Th/H bienn					X		
Lamiaceae Lindl.	Hyptis suaveolens (L.) Poit. = Mesosphaerum suaveolens (L.) Kuntze	A or P	Th							
	Lamium amplexicaule L.	A	Th			X		X		
	Lamium purpureum L.	A	Th							
	Leonurus cardiaca L.	P	H scap						X	
Malvaceae Juss.	Abutilon theophrasti Medik.	A	Th						X	
	Malva neglecta Wallr.	A	Th							
	Malva sylvestris L	P, rar A	H scap/Th					X		X
Nyctaginaceae Juss.	Boerhavia diffusa L.	A, rar P	Th/Ch rept							

(continued)

Table 1 (continued)

		Hab[a]	LF[b]	Nepeta cataria L. (8)	Nigella sativa L. (11, 12)	Origanum vulgare L. (13)	Plantago ovata Forsk (14)	Rosmarinus officinalis L. (15, 16)	Silybum marianum Gaertn. (17, 18)	Thymus vulgaris L. (19)
Oxalidaceae	Oxalis corymbosa DC	P	G bulb							
Papaveraceae Juss.	Argemone mexicana L.	A	Ch				X			
	Fumaria officinalis L.	A	Th		X			X		X
	Papaver rhoeas L.	A	Th					X		
Phyllanthaceae	Phyllanthus amarus Schum. et Thonn.	A	Th/Ch							
	Phyllanthus niruri L.	A	Th				X			
Poaceae Barnhart	Aristida adscensionis L.	P	H caesp							
	Avena fatua L.	A	Th							
	Chloris barbata Sw	A or P	H caesp							
	Cynodon dactylon (L.) Pers.	P	G rhiz/H rept				X	X	X	
	Digitaria ciliaris (Retz.) Koeler	A	Th							
	Digitaria ischaemum (Schreb.) Muhl.	A	Th							
	Digitaria radicosa (J.Presl.) Miq. = D. timorensis (Kunth) Balansa	A	Th							
	Digitaria sanguinalis (L.) Scop.	A	Th				X		X	
	Echinochloa crus-galli L. (P. Beauv.)	A	Th		X				X	
	Eleusine indica (L.) Gaertn.	A	Th							
	Elytrigia repens (L.) Nevski = Elymus repens (L.) Gould	P	G rhiz/H caesp						X	

(continued)

Table 1 (continued)

		Hab[a]	LF[b]	Nepeta cataria L. (8)	Nigella sativa L. (11, 12)	Origanum vulgare L. (13)	Plantago ovata Forsk (14)	Rosmarinus officinalis L. (15, 16)	Silybum marianum Gaertn. (17, 18)	Thymus vulgaris L. (19)
	Lolium multiflorum Lam.	A or P	Th/H scap							
	Ochlopoa annua (L.) H. Scholz = Poa annua L.	A	Th							
	Panicum capillare L.	A	Th							
	Panicum repens L.	P	G rhiz							
	Paspalum distichum L.	P	G rhiz							
	Phalaris spp.	A	Th					X		
	Rottboellia exaltata L.f. = R. cochinchinensis (Lour.) W.D. Clayton	A	Th							
	Setaria viridis (L.) P. Beauv.	A	Th						X	
	Setaria pumila (Poir.) Roem.& Schult. = Setaria glauca L.	A	Th							
	Setaria faberi Herrm.	A	Th	X						
	Sorghum halepense (L.) Pers.	P	G rhiz						X	
Polygonaceae	Polygonum aviculare L.	A	Th		X			X	X	
	Polygonum convolvulus L.	A	Th					X	X	
	Rumex crispus L.	P	H scap							
Portulacaceae Juss.	Portulaca oleracea L.	A	Th		X		X	X		
Primulaceae Vent.	Anagallis arvensis L.	A	Th						X	
Resedaceae Gray	Reseda luteola L.	B, rar P or A	H scap/Th							
Rubiaceae	Galium aparine L.	A	Th					X		

(continued)

Table 1 (continued)

		Hab[a]	LF[b]	Nepeta cataria L. (8)	Nigella sativa L. (11, 12)	Origanum vulgare L. (13)	Plantago ovata Forsk (14)	Rosmarinus officinalis L. (15, 16)	Silybum marianum Gaertn. (17, 18)	Thymus vulgaris L. (19)
Scrophulariaceae Juss.	Veronica hederifolia L.	A	Th							
	Veronica persica Poir.	A	Th		X			X		
Solanaceae	Datura metel L.	A	Th							
	Datura stramonium L.	A	Th						X	
	Physalis subglabrata Mack. and Bush.	P	Th							
	Solanum nigrum L.	A	Th		X				XX	
	Solanum carolinense L.	P	H scap							
Xanthorrhoeaceae	Asphodelus tenuifolius L.	B	H				X			
Zygophyllaceae R.Br.	Tribulus terrestris L.	A	Th							

Habitus[a]: A- annual; P-perennial; B- biennial

Life form[b]: Ch- Chamaephytes (rept: reptant; suff: suffrutescent); Th- Therophytes; H- Hemicryptophytes (scap: scapose; caesp: caespitose); G-Geophytes (bulb: bulbose; rad: root-budding; rhiz: rhizome); P- Phanerophytes (caesp: caespitose)

References: (1) Frabboni et al. 2008; (2) EPPO 2001; (3) Rajeswara Rao et al. 2007; (4) Hossain 2005; (5) Rahimi et al. 2015; (6) Yousefi and Rahimi 2014; (7) Mubeen et al. 2009; (8) Duppong et al. 2004; (9) Singh and Saini 2008; (10) Bonasia et al. 2012; (11) Seyyedi et al. 2016; (12) Nadeem et al. 2013; (13) Zumelzù et al. 1999; (14) Salvi et al. 2015; (15) Frabboni et al. 2009b; (16) Usano-Alemany et al. 2008; (17) Zheljazkov et al. 2006; (18) Tyr and Veres, 2011; (19) Frabboni et al. 2009a towards the prevalence of long-lasting weed species; in general terms, the adoption of wide inter-plant spacings, the reduced use of herbicides and a crop duration longer than one year may be crucial factors in assessing this condition.

Effects of Weeds on Yield, Growth and Development of Medicinal Plants

The necessity to remove weeds as completely as possible is well assessed by almost all experiments performed on MPs. Whenever trials have been performed including a weed-free treatment, independently upon the specific experimental goals, yield results are always inversely correlated to the incidence of weeds. Table 2 reports some of the published data about the variations in yield performance in several MPs attributable to the presence or the removal of weeds. As shown, irrespective of which kind of "yield" is considered, losses due to the presence of weeds may take relevant values. Under the different growth conditions of crops, seed yields decreases were found to range from 34% in Garden cress (Shehzad et al. 2011) to more than 90% in coriander, fennel and psyllium (Carrubba and Militello 2013). Also yield losses in biomass and herbage yields were high: they ranged from about 30% in cornmint (Singh and Saini 2008) to 80–90% and more in coriander, fennel and psyllium (Carrubba and Militello 2013). These figures do not vary when the harvestable part is a fraction of total plant biomass: yield losses have been calculated from 51 to 64% in flowers of saffron (Norouzzadeh et al. 2007), more than 20% in bulbs of Tassel hyacinth (Bonasia et al. 2012), 49–55% in leaves of Rose-scented geranium (Kothari et al. 2002) and 75% in shoots of sage (Satvati Niri et al. 2015).

Under some aspects, the outcome of crop-weed interaction is similar to that assessed for inter-species competition, and the effect of weed removal is similar to that obtained with reducing plant population: weeding allows obtaining bigger plants, with a higher number of ramifications and consequently more flowers and seeds. In *Nigella sativa*, weed control increased yield and yield components such as number of plants, number of branches, number of capsules, number of seeds per capsule, seed weight and total biomass (Nadeem et al. 2013; Seyyedi et al. 2016); in *Lepidium sativum*, removal of weeds gave higher plants, number of branches and number of seeds per plant (Shehzad et al. 2011).

For the same reason, when competition is high and shading becomes intense, plants are expected to grow taller and thinner (Ballaré and Casal 2000). Data from the literature (Table 3) show that this occurs in many cases, and very often MPs experience an increase in height when grown in presence of weeds. This increase in height however, being not associated with an increase in size, in most cases does not determine any increase in biomass yield.

Total weed biomass, however, is not the only factor affecting the outcome of cultivation. Competition with weeds may assume a different severity according to the time of competition period (i.e. the crop growth stage when it occurs), as well as its duration. In the starting phases of cultivation, when crop canopy is sparse, the

Table 2 Literature data about effect of weeding on yields of seeds, biomass and special plant parts in several MPs. Relative Losses (RL) % = (F-W)/F × 100

	Weed-free (F)	Unweeded (W)	RL%
Seeds (kg ha^{-1})	Coriander (Carrubba and Militello 2013; Choudhary et al. 2014)		
	1842.2	912.1	50.5
	1366.2	702.8	48.6
	1364.5	313.4	77.0
	1372.9	79.8	94.2
	1230.0	510.0	58.5
	1511.0	525.0	65.3
	Fennel (Carrubba and Militello 2013; Yousefi and Rahimi 2014; Mubeen et al. 2009)		
	3697.8	2200.1	40.5
	2756.6	79.2	97.1
	203.5	25.4	87.5
	637.9	42.8	93.3
	3816.0	80.0	97.9
	3938.0	52.0	98.7
	2336.0	1472.0	37.0
	Psyllium (Carrubba and Militello 2013)		
	1380.3	367.4	73.4
	541.2	245.7	54.6
	1542.7	36.5	97.6
	336.4	19.4	94.2
	Milk thistle (Zheljazkov et al. 2006)		
	1133.2	655.3	42.2
	1273.1	737.5	42.1
	Garden cress (Shehzad et al. 2011)		
	305.9	201.6	34.1
	Black cumin (Nadeem et al. 2013; Seyyedi et al. 2016)		
	1720.0	526.2	69.4
	1558.2	266.9	82.9
	686.4	87.3	87.3
	Isabgol (Salvi et al. 2015)		
	1225.0	381.0	68.9
	Fenugreek (Baradaran and Ghahhari 2016)		
	280.5	257.5	8.2

(continued)

Table 2 (continued)

	Weed-free (F)	Unweeded (W)	RL%
Biomass and herbage (kg ha^{-1})	Coriander (Carrubba and Militello 2013; Choudhary et al. 2014)		
	2545.1	601.1	76.4
	3923.7	1780.2	54.6
	4076.1	549.4	86.5
	1362.7	115.9	91.5
	2193.0	1141.0	48.0
	2972.0	1217.0	59.1
	Fennel (Carrubba and Militello 2013; Yousefi and Rahimi 2014; Mubeen et al. 2009)		
	3520.7	1093.1	69.0
	6653.1	501.6	92.5
	1792.1	242.7	86.5
	1169.4	37.1	96.8
	10718.0	8243.0	23.1
	Psyllium (Carrubba and Militello 2013)		
	3254.6	1287.2	60.4
	3885.2	1576.3	59.4
	4364.1	1795.9	58.8
	4487.4	375.4	91.6
	Garden cress (Shehzad et al. 2011)		
	3750.0	3472.0	7.4
	Isabgol (Salvi et al. 2015)		
	2930.0	621.0	78.8
	Cornmint (Singh and Saini 2008)		
	13820.0	9462.0	31.5
	12391.0	6307.0	49.1
	Catnip (Duppong et al. 2004)		
	600.0	278.0	53.7
	4138.0	2558.0	38.2
	St John's Wort (Duppong et al. 2004)		
	1903.0	1650.0	13.3
	Basil (Sarrou et al. 2016)		
	16.1	10.8	33.0
Special plant parts (kg ha^{-1})	Flowers of Saffron (Norouzzadeh et al. 2007)		
	1100.0	402.0	63.5
	510.0	250.0	51.0
	580.0	220.0	62.1
	Leaves of Rose-scented geranium (Kothari et al. 2002)		
	120.0	61.0	49.2
	123.0	55.0	55.3

(continued)

Table 2 (continued)

	Weed-free (F)	Unweeded (W)	RL%
	Leaves of Moldavian balm (Janmohammadi et al. 2016)*		
	250.0		
	99.0	60.4	
	Bulbs of Tassel hyacinth (Bonasia et al. 2012)		
	12.7	9.5	25.2
	11.1	8.7	21.6
	Shoots of Sage (Satvati Niri et al. 2015)		
2000.0	500.0	75.0	

*Approximate figures

Table 3 Literature data about effect of weeding on measured height of plants (cm) in several MPs. Relative Losses (RL) % = (F−W)/F × 100

Weed-free (F)	Unweeded (W)	RL%
Black cumin (Nadeem et al. 2013; Seyyedi et al. 2016)		
80.8	62.0	23.2
99.1	76.2	23.1
32.9	43.0	−30.8
Catnip (Duppong et al. 2004)		
30.0	35.0	−16.7
Coriander (Carrubba and Militello 2013)		
131.2	133.8	−2.0
120.9	123.7	−2.3
85.8	91.5	−6.6
144.6	124.4	14.0
Cornmint (Singh and Saini 2008)		
48.7	46.9	3.7
60.3	56.3	6.6
Fennel (Carrubba and Militello 2013; Mirshekari 2014; Mubeen et al. 2009)		
99.1	93.3	5.9
80.5	91.3	−13.4
57.6	59.3	−3.0
100.7	105.5	−4.8
76.0	61.0	19.7
270.8	196.5	27.4
133.6	140.1	−4.9
Garden cress (Shehzad et al. 2011)		
118.8	107.4	9.6
Psyllium (Carrubba and Militello 2013)		
91.0	92.1	−1.2
91.1	95.3	−4.6
81.4	84.2	−3.4
90.1	110.3	−22.4
St John’s Wort (Duppong et al. 2004)		
32.0	38.0	−18.8

outcome of an early weed infestation is expected to be severe. As a matter of fact, very often weeds grow much faster than crops; initial plant growth is slow in perennial crops, such as gentian (Radanović et al. 2014), oregano (Zumelzù et al. 1999) or sage (Karamanos 2000), but this issue has been reported also in annual or biennial herbs such as fennel (Yousefi and Rahimi 2014) or coriander, especially in genotypes not forming a dense basal rosette (Diederichsen 1996). When weeds sprout later (i.e. after crop canopy closure), their competition effects are generally lower. Hence, many works, especially in perennial or long-lasting MPs, strongly advocate for an early intervention against weeds. What is "early", and therefore the exact timing for weeding, is rather a crucial issue. As previously mentioned, to extend also to MPs the concepts and definitions that are established for the other crops is not easy, due to the preliminary necessity to give an univocal definition of what, in these crops, is termed as "yield".

As a rule, timing should be stated based on the sensitivity of crop to the presence of weeds; values for maximum tolerance period, corresponding to the moment when weeding operations should be started, have been found about 40 days after sowing (DAS) for *Nigella sativa* (Nadeem et al. 2013); 30–45 DAS for *Zingiber officinale* (Kifelew et al. 2015), 24–38 DAS for *Cuminum cyminum.* A great importance is therefore attributed to the critical period for weed control (CPWC), i.e. the time span in which "weeds must be controlled to prevent yield losses" (Knezevic et al. 2002). In some cases, one or two thresholds are set for acceptable seed yields losses (generally 5% or 10% of the maximum attainable yield, usually defined as that measured in the weed-free controls). In Table 4, some of the CPWC

Table 4 Calculated values for critical period for weed control (CPWC) in some MPs

References	MP species and measured yield	Threshold loss value (%)	CPWC		
			Start (DAS*)	End (DAS*)	Total (dd)
Seyyedi et al. (2016)	Black cumin (seeds)	5	13	75	62
			11	71	60
		10	17	64	47
			14	57	43
Hosseini et al. (2006)	Cumin (seeds)		24	38	14
Sharifi Nouri (2005)	Psyllium (seeds)	5	21	52	31
		10	26	38	12
Satvati Niri et al. (2015)	Sage (biomass)	5	22	98	76
		10	25	85	60
		15	29	82	53
Mojtaba et al. (2015)	Thyme (Fresh biomass)	5	41	90	49
		10	54	76	22
	Thyme (Dry biomass)	5	12	94	82
		10	29	78	49
	Thyme (Essential oil)	5	20	105	85
		10	30	86	56

*Days After Sowing (transplant in poliennials) values obtained in the sparse experiments on MPs are reported. Expectedly, total CPWC values are longer when a lower threshold value is set, i.e. when the allowed loss values are lower, and it is interesting to observe that in annual crops, the time span when fields must be kept weed-free shares a rather large part (more than 60%) of the reasonable duration of entire crop cycle.

Effects of Weeds on Quality Features of Medicinal Plants

Quality of MPs may be affected by weeds in many ways. The first issue to be considered is botanical identity of drugs (Zhang et al. 2010). Whoever the buyer, medicinal plants are graded according to their chemical characteristics, that are linked to the botanical uniformity of the traded biomass. The presence of weeds in the harvested biomass is therefore an important factor that affects negatively the market end value of the herbal product (Upadhyay et al. 2011). It is not, however, the only one. Even when weeds have been removed from the herbal products before sale, modifications in MPs chemical patterns due to the persistence of weeds during MPs cultivation cannot be excluded. Indeed, the presence of weeds is often believed to exert a significant effect on plant metabolic pathways, in this way causing variations in the content and quality of active metabolites (Gil et al. 1998). For example, weed infestation has been claimed to affect essential oil yields. On this regard, data from the literature are controversial. The EO content was reported to decrease of 20–28.6% in leaves of unweeded rose-scented geranium (Kothari et al. 2002) and coriander (Pouryousef et al. 2015), but oppositely an increase in EO was found in basil (Sarrou et al. 2016) such as in fatty oil from Milk thistle (Zheljazkov et al. 2006) (Table 5). When the oil yield per unit area is considered, a decrease is generally found, although probably this is due to the strong influence of the decrease of biomass yield.

The quantitative variations of EO yields, however, are not the only issue, and apart from them, the occurrence of weeds may also alter the chemical EO profile of herbs (Rajeswara Rao et al. 2007). The simplest case is when weeds are mixed with the plant material to be distilled: although weeds do not yield any essential oil, their presence may alter the quality feature of crop essential oil conferring it an unwanted off-flavour (Rajeswara Rao et al. 2007). However, also modifications of the EO chemical characteristics due to the changes in plant secondary metabolism because of weeds, have been reported by several Authors. In *Satureja sahendica* grown together with *Amaranthus retroflexus*, the effect of competition induced a decrease in thymol and an increase in p-cymene content (Hossaini et al. 2015). In basil, the highest yield of linalool (63.88%) and consequently of the oxygenated monoterpenes (75.05%), was achieved in the weeded treatment (Sarrou et al. 2016). In Milk thistle, a decrease in silymarin content was reported as a consequence of weed presence (Zheljazkov et al 2006). Although in some other cases, the EO obtained from MPs grown in weedy conditions had similar characteristics to that obtained in absence of competition, the unpredictability of chemical characteristics of the obtained EO must be considered as a major issue for trading.

Table 5 Literature data about effect of weeding on quality traits in several MPs. Relative Losses (RL) % = (F −W)/F × 100

Weed-free (F)	Unweeded (W)	RL%
Oil/Essential oil content (%)		
Basil (Sarrou et al. 2016)		
1.06	1.22	−15.1
Black cumin (Seyyedi et al. 2016)		
31.04	31.04	0
Coriander (Pouryousef et al. 2015)		
0.92	0.76	17.4
0.86	0.72	16.3
Cornmint (Singh and Saini 2008)		
0.73	0.82	−12.3
0.78	0.77	1.3
Milk thistle (Zheljazkov et al. 2006)		
19.50	24.60	−26.2
18.40	25.90	−40.8
Moldavian balm (Janmohammadi et al. 2016)*		
0.51	0.32	37.3
Rose-scented geranium (Kothari et al. 2002)		
0.20	0.16	20.0
0.21	0.15	28.6
Oil yield (kg ha^{-1})		
Black cumin (Seyyedi et al. 2016)		
214.0	27.3	87.2
Cornmint (Singh and Saini 2008)		
99.9	77.5	22.4
96.6	49.2	49.0
Fennel (Mirshekari 2014)		
270.8	196.5	27.4
Special oil components		
Silymarin % in Milk thistle (Zheljazkov et al. 2006)		
2.90	2.30	20.7
3.20	2.00	37.5
Thymol% in EO of Sahendi savory (Hossaini et al. 2015)		
45.18	38.60	14.6

*Approximate figures

Effects of Weeding Tools on MPs

Although the removal of weeds is surely beneficial to biomass and quality features of MPs, the tools that are used with this purpose may affect MPs production in many ways. Much work in this sense has been focussed on chemical weed control. Chemicals have been tested for many MPs, including caraway (*Carum carvi*), clary sage (*Salvia sclarea*), coriander (*Coriandrum sativum*), chamomile (*Matricaria recutita*), mint (*Mentha piperita*, *M. arvensis*, *M. spicata* and others), milk thistle (*Silybum marianum*), Moldavian balm (*Dracocephalum moldavica*), fennel (*Foeniculum vulgare*), sage (*Salvia officinalis*), savory (*Satureja officinalis*), oregano (*Origanum vulgare*), thyme (*Thymus vulgare*) and many others (Mitchell and Abernethy 1993; Mitchell et al. 1995; Pank 1992; Singh et al. 2011; Zheljazkov et al. 2006, 2010; Zumelzù et al. 1999). Following these Authors, if phytotoxic effects were avoided, the use of pendimethalin, fluchloralin, and other chemicals did not affect seed oil yields or oil quality. Otherwise, other experiments gave different results, and several modifications of essential oil components were recorded; e.g. in chamomile, chemically treated crop had lower chamazulene content (Singh et al. 2011), whereas plants of Moldavian balm treated with trifluralin showed a higher geraniol content (Janmohammadi et al. 2016).

As it seems, an interference of herbicides with the metabolism of secondary products cannot be always excluded. An additional constraint is that, although a number of exception may be found, the interest in cultivation of MPs making recourse to usual herbicides is generally rather low. A large number of growers, in fact, is oriented to cultivate MPs with organic management, that, according to EU regulations (EU Reg. 2092/91 and 2078/92 and, more recently, EU Reg. 834/2007 and 1107/09, and EU Dir. 128/2009), does not permit the use of chemicals. Under organic cropping management, weeds are a major issue for cultivation: in a survey on herb and vegetable organic production in Australia (Kristiansen et al. 2001), 80% of growers defined weeds as “a problem”, and the acknowledgement of this has pushed towards a big effort in studying alternative and non-chemical tools for weed control.

The most effective method is surely hand-removal of weeds (Carrubba and Militello 2013; Kristiansen et al. 2003); it is however a time- and labor-consuming method, and a need for 200–600 manpower hours per hectare has been estimated for manual weed management in many aromatic and medicinal plants (Pank 1992). Hence, it is considered suitable only to small areas, and other tools and strategies are continuously studied. Among these, mechanical weeding is one of the preferred methods by the majority of growers, especially for soil preparation before sowing or transplanting. One or two thorough preliminary mechanical works, with the goal to keep the field totally weed-free, are recommended for most of perennial MPs, such as sage (Karamanos 2000). It must be said, however, that many perennial weeds may take advantage of this practice, because mechanical equipments rather than excavate and dry the underground propagating organs of weeds, very often can split them and facilitate their diffusion (Zanin et al. 2001). The use of transplant instead of direct sowing, with the purpose to give to the plants some competitive advantage, is adopted in some high-income MPs such as gentian (Radanović et al. 2014), lemon balm, catnip, stinging nettle, and globemallow (Kleitz et al. 2008) but of course the costs of this additional step must be justified by a significantly higher value of crop.

After crop establishment, the effects of weeds are generally lower, and although there is always the necessity for further interventions, they are normally reduced to a few works between the rows. Alternative methods for weed control have been studied for many crops and growth conditions. In *Aloe vera*, in some regions of Mexico, the farmers allow goats and lambs grazing on weeds, since these animals do not feed on aloe (Cristiano et al. 2016). In lack of any other more exact indication, many MPs growers try to keep their fields weed free by means of one or two mechanical treatments after sowing/transplant. A special attention must be paid, in this case, to the proper choice of timing and equipment, since many crops may be sensitive to mechanical injuries (Bond and Grundy 2001).

Many other alternative methods, such as mulching or flaming, have been developed and suggested in time, both alone and in combinations, with variable and often controversial results. So far, it seems possible to suggest, in agreement with some Authors (Satvati Niri et al. 2015; Upadhyay et al. 2011; Dajic-Stevanovic and Pljevljakusic 2015), that an integrated approach to this issue, i.e. the recourse to different and integrated methods, tools and strategies, according to local conditions and requests from the buyers, would be preferable to the search for one specific weeding method. Specific trials concerning a larger number of MPs, also including the effects of different weed management strategies on yield and chemical traits of herbal products, seem however to be lacking, and there is increasing need for a detailed experimental activity concerning all aspects of the complex interactions between MPs and weeds.

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Medicinal Plant Diseases Caused by Nematodes

Faezehossadat Abtahi and Mansoureh Bakooie

Abstract The agents that cause infectious disease in plants are pathogenic microorganisms, such as viruses, bacteria, fungi, protozoa, and nematodes. Several hundred species of nematodes, however, are known to feed on living plants, obtaining their food with spears or stylets and causing a variety of plant diseases worldwide. Almost all plant pathogenic nematodes live part of their lives in the soil. Many live freely in the soil, feeding superficially on roots and underground stems, and in all, even in the specialized sedentary parasites, the eggs, the preparasitic juvenile stages, and the males are found in the soil for all or part of their lives. Nematodes occur in greatest abundance in the top 15 to 30 centimeters of soil. A few nematodes that attack the aboveground parts of plants not only spread through the soil as described earlier, but they are also splashed to the plants by falling rain or overhead watering. All plant parasitic nematodes belong to the phylum *Nematoda*. In this chapter, considering the importance of plant diseases caused by nematodes, we review some of the most important diseases of some of the medicinal plants.

Keywords Medicinal Plants · Pathogenic microorganisms · Loss
Nematodes

Introduction

Nematodes belong to the kingdom *Animalia*. Nematodes are wormlike in appearance but quite distinct taxonomically from the true worms. Several hundred species are known to feed on living plants, obtaining their food with spears or stylets and

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causing a variety of plant diseases worldwide. Almost all plant pathogenic nematodes live part of their lives in the soil. Soil temperature, moisture, and aeration affect survival and movement of nematodes in the soil. Nematodes occur in greatest abundance in the top 15–30 cm of soil. The overall distance traveled by a nematode probably does not exceed a few meters per season. Nematode infections of plants result in the appearance of symptoms on roots as well as on the aboveground parts of plants. Root symptoms may appear as root lesions, root knots or root galls, excessive root branching, injured root tips. The root symptoms are usually accompanied by symptoms in the aboveground parts of plants, appearing primarily as reduced growth, symptoms of nutrient deficiencies such as yellowing of foliage, excessive wilting in hot or dry weather, reduced yields, and poor quality of products. All plant parasitic nematodes belong to the phylum *Nematoda*. Most of the important parasitic genera belong to the order *Tylenchida*, but a few belong to the order *Dorylaimida*.

Scientific name: *Achillea millefolium* L.
Common name: Yarrow

Mesoanguina millefolii (Low 1874) Chizhov and Subbotin (1985) induce small galls on the leaves of yarrow (Goodey 1938; Hooper and Doncaster 1972; Evtushenko et al. 1994). The milfoil cyst nematode, *Heterodera millefolii*, and yarrow cyst nematode, *Heterodera achilleae* were reported from this plant in Russia and Yugoslavia, respectively. Host tests at Sarajevo; Yugoslavi showed that yarrow, *Achillea millefolium* L. was heavily attacked (Fig. 1) (Golden and Klindic 1973). *Meloidogyne* sp., *Helicotylenchus* sp., *Xiphinema* sp. and Dorylaimid were reported on yarrow in Isfahan, Iran (Nasresfahani et al. 2015).

Scientific name: *Atropa belladonna L.*
Common name: Belladonna, Deadly nightshade

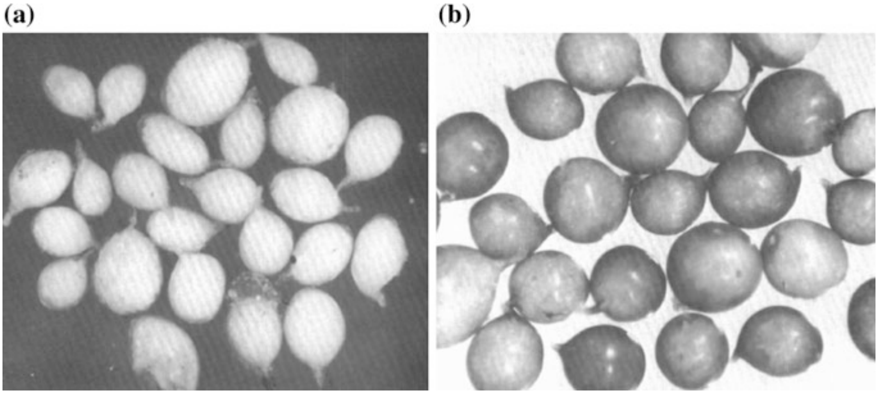

Fig. 1 Phomicrographs of whole specimens of *Heterodera achilleae* **a** White females (note egg mass attached to one specimen), **b** Cysts × 30 (after Golden and Klindic 1973)

Longidorus spp. was identified on *Atropa belladonna* in Uttar Pradesh, India. Four larvae of this parasitic nematode were collected from soil sample of 5–30 cm depths (Rashid et al. 1973).

Scientific name: *Berberis vulgaris* L. *var. sperma*
Common name: Berberry, Common berberry

Plant parasitic nematodes; *Merlinius acuminatus* (Minagawa 1985; Brzeski 1991, *Geocenamus dobroticus* (Budurova et al. 1996), *Paratylenchus vandenbrandei* (De Grisse 1962), *Criconemoides morgensis* (Hofmanner and Menzel 1914) and *Boleodorus* spp. (*B. typicus* (Husain and Khan 1968), *B. impar* (Khan and Basir 1964), *B. volutus* (Lima and Siddiqi 1963) and *B. thylactus* (Thorne 1941)) were collected from the rhizosphere of *Berberis vulgaris* crop in the South Khorasan Provine of Iran. The male nematodes was founded only in *B. typicus* species (Alvani et al. 2013, 2016). Two species *Irantylenchus vicinus* and *Neopsilenchus magnidens* were reported on this plant, also (Alvani et al. 2015).

Scientific name: *Calendula officinalis* L.
Common name: Marigold

Meloidogyne incognita (Kofoid and White) Chitwood race 1 (Magbool et al. 1985) reported on this flower plant from Pakistan. Moreover, the root system of *Calendula officinalis* showed clear symptoms of attack by root-knot nematode *Meloidogyne incognita* in Italy (Fig. 2). However, the low degree of infestation and tolerable damage is related with the cultivation period of this plant from the end of September to the beginning of March. Therefore, a treatment with non-volatile nematicide has been recommended for nematode control (Russo et al. 2008). *Meloidogyne incognita*, *M. javanica* and *Rotylenchus reniformis* were also recorded on marigold in India (Bhat et al. 2014). In New Zealand *Meloidogyne hapla* recognized on marigold (Dale 1971). It is recently reported that the peach root knot

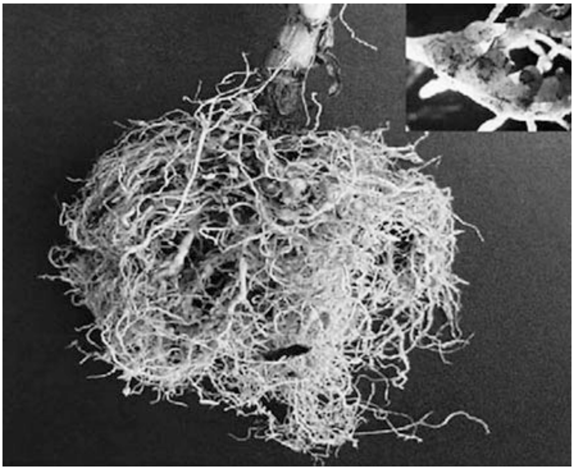

Fig. 2 Roots of *Caendula officinalis* infested by *Meloidogyne incognita* (After Russo et al. 2008)

nematode *Meloidogyne floridensis* (Handoo et al. 2004) in addition to peach, infects many important crops including *Calendula officnalis* cv. Oktoberfest (Brito et al. 2015). *Meloidogyne* sp., *Helicotylenchus* sp., *Xiphinema* sp. and *Ditylenchus* sp. were collected on marigold in Isfahan, Iran (Nasresfahani et al. 2015). *Aphelenchoides besseyi* and *A. ritzemabosi* were detected on *Calendula officinalis* L. (Sanchez-Monge et al. 2015). In South Africa, *Hemicycliophora litorea* Van den Berg found in the rhizosphere of wild calendulas (Van Den Berg 1987) (Fig. 2).

Scientific name: *Carum carvi* L.
Common name: Caraway

Meloidogyne trifoliophila Bernard and Eisenback (Bernard and Eisenback 1997) produced numerous galls on caraway in greenhouse experiment and 50 to 75% of the root system not functioning (Bernard and Jennings 1997).

Scientific name: *Cassia angustifolia Vahl.*
Common name: Senna, Alexandria senna

The infection of root-knot nematode, *Meloidogyne incognita* on senna was recorded. The management of this parasite by different bioagents, organic amendments and humic acid treatments showed that the use of plant growth promoting *Rhizobacterium*, *Pseudomonas fluorescens* as soil application has the lowest nematode population accompanied with highest economic yield (Ramakrishnan and Senthilkumar 2009).

Scientific name: *Catharanthus roseus* L.
Common name: Periwinkle, Madagascar Periwinkle

The results of the host suitability investigations in greenhouse condition were illustrated that Madagascar Periwinkle cultivars; Carpet Dawn, Cooler Grape, Little Blanche, Little Delicata and Polka Dot, ranked slightly susceptible to *M. incognita* race 3 (Walker et al. 1994) and cultivars; Blush Cooler, Little Bright Eyes, Little Mixed Colors and Peppermint rated as a generally poor hosts of *M. incognita* race 1 and *M. javanica* (McSorley and Frederick 1994, 2001). The identification of nematodes collected from Narendrapur medicinal plants garden revealed that *Catharanthus roseus* infected with the plant parasitic nematode, *Hoplolaimus* sp (Haldar and Gupta 2015). This medicinal species was introduced as one of the hosts of the rice-knot nematode *M. graminicola* (Golden and Birchfield 1965; MacGowan and Langdon 1989) and *Pratylenchus roseus* (Zarina and Maqbool 1998; Castillo and Vovlas 2007).

Scientific name: *Crocus sativus* L.
Common name: Saffron

Plant parasitic nematodes, *Aphelenchoides subtenuis* (Cobb 1926; Steiner and Buhrer 1932; Koliopanos and Kalyviotis-Gazelas 1979; Decker 1989; Southey 1993; Ortuno and Oros 2002; McCuiston et al. 2007), *Pratylenchus penetrans* and *P. pratensis* (Metcalf 1903; Schenk 1970), *Ditylenchus destructor* (Ortuno and Oros 2002), *Psilenchus hilarulus* (Alvani et al. 2015a), *Tylenchus* sp., *Hirschmaniella* sp., *Psilenchus* sp., *Pratylenchus* sp. and *Helicotylenchus chishtii* (Sheikh et al. 2014), *Amplimerlinius icarus*, *A. macrurus*, *A. socialis*, *Aphelenchoides asterocaudatus*, *A. besseyi*, *A. curiolis*, *Criconemoides deccipiens*, *Ditylenchus dipsaci*, *D. myceliophagus*, *Helicotylenchus crassatus*, *H. exallus*, *Merlinius bavaricus*, *M. brevidens*, *M. graminicola*, *M. microdorus*, *M. nanus*, *M. pseudobavaricus*, *Paratylenchus coronatus*, *Pratylenchus coffeae*, *P. loosi*, *P. penetrans*, *P. thornei*, *Psilenchus elegans*, *P. minor*, *Tylenchorhynchus brassicae*, *Tylenchus parvus*, *Geocenamus squamatus*, *G. tenuidens*, and *Filenchus pratensis*, *F. hamatus* (Mahdikhani and Alvani 2013), *Pratylenchus crenatus* (Kasimova and Atakishieva 1980), *Aerotylenchus safroni* (Fotedar and Handoo 1977) have been identified in *C. sativus*.

Scientific name: *Cuminum cyminum* L.
Common name: Cumin

Occurrence of root-knot nematodes (*Meloidogyne* spp.) on this crop were reported in India (Shah and Patel 1979; Midha and Trivedi 1989).

Scientific name: *Curcuma longa* L.
Common name: Turmeric, Indian saffron, Yellow ginger

The different plant parasitic nematodes like *Meloidogyne* sp., *Rotylenchulus reniformis*, *Helicotylenchus multicinctus*, *Hoplolaimus seinhorstii*, *H. columbus*, *H. indicus*, *Tylenchorhynchus martini*, *Radopholus similis*, *Pratylenchus delattrei*, *P. coffeae*, *Calloosia* sp., *Tylenchus* sp., *Aphelenchus* sp., *Criconemoides ornatus*, *Longidorus elongidorus* and *Xiphinema elongatum* were recorded from the rhizosphere of turmeric of which *Meloidogyne* sp., *Radopholus similis* and *Pratylenchus coffeae* are of the economic important and caused yield losses (Ayyar 1926; Nirula and Kumar 1963; Nadakal and Thomas 1964; Koshy and Sosamma 1975; Vilsoni et al. 1976; Sosamma et al. 1979; Venkitesan and Charles 1980; Bhardwaj and Hogger 1984; Bai et al. 1995; Ray et al. 1995; Poornima and Sivagami 1998, 1999; Haidar et al. 1998; Koshy et al. 2005; Udo and Nwagwu 2007; Idorenyin and Ugwuoke 2010; Aminu-Taiwo et al. 2015). The results of root-knot nematode management experiment in turmeric was indicated that the soil solarization and the organic soil amendment with poultry manure were founded the effective treatment in controlling disease (Patel et al. 2008; Idorenyin and Ugwuoke 2010). The results of the resistant screening revealed that several cultivars and accessions of this crop were resistant to root-knot nematode (Sasser et al. 1984; Gunasekharan et al. 1987; Mani et al. 1987; Eapen et al. 1999; Mohanta et al. 2015; Prasath et al. 2016).

Scientific name: *Datura stramonium* L.
Common name: Thorn apple, Jimson weed

Datuta stramonium was recorded as a good weed host of *Ditylenchus destructor* Thorne in peanut fields in South Africa (Waele et al. 1990) and of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* in Khorasan Province (Iran) and South Carolina (Tedford and Fortum 1988; Gharabadiyan et al. 2012). *M. ethiopica* (Whitehead 1968) was also recognized on *D. stramonium* (O'Bannon 1975).

Scientific name: *Digitalis purpurea* L.
Common name: Foxglove, Common foxglove, Digitalis

Paratylenchus spp. (Rashid et al. 1973) and *Pratylenchus penetrans* (Seinhorst 1998) were reported on *Digitalis purpurea*.

Scientific name: *Foeniculum vulgare* Mill
Common name: Sweet fennel, Common fennel

Root-knot nematode is a common problem in fennel (Walker 1995; Patel et al. 1995; Park et al. 2007; Ibrahim and Mokbel 2009; Khare et al. 2014; Nasresfahani et al. 2015; Kumar et al. 2016). This parasite can be managed by the application of oil cakes like neem cake (1000 kg/ha) + phorate (1 kg/ha) or 1000 kg castor cake + 1 kg phorate (Patel et al. 2005) and soil solarization with 25µ LLDPE film and rabbing with castor husk at 7 kg/m^2 (Patel et al. 2002). The other plant parasitic nematodes, *Aphelenchoides siddiqii* in Sudan (Fortuner 1970) and *Helicotylenchus* sp., *Xiphinema* sp., *Tylenchus* sp. and *Tylenchorhynchus* sp. in Iran (Nasresfahani et al. 2015) are described from *Foeniculum vulgare* Mill.

Scientific name: *Glycyrrhiza glabra* L. var. *glabra*
Common name: Licorice, liquorice

Aphelenchus avenae and *Pratylenchus* spp. infections were identified on the imported rhizomes of *Glycyrrhiza glabra* from Italy, Polans and Pakistan into New Dehli, India (Mathur et al. 1980).

Scientific name: *Humulus lupulus* L.
Common name: Hops, Common hop

The important plant parasitic nematodes including *Ditylenchus dipsaci*, *D. destructor*, *Bitylenchus dubius*, *Merlinius brevidens*, *Rotylenchus robustus*, *Helicotylenchus canadensis*, *H. digonicus*, *H. labiatus*, *Pratylenchus penetrans*, *P. pratensis*, *P. neglectus*, *P. thornei*, *Paratylenchus bukowinensis*, *Geocenamus* sp., *Trichodorus* sp., *Criconema* sp., *Xenocriconemella* sp., *Mesocriconema* sp., *Longidorus elongates*, *Xiphinema diversicaudatum* (plant virus-vectore nematodes), *Heterodera humuli* (cyst nematode) and *Meloidogyne* spp. (root-knot nematode) were reported from hop plant in India, USA, Czechslovakia, Africa, New Zealand, Australia, Belgium, Canada, England, Holland (Voigt 1894; Percival 1895; Šály and Kříž 1961; Maggenti 1962; Wasilewska 1979; Foot and Wood 1982; Esser 1985; Hogger 1988; McNamara and Eppler 1989; Malan et al. 1991; Hay and Close 1992; Yeates and Wouts 1992; Lopez-Robles 1995; Mende and Mcnamara 1995a, b; Eppler 1999; Hafez et al. 1999; Reis et al. 1986; Hay and Pethybridge 2003; Hanel 2003, 2010; Liskova and Renco 2007; Grasswitz and James 2008; Mahaffee et al. 2009; Hafez et al. 2010; Renco et al. 2010, 2011). For cyst-forming nematode management has been recommended to prevent the transfer of the nematode to younger plants, washing or cleansing the hop cones and agricultural tools to prevent the possible transfer of adhering cysts and plant stock be taken from healthy specimens (Decker 1981).

Scientific name: *Hyoscyamus niger* L.
Common name: Henbane, Hyoscyamus

It is known that two species *Meloidogyne incognita* and *M. javanica* attacks to *Hyoscyamus niger* and cause agent of the significant reduction in different plant characters (Haseeb and Pandey 1989; Janardhanan 2002). The other parasitic nematodes; *Hoplolaimus* sp., *Helicotylenchus* sp., *Tylenchorhynchus vulgaris*, *Pratylenchus thornei*, *Rotylenchulus reniformis*, *Xiphinema* sp., *Longidorus* sp. and *Trichodorus* sp. were also isolated from rhizosphere of this plant (Southey 1970; Akhtar et al. 2000). The protecting effects of three vesicular-arbuscular mycorrhizal (VAM) fungi against the root-knot nematode infection in *Hyoscyamus niger* indicated that *Meloidogyne incognita* populations in both soil and roots decreased in pot experiments and thus the inoculation of VAM fungi was recommend as a wise option instead of nematicides for reducing root-knot diseases in henbane plant (Pandey et al. 1999).

Scientific name: *Levisticum officinale* W.D.J. Koch
Common name: Lovage

The migratory endoparasitic nematodes *Zygotylenchus guevarai* and *Pratylenchus sudanensis* were collected from lovage farm at Dasht-e-Room, Boyer-Ahmad, Iran (Ansari et al. 2016).

Scientific name: *Matricaria chamomilla L.*
Common name: German chamomile, True chamomile, Common chamomile

The stunt nematode; *Tylenchorhynchus* spp., root lesion nematode; *Pratylenchus* spp. and root-knot nematode; *Meloidogyne* spp. were the major parasitic nematodes on chamomile showed an infection of 81.9, 56 and 45.5%, respectively (Ismail et al. 2002).

Scientific name: *Mentha piperita* L.
Common name: Pepper mint, Black mint, Candy mint

Information was showed the occurrence and distribution of phytoparasitic nematodes such as *Helicotylenchus digonicus*, *H. pseudorobustus*, *Mesocriconema ornatum*, *Paratylenchus projectus*, *P. tenuicaudatus*, *Tylenchorhynchus clarus*, *Pratylenchus coffeae*, *Rotylenchulus reniformis*, *Xiphinema* sp., *Longidorus elongates*, *Pratylenchus thornei*, *P. penetrans*, *Amplimerlinius gelobigerus*, *Criconema mutabil*, *Criconemella xenoplax*, *Meloidogyne* spp. from India, Iran, Egypt, Pakistan, USA (Bergeson and Green 1979; Haseeb 1994; Haseeb and Shukla 1996; Merrifield and Ingham 1996; Shukla et al. 1998; Hashemi and Akbarinia 2009; Hafez et al. 2010; Khanzada et al. 2012; Ibrahim and Handoo 2016) on pepper mint influence the plant growth, oil yield, physiological and biochemical changes in plant. The survey of the chemical control of *Longidorus elongates* with nonvolatile nematicides was indicated that foliar sprays of oxamyl and later with granular incorporation of aldicarb and oxamyl in soil resulted the greatest yield response (Pinkerton and Jensen 1983).

Scientific name: *Nigella sativa L.*
Common name: Black cumin, Black seed

Meloidogyne incognita was recorded on *Nigella sativa* (Haidar et al. 2001).

Scientific name: *Panax ginseng* L.
Common name: Chinese ginseng, Korean ginseng, Oriental ginseng

The several studies were revealed presence of plant parasitic nematode namely, *Criconemoides morgensis*, *Ditylenchus destructor*, *Helicotylenchus dihystera*, *Meloidogyne incognita*, *M. hapla*, *Paratylenchus lepidus*, *Pratylenchus penetrans*, *P. subpenetrans*, *Psilenchus hilarulus*, *Trichodorus similis*, *Tylenchorhynchus claytoni*, *Xiphinema americanum* on ginseng (Choi 1976; Ahn et al. 1983; Janardhanan 2002; Chung et al. 2004; Kim et al. 2006). *Meloidogyne* spp. induce root galls, *Ditylenchus destructor* attacks tubers and rhizomes, stem-like underground parts, causing the formation of necrosis. *D. destructor* causing root rot disease of ginseng, infected roots exhibit small discolored spots. In heavy attacks, the epidermis becomes grayish-black, abnormally thin, dry and cracked; underlying tissues are spongy and brownish, forming lumpy masses. *P. penetrans* is a migratory endoparasite of roots, its infection make fine reddish-brown lesions on rootlets. The typical symptoms resulted of *P. subpenetrans* observed took the form of lesions on the main root of 1-year-old seedlings, the lesions being sunken and the roots constricted in places. Sometimes, severe constrictions occurred at several sites on a single root, producing a bead-like appearance. The lower portion of severely rotted roots was sometimes missing. The control practices for the important nematodes have been suggested. If root lesion nematode populations exceed 100 per 100 cc of soil, when sampled in late summer, soil fumigation prior to planting should be treated. In fields infected by *Ditylenchus destructor*, the soil fumigation by cylon and treated with ethoprop or triazophos suppressed the nematode populations (Ohh et al. 1983, 1986; Chung et al. 2004; Kim et al. 2006).

Scientific name: *Papaver somniferum L.*
Common name: Opium poppy, Common poppy

Different plant parasitic nematodes; *Basirolaimus saccharis*, *Meloidogyne incognita*, *Boleodorus thylactus*, *Ditylenchus anchilisposomus*, *D. dipsaci*, *Filenchus filiformis*, *Helicotylenchus digonicus*, *Pratylenchus alkani*, *P. coffeae*, *P. pratensis*, *Pratylenchoides conincki*, *Tylenchorhynchus latus*, *T. striatus*, *Zygotylenchus guevarai* were associated with poppy crop (*Papaver somniferum* L.) (Schmitt and Lipscomb 1975; Shamsi 1979; Rakesh et al. 1999; Akgul and Okten 2001).

Scientific name: *Pimpinella anisum L.*
Common name: Anise, Anise seed, Aniseed, Sweet cumin

In the survey carried out in anise growing areas in Burdur province, Turkey 15 species; *Sakia propora*, *Irantylenchus clavidorus*, *Boleodorus mirus*, *Tylenchorhynchus annulatus*, *Bitylenchus maximus*, *Quinisulcius acutus*, *Negelus affinis*, *Hoplolaimus geleatus*, *Helicotylenchus dihystera*, *Pratylenchus zeae*, *P. alkani*, *Meloidogyne arenaria*, *Loofia thienemanni*, *Ditylenchus dipsaci*, *Safianema anchilisposoma* of the families Tylenchidae, Dolichodoridae, Hoplolaimidae, Pratylenchidae, Meloidogynidae, Hemicycliophoridae and Anguinidae were isolated (Kepenekci 2003).

Scientific name: *Plantago ovata* Forssk.
Common name: Plantago, Blond psyllium, Isabgol

Root-knot nematode, *Meloidogyne incognita* was significantly reduced in all growth parameters viz., root shoot length, fresh and dry root-shoot weights, number of spikes/plant and seed weight/plant on plantago (Kumar and Haseeb 2011).

Scientific name: *Rosmarinus officinalis* L.
Common name: Rosemary

Plant parasitic nematodes; *Helicotylenchus pseudorobustus*, *H. californicus*, *H. indicus*, *H. nigeriensis*, *Merlinus microdorus*, *M. indicus*, *Boleodorus thylactus*, *Psilenchus minor*, *P. hilarulus* from Iran (Mahdikhani and Mokaram 2011) and different root-knot nematodes; *Meloidogyne* spp. from Brazil and Iran (Pauletti and Echeverrigaray 2002; Nasresfahani et al. 2015), *M. incognita* race 3 (Walker 1995) and *M. javanica* from Israel (Gamliel and Yarden 1998) were collected on rosemary.

Scientific name: *Valeriana officinalis* L.
Common name: Valerian

The root lesion nematode; *Pratylenchus pratensis* from Germany (Pavlyuk 1972), migratory endoparasite nematode; *Ditylenchus* sp. from Russia (Sturhan and Brzeski 1991) and *Meloidogyne* sp., *Helicotylenchus* sp., *Tylenchus* sp. and *Tylenchorhynchus* sp. (Nasresfahani et al. 2015) were reported on valerian.

Scientific name: *Vinca minor* L.
Common name: Periwinkle, Lesser periwinkle, Small periwinkle

Meloidogyne ardenensis (Susana and Santos 1967), *M. hapla* (Walker 1965; Piron 1975), *Heterodera* sp. (Walker 1965) and *Xiphinema americanum* (Epstein and Barker 1966) were reported from periwinkle.

Scientific name: *Thymus vulgaris* L.
Common name: thyme, garden thyme

Two species, *Criconemella antipolitana* and *Geocenamus brevidens* were identified on thyme in Iran (Hashemi and Akbarinia 2009).

There were no reports about the medicinal plants susceptibility of *Angelica archangelica* L. (angelica, garden angelica), *Artemisia dracunculus* L. (tarragon), *Althea officinalis* L. (marsh mallow), *Bunium persicum* (Boiss.) Fedtsch. (black caraway) to plant parasitic nematodes in the literature.

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Plant Derived Essential Oils Against *Aedes aegypti* L. and Their Biotechnological Production

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Abstract *Aedes aegyti* L. is the main vector of important viruses like Dengue, Yellow, Zika and Chikungunya fevers. In many countries, these diseases are considered as great public health problems due to the serious consequences they can cause such as Guillain-Barré syndrome, neurological disorders and internal hemorrhagic problems, which may lead to death. The transmission of these diseases occurs through the bite of infected female of *A. aegypti*. According to World Health Organization, the major way to avoid the spread of these diseases is to control the vector by using larvicides and insecticides products. Many efforts have been done to find natural products that can be used to eliminate different phases of the life cycle of this mosquito. Among these, essential oils are recognized as important plant-derived products to control *A. aegypti*. Essential oils are complex mixture of mono, sesquiterpenes and phenylpropanoids and normally have a nice odor. Several studies have shown the larvicidal and insecticides properties of essential oils in plants belonging to different families. The literature studies indicated that in most of the cases monoterpenes like geraniol and citronellol, and phenylpropanoids; eugenol were the main components of essential oils that can control *A. aegypti* spread. In vitro studies suggested that composition and production of essential oils could be manipulated by the use of different concentrations and combinations of growth regulators and elicitors.

Keywords Medicinal plants · Mosquitoes-borne diseases · Plant derived natural compounds · Larvicides · Insecticides

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Introduction

Mosquitoes-borne diseases have become a very serious public health problem worldwide in recent decades with more than one million deaths annually (Zhang et al. 2016). Among these insects capable for transmission of infectious diseases, *Aedes (Stegomyia) aegypti* L. (Diptera: Culicidae) has been playing a role as the most dangerous vector for the human being. This mosquito is considered as the main vector transmitting several kinds of viruses which cause Dengue, Yellow, Zika and Chikungunya fevers (WHO 2017a).

The incidence of Dengue fever has increased in the past decades worldwide. Dengue is still considered as an important mosquito-borne viral disease, being endemic in more than one hundred countries. It is most common in tropical areas, being influenced by several factors such as temperature, relative humidity, urbanization and effectiveness of vector control in urban places. The dengue virus (DEN) belongs to Flavivirus genus and comprises four serotypes, each one among them causes the severe dengue (dengue haemorrhagic) fever (WHO 2012a, b).

The Zika virus (ZIKV) causes a very serious disease and its geographic distribution occurs globally although it is most frequent in America (WHO 2017a). This virus is primarily transmitted by mosquito vector bite and *A. aegypti* is its main vector. However, this virus is found in different body fluids but can also be transmitted by sexual contact or during the pregnancy. Recently, the infection with ZIKV was linked with microcephaly among newborns and besides the cases of Guillain-Barré syndrome and other neurological disorders, the concern about this virus is also increasing and there is need to control the vector (WHO 2016).

Chikungunya fever is another viral disease that can be transmitted by *A. aegypti*. Its main symptoms are fever, severe joint pain, muscle pain, nausea, fatigue and rash. Due to similar clinical signs and symptoms, Dengue and Zika may be misdiagnosed with Chikungunya. The treatment is based on relieving its symptoms and signs as these other viral diseases. The main outbreak of this disease was noticed in America in 2015. However, it is also reported in Africa, Asia, Europe and India areas (WHO 2017b).

Yellow fever is another vector-borne disease with major vector mosquitoes from genera *Haemagogus*. The resurgence of *Aedes aegypti* in urban areas after failing of vector control programs in several countries during the last decades of previous century has allowed the re-urbanization of Yellow fever (Vasconcelos et al. 1999). Since 2016, many new cases of Yellow fever have been reported in the southeast of Brazil. In the states of Minas Gerais, Espírito Santo, São Paulo, Rio de Janeiro and Bahia, yellow fever cases were confirmed by Brazilian authorities and many patients died due to this fever (WHO 2017b). Based on these reports, it became quite important to find new strategies to achieve *A. aegypti* control.

A. aegypti is a highly anthropophilic species. It has adapted to the urban environment and uses containers with water for oviposition, both artificial as bottle, tires, flowerpots, buckets, glasses, cans etc. or natural ones such as treeholes, flowers and plant axils. The eggs hatches and it starts the development of the aquatic stages of its life cycle until it becomes an insect. Its adult form is a small and black mosquito with white lyre shaped, which is used to differentiate from another insets. The eggs may survive during long time when they are in dry environment, allowing the easy spread to new places. When it rains, the life cycle of the mosquitoes may start. Both female and male adult form of *A. aegypti* feed on organic material, like nectar of plants, however female need blood in order to develop eggs (Dias and Moraes 2014; Klowden 2007).

The transmission of virus to humans occurs through the bites of infected female mosquitoes, which are daytime feeders. Unfortunately, there is no effective vaccine against Zika and Chikungunya fever and there is no report about effectiveness of Dengue vaccine. On the other hand, there are no specific drugs available to prevent viral propagation nor as antiviral action. Thus the control programs to avoid the spread of these diseases are targeted directly to the mosquitos' vectors at different phases of their life cycle.

The larvicides are employed against the development of the mosquito at various stages, where the transmission occurs. The organophosphate temephos is the most appropriate larvicide to be used in public health programmes (Tikar et al. 2008; WHO 2009). However, the continued and repeated use of temephos has contributed to the development of resistance besides the death of no target organisms. The use of spraying insecticides in reducing populations of *A. aegypti* is considered as a primary method of controlling virus transmission. Although pyrethroids and organophosphates are used worldwide in order to control the virus transmission but *A. aegypti* is becoming resistant to these compounds (Darbro et al. 2017).

Although the strategies used to eliminate *A. aegypti* normally rely on pesticides (larvicides and insecticides) but the results have not revealed the decrease of these viruses. In 2015, there was 1–6 million cases of dengue fever, including 863 deaths. In 2016, the microcephaly cases warned the whole world about this mosquito. The Chikungunya may cause the incapacity or problems of mobility. Thus it is necessary to find out more effective vector control to reduce mortality and morbidity attributable to avoid environment pollution mainly because there are no specific drugs available to treat these viruses. The huge efforts have been conducted in search of new active natural products such as plant derived bioactive compounds. In this field, essential oils represent a special alternative against *A. aegypti*. Many essentials oils as well their isolated compounds have been proved to possess repellent activity against mosquitoes.

Essential Oils Against *Aedes aegypti* and Their Chemical Composition

Essential oils are complex mixtures of low molecular weight lipophilic organic compounds. Their main groups of secondary metabolites are sesquiterpenes; derived from the mevalonate biosynthetic pathway, mono- and diterpenes from the methyl-erythritol pathway and products from the shikimic acid pathway, phenylpropanoids (Baser and Buchbauer 2015).

Several literature reports describe the plant families and species producing essential oils to prevent mosquito bites. Tisgratog et al. (2016) recognized 37 promising plant species within 14 families with this property. Preparations from members of the Myrtaceae, Lauraceae, Rutaceae, Lamiaceae, Asteraceae, Apiaceae, Cupressaceae, Poaceae, Zingeberaceae and Piperaceae are commonly known as insect repellents (Maia and Moore 2011; Rehman et al. 2014). The essential oils extracted from species of the genera *Cymbopogon* Spreng., *Ocimum* L. and *Eucalyptus* L'Hér are the most widely considered for their repellency effect. Figure 1 depicts the chemical structures of compounds studied for their repellent activity against *A. aegypti*. Combination of monoterpenes and sesquiterpenes in these oils attribute to repellent activity (Akhtar et al. 2012; Ravi Kiran and Sita 2007). Among the individual chemicals present in mixtures with high biological activity, the major compounds include mainly: monoterpenes, such as geraniol (1), citronellol (2), citronellal (3), limonene (4), terpinolene (5), p-menthane-3,8-diol (PMD) (6), 1,8-cineol (7), thymol (8), α and β-pinenes (9 and 10), and camphor (11); and a phenylpropene eugenol (12) (Nerio et al. 2010; Pohlit et al. 2011).

Although a large number of papers show the repellent potential of essential oils, some of them do not define their chemical composition (Sritabutra et al. 2011). Even considering that the constitution of most of these essential oils, viz. *Allium sativum* bulb and *Eucalyptus globulus* leaf are well reported in the literature, qualitative and quantitative variations may exist in genuine samples according to biotic and abiotic factors.

Insect repellent formulations, like candles, gels, body cream and diffusers, which contain plant extracts are commercially available and include peppermint, thyme, eucalypt and clove oils. As an example, candles prepared with 5% geraniol can protect against mosquitoes for 3 h (Müller et al. 2009; Trongtokit et al. 2005). However, this protection is not identical between different species; in general, *A. aegypti* is the most difficult species to repel, being tolerant to compounds that are effective on other species (Lupi et al. 2013). The following repellency effects of essential oils are specified to adult female of *A. aegypti*.

The protective activity of *Cymbopogon citratus* (DC.) Stapf (lemon grass) against mosquito bite is due to presence of geranial (13) and neral (14) in its leaves (Leal and Uchida 1998). A most effective action was observed for other species of this genus e.g. *C. nardus* (L.) Rendle (citronella); provided 100% protection to the human skin for 2 h (Trongtokit et al. 2005). This oil was observed to have high levels of geraniol (1), citronellol (2) and citronellal (3). An aqueous cream was prepared with 15% of *C. nardus* oil, together with the same percentage of *Litsea elliptica* Blume (rich in 10-undecen-2-one—15) and *Cinnamomum mollissimum* Hook. f. (rich in benzyl benzoate—16). This cream, when used in field trials was found to active during all the experiment time (3 h) (Jantan and Mohd 1999). *Pelargonium citrosum* Voigt ex Breiter essential oil, also rich in geraniol (1), was able to repel from oil emission area (Matsuda et al. 1996).

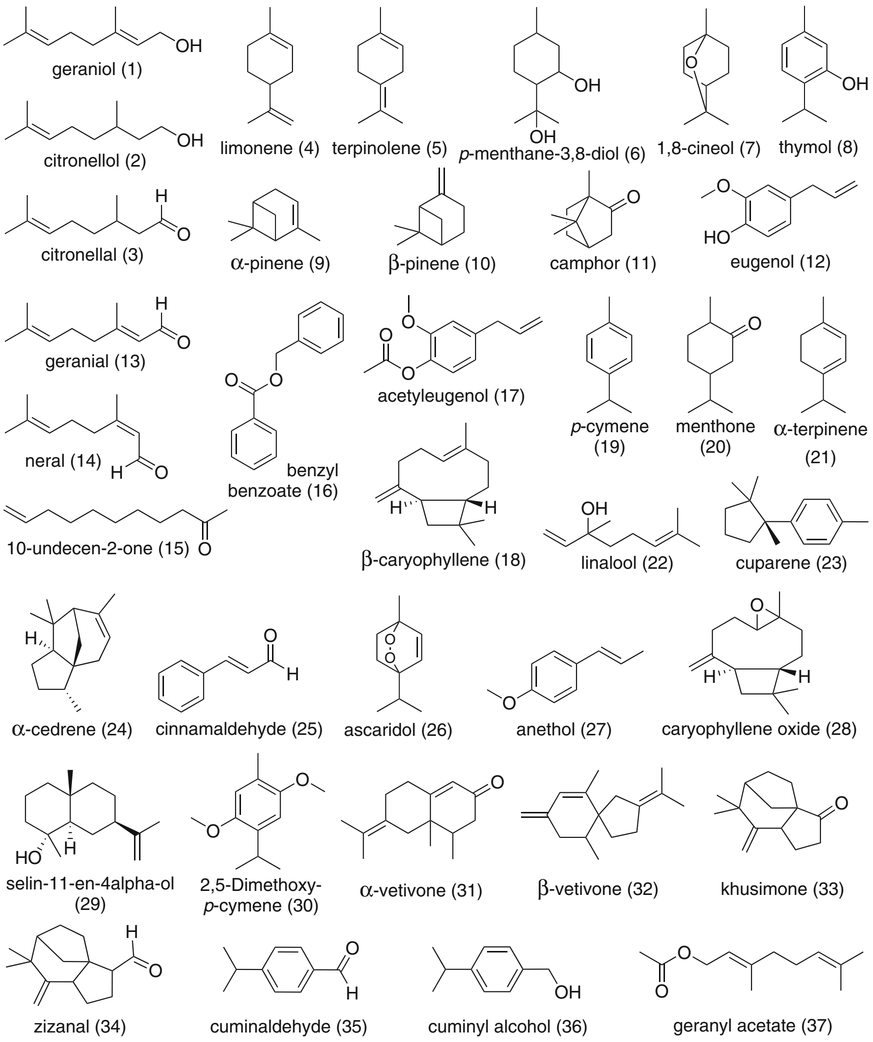

Fig. 1 Chemical structures of compounds studied for their repellent activity against *Aedes aegypti* L. (Diptera: Culicidae)

Barnard (1999) reported the repellency of serial dilutions (100 to 5%) of some essential oils. None of them was effective when tested at less than 10% concentration. The most active was the *Syzygium aromaticum* (L.) Merr. & L.M. Perry oil (clove), protecting for about 4 h against *A. aegypti* bites. Eugenol (12), acetyleugenol (17) and beta-caryophyllene (18) are the major constituents of this extract and they may be responsible for this activity. Similarly, *Thymus vulgaris* L. (thyme) and *Mentha piperita* L. (peppermint) exhibited complete protection for more than 2 h and they are rich in *p*-cymene (19), geraniol (1); and menthone (20), respectively. Another work confirmed the significant repellent potential of *M. piperita* oil;

0.1 mL oil on 25 cm^2 skin arm was able to completely protect the area for 2.5 h (Kumar et al. 2011).

An essential oil highly concentrated in α-pinene (9), α-terpinene (21) and linalool (22) from *Juniperus communis* L. leaf provided protection against adult mosquitoes. On the other hand, *J. chinensis* L. and *Cupressus funebris* Endl. wood oils had no repellent effect on female *A. aegypti* and had high levels of cuparene (23) and α-cedrene (24), respectively (Carroll et al. 2011). The fruit essential oil from other species of *Juniperus* genus, *J. macropoda* Boiss., had promising results in the repellent assay. In this study, the highest repellent activity was achieved by the bark oil of *Cinnamomum zeylanicum* L., which is known to be rich in eugenol (12) and cinnamaldehyde (25) (Prajapati et al. 2005). Eugenol was also the main compound of *Atalantia monophylla* DC. essential oil and a formulation of this oil exhibit repellent potential (Baskar et al. 2017). This indicates the interesting potential of this phenylpropanoid in vector-borne diseases.

It is suggested that essential oils rich in limonene (4) and camphor (11) prevent female *A. aegpypti* from biting. The main compound in *Rosmarinus officinalis* and *Baccharis spartioides* oil is camphor (11) with 34 and 51% concentrations, respectively (Boix et al. 2010). Oils of *Aloysia citriodora* Palau, *Tagetes minuta* L. and *Zanthoxylum piperitum* DC. showed high concentration of limonene (4). These samples had significant repellent activity that may be caused by their principal components. Ascaridol (26), corresponding to over 99% of *Chenopodium ambrosioides* L. oil, as well as 1,8-cineol (7) that comprises 93% of *Eucalyptus saligna* Sm. essential oil may play a role in the repellency effect of these plant extract. On the other hand, *Tagetes pusilla* Kunth that contains 75% anethol (27), *Hyptis mutabilis* (Rich.) Briq. and *Achyrocline satureioides* (Lam.) DC., both rich in beta-caryophyllene (18) showed no or low repellence activity. These data suggest that these compounds do not hold promise to repel insects (Choochote et al. 2007; Gillij et al. 2008), as was confirmed by the repellency test of the standard reference of beta-caryophyllene (18) and caryophyllene oxide (28) against female *A. aegypti* mosquitoes, where both of these compounds showed no activity (Demirci et al. 2013).

Flower oil of *Pluchea carolinensis* (Jacq.) G. Don is mainly composed by the oxygenated sesquiterpene selin-11-en-4alpha-ol (29) and by 2,5-dimethoxy-*p*-cymene (30). This plant essential oil has been reported to protect against mosquito bites (Kerdudo et al. 2016). The preparation of 10% *Vetiveria zizanioides* (L.) Nash oil showed interesting repellent activity. Its composition comprised hundred compounds and the most concentrated were alpha-, beta-vetivone (31 and 32), khusimone (33) and zizanal (34) (Trongtokit et al. 2005).

Citronellal (3), limonene (4), linalool (22), cuminaldehyde (35), cuminyl alcohol (36) and geranyl acetate (37) were tested on human skin as pure compounds and all of them caused repellent effect against female *A. aegypti* (Kwon et al. 2011). Essential oils are complex chemical mixtures of major and minor compounds. Biological activities of essential oils are mainly correlated with their major substances. However, minor components can interfere with the final result of the repellency potential of a natural product by the synergistic phenomenon existing among molecules (Dias and Moraes 2014).

Mode of Action

Current control strategies mainly rely on synthetic pesticides, insect growth regulators and microbial control agents (Benelli 2015). The most commonly used insect repellents for topical application are based on the synthetic ingredient repellent N, N-Diethyl-3-methylbenzamide (DEET) (Dickens and Bohbot 2013). Even though it is approved by FDA for its safety, but some authors reported the disadvantages associated with its use, such as the risk of toxicity to human beings and the development of resistance (Clem et al. 1993; Corbel et al. 2009; Stanczyk et al. 2010). Several modes of action for DEET have been proposed (Bohbot and Dickens 2010; Pellegrino et al. 2011), but these are still under considerable debate. The same thing happens with repellents derived from essential oils, which are being developed as an alternative to DEET. This is probably due to its complex mixtures of chemical compounds. Therefore, we briefly outline the main modes of action of essential oils and isolated compounds against *A. aegypti* as described in the recent literature.

The knowledge about the mode of action is important to improve the quality and sustainability of a product, besides preventing development of pesticide resistance. For this, is necessary to understand about the physiology of the insect (El-Wakeil 2013). For example, how and when a product presents a rapid mode of action (pointed to neurotoxic effect) due to interference with the octopaminergic system of insects. Octopamine receptors are present in the nervous system of invertebrates and acts as a neurotransmitter, neurohormone, and neuromodulator that influence many features of animal physiology and behavior (Enan 2001; Kostyukovsky et al. 2002).

The terpene compounds carvacrol, coumarin, eucalyptol, eugenol and geraniol were found to perform docking with octopamine receptor and acetylcholinesterase protein of *A. aegypti* and human models. Therefore, these are the probable target site of these terpene compounds (Khanikor et al. 2013).

Acetylcholinesterase (AChE), is an essential enzyme for the function of nervous system. It is involved in the termination of impulse transmission at the synaptic cleft by hydrolysis of the neurotransmitter acetylcholine. The enzyme inactivation leads to acetylcholine accumulation, and neurotransmission disruption. Hence, inhibitors of this enzyme play a key role in the *Aedes* control (Colovic et al. 2013; Otero et al. 2014).

The insect gut is another target of many repellent products. Silva et al. 2005a showed that the essential oil of *Microlobius foetidus* possess insecticide potential against *A. aepypti* by retarding the overall growth. *M. foetidus* essential oil inhibited acetylcholinesterase as well as change the PM by inhibition of digestive enzymes, trypsin and chymotrypsin. Edwin et al. (2016) observed that the treatment with the bioactive compound andrographolide from *Andrographis paniculate,* severely affected the midgut epithelium of *A. aegypti* larvae.

The peritrophic matrix (PM) consists of chitin and glycoprotein that lines the invertebrate midgut epithelium. The mainly functions of PM in insects are:

separation of midgut cavity into three distinct compartments, protection of ingested toxins, physical barrier, protecting the midgut epithelium from damage by abrasive food particles, bacteria, virus and parasites (Hegedus et al. 2009). Although the repellent activity of essential oils against *A. aegypti* is well described in the literature and repellents derived from essential oils are available in market but still only a few studies about the structure-activity relationship suggesting their mode of action have been reported.

Wang et al. (2008) used QSAR model to show how the chemical structure of terpenoids affect repellency. The features that influenced the activity were boiling point, dipole moment, the presence of oxygen-containing substituent groups. All repellents synthesized from α- and β-pinene tested had two functional/substituent groups, one negatively charged end containing either ester/ether bonds or an ethanol hydroxyl group and other positively charged end containing alkane groups. The positive end was more favorable to receptor interactions, indicating that the repellent–receptor interactions are most likely related to electrophilic interactions.

Another important aspect to consider about the effectiveness of essential oil repellent action is the synergism. Many studies have shown that synthetic compounds or blends of pure compounds are less effective when compared to the activity of their corresponding essential oil (Rehman et al. 2014). Indeed, the synergistic activity of different metabolites may result in an enhanced bioactivity than the single metabolite (Gillij et al. 2008). Because of its synergistic activity, some substances are included in the formula to provide an improvement in the efficacy (Choochote et al. 2007; Kiplang'at Mwangi 2014).

In Vitro Culture of Plants Producing Essential Oils Affective Against *A. aegypti*

In vitro culture offers an alternate means for fast multiplication of rare and endangered plant species (Malik et al. 2009, 2016, 2010). There are different methods for multiplication of plants under in vitro conditions, which may include shoot, root, callus or embryo culture. Using plant cell culture, it is possible to obtain high value secondary compounds including essential oils and optimize their production through use of elicitors and other methods, without harvesting the plants from nature (Rodrigues et al. 2014). *C. shoenanthus*, commonly known as camel grass possesses essential oil active against *Aedes aegypti*. Overcollection from the natural habitat has threatened this plant species to the edge of extinction. El-Bakry and Abdel-Salam (2012) developed regeneration protocols from callus and suspension cultures of *Cymbopogon shoenanthus* subsp. Proximus. The different stages of somatic embryogenesis were obtained by varying growth regulators, phosphorus concentration and different nitrogen sources. The composition of essential oils depends greatly on the presence of growth regulators in *Melissa officinalis*. Inclusion of indole-3-acetic acid (11.42 μmol L^{-1}) and benzylaminopurine (8.87 μmol L^{-1}) led to 1.7 and 2.2 fold in proportion of nerol and geraniol, respectively in 60-day-old whole plants. However, plantlets grown on MS medium showed 1.4 and 4.1 times in proportion of nerol and geraniol, respectively, when compared with *ex vitro* plants (Silva et al. 2005b). A protocol for callus induction and an identification of the callus growth pattern in *Piper permucronatum* leaves, which are source of essential oils against *A. aegypti* larvae, have been studied by Santos et al. (2016) Combination of auxin, 2,4-Dichlorophenoxyacetic acid (4.52 μM) and cytokinin, benzylaminopurine (4.44 μM) favoured highest percentage of callus induction. The growth pattern of callus showed sigmoid shape with deceleration phase started on 56th day of culture. Cytokinins; benzylaminopurine, kinetin and thiadiazuron triggered the induction of adventitious shoot production from young leaf and petiole explants in *Pelargonium X Citrosum* (Zhou et al. 2007). *Pelargonium X Citrosum*, also called mosquito plant, is a genetically engineered hybrid by cell fusion that incorporated the characteristics of scented geranium and citronella grass. The mosquito repelling activity along with attractive foliage and sweet lemony citronella smell make this plant a very popular all over the world. In *Artemisia vulgaris*, in vitro method for the steady production of a large number of plants for essential oil production has been developed (Govindaraj et al. 2008). GC-MS analyses of leaves from acclimatized plants showed the presence of 88 components with camphor, alpha-thujone, germacrene D, camphene, 1,8-cineole and beta-caryophyllene as main components.

Chemical agent or elicitors modulate biochemical signaling of the different metabolic pathways and hence the production of bioactive compounds (Zhao et al. 2005). The perception of elicitor signal directs the activation of transduction factors regulating expression of the genes involved in the biosynthesis of secondary metabolites. Concentration and type of elicitor used in the medium affect the accumulation of cell biomass, chemical composition and production of essential oils in plants. Elicitors, methyl jasmonate and chitosan have been studied to see their effects on growth characteristics and induction of phytochemicals in *Ocimum basilicum* L., *Ocimum sanctum* L. and *Ocimum gratissimum* L. cell suspension cultures (Mathew and Sankar 2012). Both the elicitors enhanced the accumulation of cell biomass when used individually as compared to use in combination. Highest biomass in *O. basilicum* and *O. sanctum* was found at 12 h for and 48 h for with 25 μM methyl jasmonate, while maximum enhancement of biomass for *O. gratissimum* has been achieved at 8 h by using 50 μM of methyl jasmonate. On the other hand, chitosan at 200 mg/L and 50 mg/L concentrations has been observed to be optimum for *O. basilicum* and *O. sanctum*, *O. gratissimum*, respectively after 24 h. In *Azadirachta indica*, highest concentration of azadirachtin (0.2470 μg g^{-1}) was produced from cell mass grown in a medium with glucose (2%, v/v), hydrolyzed casein (500 mg L-1) and 100 μM methyl jasmonate (Rodrigues et al. 2014).

The biosynthetic disposition of essential oils in genetically transformed cultures has been examined in several plant species. It is established that essential oil content increases with the increase in organizational level. Hairy roots, also called genetically transformed roots are characterized by the fast growth, profuse branching and able to produce biomass and bioactive compounds comparable or exceeding that of, the intact plant roots or fastest growing cell cultures. Hairy roots obtained after transformation of *Matricaria recutita* (Chamomile) plants with *Agrobacterium rhizogenes* strain A4-Y were able to produce essential oil as found in intact plant. The composition of essential oil in hairy roots differed in proportion when cultured on liquid and solid MS and G5 media. The main component of essential oil was trans-β-famesene as found in the intact roots (Maday et al. 1999).

Conclusion

Mosquito-borne diseases cause threat for millions of people worldwide. *Aedes aegypti* have been implicated in recent large outbreaks of dengue, zika, chikungunya and yellow fever, which generated great community interest in the most effective and ecofriendly way to avoid mosquito bites. Monoterpenes such as geraniol and citronellol and the phenylpropanoid eugenol are commonly the main compounds of plant essential oils with high repellent activity against *A. aegypti*. In vitro production of essential oils from plants could act as an alternative for safe and ecofriendly synthesis of natural mosquito repellents. Literature studies suggest that this attempt has been carried out in many plant species but still more studies are required to focus on genetic level to understand the synthesis of these compounds in plants and their mode of action.

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Adaptation Strategies and Defence Mechanisms of Plants During Environmental Stress

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Abstract Several biotic and abiotic stresses affect plant growth, development and crop productivity. To cope up all these stresses, plant develops certain efficient strategies to avoid or tolerate the stresses which allow them to adapt and defense themselves from stress situations. Such adaptation strategies are at morphological, anatomical, biochemical and molecular levels. Molecular crosstalk, epigenetic memories, reactive oxygen species (ROS) signaling, accumulation of plant hormones such as salicylic acid, ethylene, jasmonic acid and abscisic acid, change in redox status and inorganic ion fluxes, R-gene resistance and systemic acquired resistance (SAR) are some of the modifications/mechanisms adopted by plants to adapt and defense themselves from the environmental stress. The novel "omics" technologies allow the researchers to identify the genetics behind plant stress

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response and adaptation and provide unbiased data that can be precisely used to investigate the complex interplay between the plants, its metabolism and the stress environment.

Keywords Stress · Adaptation strategies · Molecular crosstalk · Epigenetic memories

Introduction

Stress can be defined as a stimulus or influence that is outside the normal range of homeostatic control in a given organism. If a plant is exposed to extreme stress conditions that are beyond the tolerance level, mechanisms are activated at morphological, physiological, biochemical and molecular levels. Once the stress is released, a new physiological state is established and the plant may return to the original state thereby, reestablishing the homeostasis (Fraire-Velazquez et al. 2011). Plants being a sessile organism faced many environmental challenges including biotic and abiotic factors. In the course of evolution, plants have developed highly sophisticated and efficient strategies to cope up environmental stress imposed by the nature. Even though plant species vary in their sensitivity and response to various environmental stresses, they have developed various adaptation mechanisms to encode stress perception, signaling cascades and gene transcription networks in response to environmental cues (Fu and Dong 2013; Sanghera et al. 2011; Joshi et al. 2016) and developed a multitude of defense mechanisms to adapt and survive during the harsh environmental conditions.

Major mechanisms underlying environmental stress adaptation and defense include reactive oxygen species (ROS) signaling; accumulation of plant hormones such as salicylic acid, ethylene, jasmonic acid and abscisic acid (Wani and Kumar 2015); change in redox status and inorganic ion fluxes; molecular crosstalks; epigenetic modifications; R-gene resistance and systemic acquired resistance (SAR), etc. (Kissoudis et al. 2014). Physical barriers such as the cuticle, stomata and cell walls are also important for timely pathogen recognition and interception (Asselbergh et al. 2007). Biochemical studies revealed processes induced by stress that lead to accumulation of metabolites like nitrogen-containing compounds such as proline, quaternary amino compounds and polyamines; hydroxyl compounds like sucrose, polyols and oligosaccharides (McCue and Hanson 1990). Through "omics" technologies, it has been found out that plant responses to stress are mediated through profound changes in gene expression resulting in changes in composition of plant transcriptome, proteome and metabolome (Perez-Alfocea et al. 2011; Shriram et al. 2016). These novel techniques allow researchers to identify the genetics behind plant stress responses and enable to investigate the complex interplay between the plant, its metabolism and the stresses imposed to them (Perez-Clemente et al. 2013).

In this article, we will elaborate on the morphological, anatomical, biochemical and molecular adaptation strategies against biotic and abiotic stresses. We will also discuss molecular crosstalks and epigenetic modifications for stress response and adaptations in plants. Passive and active defense mechanisms of plant [including hypersensitive response, systemic acquired resistance (SAR) etc.] against pathogen and mechanisms against insect pests like antixenosis, antibiosis and tolerance are also highlighted in the work.

Adaptation Strategies of Plants During Environmental Stress

Morphological Adaptation Strategies

The productivity of crop is reducing due to harmful effects of various biotic and abiotic stresses. Minimizing these losses is a major area of concern to ensure food security under changing climate condition of the growing global population. Somehow, the plant changes their morphological structure to adopt the adverse climate condition to sustain in the existing environment. Abiotic stresses, such as drought, extreme temperature, cold, heavy metals, or high salinity, severely impair in plant growth, development and eventually in the productivity of the crops (Wani et al. 2016a; Hossain et al. 2016; Sah et al. 2016). These stresses reduced the yield of crops, depending on the type of crop and stress period. Drought, being an important environmental stress as it severely impairs plant growth and development (Shao et al. 2008a, b; Wani et al. 2016b; Telem et al. 2016; Dar et al. 2016). Drought impaired in germination and poor crop establishment. It also induced reduction in leaf area is ascribed to suppression of leaf expansion through reduction in photosynthesis (Rucker et al. 1995). Drought related reduction in yield and yield components of plants could be ascribed to stomata closure in response to low soil water content, which decreased the intake of CO_2 and as a result, photosynthesis decreased (Cornic 2000; Flexas et al. 2004). The presence of small roots to the lateral roots is considered as an adaptive strategy to increase water uptake by providing more absorptive surface. However, the growth of lateral roots is significantly reduced, mainly by suppression of the activation of the lateral root meristems (Deak and Malamy 2005). Plants growing in dry areas have developed xeromorphic traits such as leaf shedding as well as decrease in leaf number, leaf size, and branching. Presence of specialized tissues like rhizodermis, with a thickened outer cell wall or suberized exodermis, or reduction in the number of cortical layers is considered an adaptive advantage for drought stress survival. Another adaptation to counter drought stress is sclerophylly, where plants form hard leaves that will not suffer permanent damage due to wilting and can be restored to full functionality when normal conditions resume (Micco and Aronne 2002). Recent research has shown that decreased stomatal conductance in response to drought stress is related not only to reduced expression of aquaporin genes but also to anatomical traits leading to reduction of chloroplast surface area exposed to intercellular space per unit leaf area (Miyazawa et al. 2008; Howe and Jander 2008).

Heavy metal contamination may rigorously hinder the plant growth and its productivity, and also increase the risk of harm in animal and human health via the bio magnification process (Sharma 2012). Arsenic (As) has great potential to block various metabolic processes in cell, and may interact with sulphydryl groups of the proteins, and also replace the phosphate group of ATP. Similar to As, Mercury (Hg) also interacts with the -SH groups and form the S-Hg-S bridge, disrupting the stability of the group and consequently affects seed's germination and embryo's growth. The replacement of central atom (Mg) of chlorophyll molecule with As leading to the dismantle of chlorophyll resulting in the breakdown of photosynthesis and growth of the sunflower seedlings under As stress (Patra et al. 2004; Yadav et al. 2014). Chromium (Cr) stress declined biomass accumulation in barley plants by causing ultrastructure disorders in leaves such as uneven thickening and swelling of chloroplast, increased amount of plastoglobuli and disintegrated thylakoid membrane which resulted into decline in net stomatal conductance, cellular CO_2 concentration, transpiration rate, photochemical efficiency and net photosynthetic rate (Ali et al. 2013). In another finding, Hg reduces the plant growth due to its higher affinity to bind with-SH groups of proteins and causes uneven modification in protein structure (Cui et al. 2014). It has been observed that brinjal plant when exposed to Cadmium (Cd) showed restricted growth, leaf area, photosynthetic pigment and chlorophyll fluorescence (Singh and Prasad 2014). Similarly, Shahid et al. (2014) have reported that the growth and photosynthetic pigments of *Vicia faba* got inhibited under lead (Pb) stress.

The plant that grow in the cold region modified their morphological structure and escape the action of free air circulating by reducing plant size and adopting compact growth forms, thereby engineering their own microclimate in addition to sheltered habitat selection. In addition, some tropical alpine taxa have been found to perform what is called leaf super-cooling, which is retaining water in a gel-like metastable state below freezing point (to avoid nucleation). When the super-cooling capacity is exhausted around −12 °C, tissues freeze immediately and died. There is evidence that the xylem water of trees of cold regions can supercool down to −40 °C reported (Korner 2016).

Plants, animal and insects have been living together since million years ago. In co-evolution, they have evolved strategies to avoid each other's defence systems, resulted in the development of an elegant defence system in plants that has the ability to recognize the nonself molecules or signals from damaged cells, much like the animals, and activates the plant immune response against the herbivores (Howe and Jander 2008; Verhage et al. 2010; Hare 2011). Plant structures are the first line of defence against herbivory, and play an important role in host plant resistant to insects. The first line of plant defence against insect pests is the erection of a physical barrier either through the formation of a waxy cuticle, development of spines, setae, and trichomes (Hanley et al. 2007; Agrawal et al. 2009; Sharma et al.

2009; He et al. 2011). Development of structural traits such as spines and thorns, trichomes, toughened and sclerophylly nature of leaves reducing the palatability and digestibility of the tissues, incorporation of granular minerals into plant tissues, and divaricated branching play a leading role in plant protection thereby, reducing the herbivore damage (Handley et al. 2005; Hanley et al. 2007; Chamarthi et al. 2010; He et al. 2011).

Anatomical Adaptation Strategies

Physical Factors (Temperature, Light, Water, Oxygen Deprivation)

Plants are frequently exposed to many stress conditions, such as low temperature, salt, drought, flooding, heat, oxidative stress, and heavy-metal toxicity. In which, water stress is the major problem in agriculture. The ability of plants to withstand such stress is of immense economic importance (Shao et al. 2008a, b). Water stress is the state of moderate loss of water, which leads to closure of stomata and limits gas exchange. Water-stress tolerance involves subtle changes in cellular biochemistry. It appears to be the result of the accumulation of compatible solutes and of specific proteins that can be rapidly induced by osmotic stress (Shao et al. 2005). Water stress influences plant growth at various levels, from cell to community (Blumwald et al. 2004; Colom and Vazzana 2001). The quantity and quality of plant growth depend on cell-division enlargement, and differentiation, and all of these events are affected by water stress (Correia et al. 2001; Cabuslay 2002). There is evidence that stiff, leathery leaves are widespread in species adapted to drought occurring in various environments throughout the world. Sclerophyllous leaves are characterised by reinforcing tissues (e.g. thick-walled epidermal cells, sclereids, etc.) which prevent the collapse of the whole structure when water availability is scarce, thus reducing the risk for mechanical damage. Under drought conditions, a sclerophyllous leaf slightly reduces its volume thanks to thick cuticle and thick-walled epidermal cells, but thin-walled mesophyll cells severely shrink resulting in an increase of intercellular spaces. This allows photosynthesis to remain active also in conditions of severe water stress when other leaf types wilt (Shields 1950).

Heat Stress

Plants are frequently subjected to heat stress, which can severely hinder the growth and development and may even cause plant death. Heat stress is another serious threat to crop production and quality worldwide. Moreover extensive agricultural losses have been attributed to hindrance to growth due to heat (Wahid et al. 2007; Kotak et al. 2007). In general, it is evident that high temperature considerably affects anatomical structures not only at the tissue and cellular levels but also at the sub-cellular level which results in poor plant growth and productivity. The anatomical changes under high ambient temperatures are generally similar to those under drought stress (Wahid et al. 2007). Under heat stress, there is a general tendency of reduced cell size, closure of stomata and curtailed water loss, increased stomatal and trichomatous densities, and greater xylem vessels of both root and shoot (Anon et al. 2004), severely damages mesophyll cells and increased permeability of plasma membrane in grapes (Zhang et al. 2005). Different plants show varying responses under high temperature regime such as production of polymorphic leaves to reduce transpiration by showing bimodal stomatal behavior (Sayed 1996), changing the structural organization of thylakoids reducing photosynthesis (Karim et al. 1997). In grapes, chloroplasts in the mesophyll cells became round in shape, the stroma lamellae became swollen, and the contents of vacuoles formed clumps, whilst the cristae were disrupted and mitochondria became empty (Zhang et al. 2005). Lima et al. (2013) observed that coffee plants subjected to heat stress (37 °C) changed structural cell anatomy. Structural variations in the lignin contents of cell walls under abiotic stress may be the result of increased oxidative stress in wall-modifying plant tissues. Structural variations in the lignin contents of cell walls under abiotic stress may be the result of increased oxidative stress in wall-modifying plant tissues (Moura et al. 2010).

Chemical Factors (Salinity, Heavy Metals, Pollution)

Salinity

Salinity is one of the most significant environmental challenges limiting plant productivity, particularly in arid and semi-arid climates (Ashraf and Harris 2004; Hussain et al. 2009; Wani and Gosal 2011; Wani and Hossain 2015). A soil is considered to be saline when the electric conductivity (EC) of the soil solution reaches 4 dS m^{-1} (equivalent to 40 mM NaCl), generating an osmotic pressure of about 0.2 MPa and significantly reducing the yields of most crops (Munns and Tester 2008; Wani et al. 2010). As a consequence of soil salinity, ion toxicity leads to chlorosis and necrosis, mainly due to Na^+ accumulation that interferes with many physiological processes in plants (Munns 2002). Most salinity adaptive mechanisms in plants are accompanied by certain morphological and anatomical changes (Larcher 2003). Plants tolerant to NaCl implement a series of adaptations to acclimate to salinity, including morphological, physiological and biochemical changes (Pathak and Wani 2015). These changes include increase in the root/canopy ratio and in the chlorophyll content in addition to changes in the leaf anatomy that ultimately lead to preventing leaf ion toxicity, thus maintaining the water status in order to limit water loss and protect the photosynthesis process (Acosta-Motos et al. 2017). It is reported that, the cell walls of root cells of salinized plants are often unevenly thickened and convoluted (Shannon et al. 1994). Salts often promote the suberisation of the hypodermis and endodermis in woody tree roots, resulting in the formation of a well-developed casparian strip closer to the root apex, different to that found in non-salinized roots (Walker et al. 1984). Irrigation with saline water has also been found to increase root diameter and root density of *Callistemon citrinus* plants (Álvarez and Sánchez-Blanco 2014). In other plant species (*Picea* sp., *Pinus banksiana*, *Portulaca oleracea*), root diameter (hypertrophy) is found to increase in response to salinity (Franco et al. 2011; Croser et al. 2001). Under saline conditions cell wall properties change and leaf turgor and photosynthesis rates decrease, leading to a reduction in total leaf area (Franco et al. 1997; Rodríguez et al. 2005). Romero-Aranda et al. (1998) studied anatomical disturbances produced by KCl, $CaCl_2$, NaCl in Carrizo citrange and Cleopatra mandarin citrus varieties. The salt-induced declines in photosynthesis rates were linked to changes in leaf anatomical properties, such as the increase in leaf thickness and the lower area/volume ratio of mesophyll cells, increased leaf succulence and reduced intercellular air spaces, the surface/volume ratio of cells and tissue density. The increase in leaf thickness, in combination with several metabolic components such as Cl^- overloading, low Mg^{2+}, stomatal closure and chlorophyll loss, may contribute to the decline in photosynthesis. Anatomical modifications in leaves also include an increase in palisade parenchyma and intercellular spaces and a decrease in spongy parenchyma, serving to facilitate CO_2 diffusion in a situation of reduced stomatal aperture (Acosta-Motos et al. 2017).

Heavy Metals Stress

Other than moisture and salinity stress, the stress given by the heavy metals due to ever increasing population is also becoming a matter of concern. The phytotoxic effect of heavy metals in plants manifests itself through visual symptoms such as chlorosis, necrosis and wilting, and through reduced growth and biomass accumulation (Marques et al. 2000; Sanitá di Troppi and Gabbrielli 1999). Gupta and Chakrabarti (2013) observed that heavy metal (Hg, Pb and Cd) accumulations in *Bruguiera sexangula* indicated deformed vascular bundle with Hg treatment. Xylem and phloem deformation was also recorded in treatment of Cd which finally led to visible toxicity. The stem transverse section (T.S.) for Cd treatment showed diffusion of that heavy metal up to the pith region. When stem T.S. of Hg was compared with control, it was observed that the stelar region was greatly affected. Penetration of Pb inside the stem starting from epidermis to pith region was noticed. Kasim (2006) found that *Sorghum bicolor* treated with copper or cadmium applied alone or in combination caused significant reduction in root diameter, width and thickness of leaf midrib, diameter of xylem vessels of all seedling organs, parenchyma cell area in the stem, leaf midrib and pith and cortex of root, dimensions of stem vascular bundles, number of xylem arms in root, and frequency of stomata on abaxial leaf surface. The effect was more pronounced when Cu and Cd were applied together. The molecular-physiological mechanism responsible for observed changes in anatomy is not yet clear. It is also reported that exposure to heavy metals leads to a reduction in the size of mesophyll cells (Sridhar et al. 2005; Zhao et al. 2000) and the collapse of palisade and spongy parenchyma cells (Sridhar et al. 2005). According to Pasternak et al. (2005) architectural changes imposed by Cu and other abiotic stresses on roots of *Arabidopsis thaliana* may be attributed to alteration in phytohormone metabolism and local auxin accumulation near the root pericycle. Gomes et al. (2011) observed the thickened the cell layers of endodermis and exodermis in the root tissues and the cell walls of the xylem and cortical parenchyma of *Brachiaria decumbens* Stapf. due to heavy metals (Cd, Pb, Zn, Cu). In the leaf tissues, the adaxial and abaxial epidermis presented increased thickness while the leaf blade presented reduced thickness as contamination increased with consequent change in the root growth rate. In general, the effects of heavy metal increased with the metal concentration. The ill effect of moisture stress may be enhanced by other abiotic stresses like heavy metals. Similarly, Greco et al. (2012) observed additive effects of the stresses on growth and the increase in the early mortality of plants by metal contamination in combination with drought.

Pollution

Studies regarding the anatomy of the vegetative organs under polluted conditions have been carried out by various workers (Alves et al. 2008; Silva et al. 2006; Verma et al. 2006). Epidermis structures like trichomes have been reported to bear the brunt of damage from cement dust in *Cajanus cajanus* exposed to foliar dust application (Baralabai and Vivekanandan 1996). Similarly, Gostin (2009) observed the presence of the phenolic compounds (dark deposits from the epidermis, assimilatory and vascular tissues) in *Trifolium* spp. indicate that long-term exposure to air pollutants leads to enhanced accumulation of these compounds. The stomata decrease in size and increase in density in leaves from high polluted sites. In the mesophyll cells (both in palisade and in spongy parenchyma) dark phenolic deposits could be observed. The enhanced accumulation of phenolics and lignin is considered to be one of the most common reactions of plants to stress (Wild and Schmitt 1995). Verma et al. (2006) found a significant decrease of stomatal density and stomatal index in *Ipomea pes-tigridis* grown under various degrees coal-smoke pollutants. However, Ogunkunle et al. (2013) observed no anatomical modifications to the cement factory pollution in *Pennisetum purpureum*, which may indicate that the dose-response level of the pollutants has not been reached in the grass. But there were significant reduction in stomatal size and increased stomatal index in the leaves of *Sida acuta.* This could be favourable anatomical adaptations to a polluted environment. In another study, plane tree growing in urban area reduced the size and lower stomata density, thin cuticle but the other anatomical properties were unaffected suggesting that plane trees can cope with traffic exhaust in megacities (Pourkhabbaz et al. 2010).

Biological Factors (Pests, Diseases and Allelopathy)

Plants respond to herbivore attack through an intricate and dynamic defense system that includes structural barriers, toxic chemicals, and attraction of natural enemies of the target pests (War et al. 2012). Rodrigues et al. (2015) observed various anatomical changes in young coffee leaves infected by *P. syringae* pv. *garcae* which are similar to the tabtoxin damage induced by bacteria, which causes degradation of the thylakoid membrane and chloroplasts (Bender et al. 1999), and favors the tissue colonization and its use as substrate. Palmer and Bender (1995) observed that, tomato inoculated with *P. syringae* observed plasmolysis of the cellular content in tomato mesophyll, as well as cell hypertrophy and hyperplasia. Similar changes were here observed in the mesophyll of coffee leaves. Sclerophylly (reinforcing tissues) has been interpreted as a phenomenon linked to functions as protection against pathogens or as response to scarce nutrient availability (Salleo and Nardini 2000). Sclerophylls are widely distributed in arid and semiarid environments together with seasonally dimorphic species.

Molecular and Biochemical Adaptation Strategies

The understanding of the mechanism of complex nature of stress signaling and plant adaptive strategies would require the analysis of the function of enormous amount of genes involved in stress response (Wani et al. 2012). According to the result of several investigations, plant defense response genes are transcriptionally activated by pathogens as well as by different types of abiotic stress. The activation of specific defense genes against certain pathogens depend on specific environmental conditions and this suggest the interplay of certain complex signaling network that enable the plant to recognize and protect itself against different stresses including pathogens (Bansal et al. 2012; Wani et al. 2013a; Khan and Wani 2014; Jaspers and Kangasjarvi 2010).

The APETALA2 (AP2)/ethylene-responsive-element-binding factors (ERFs) proteins function as either activators or repressors of transcription of gene as response to both biotic and abiotic factors to adapt under adverse environmental conditions (Fujimoto et al. 2000). The RNA levels of specific AP2/ERF genes are known to be regulated by factors like drought, cold, pathogenic infection, salinity, wounding or treatment with salicylic acid or jasmonic acid (Onate-Sanchez and Singh 2002). These genes resulted in improved tolerance against osmotic stress and pathogen attack in tobacco (Park et al. 2001), and were also identified in rice (Datta et al. 2012), grape (Licausi et al. 2010), Arabidopsis (Kang et al. 2011), Wheat (Zhuang et al. 2011), apple (Zhao et al. 2012) and potato (Bouaziz et al. 2015). Nakano et al. (2006) and Wu et al. (2007) reported that the AP2/ERF proteins from one plant species are functional in another plant species. Therefore, these can be potentially utilized in increasing the adaptation and stress tolerance of plants.

Another important family of transcription factors is the “basic-domain leucine-zipper (bZIP) which are regulators of many important plant processes including defense against pathogen, hormone and sugar signaling, osmotic control, abiotic stress signaling, energy metabolism, etc. (Perez-Clemente et al. 2013; dos Reis et al. 2016). TGA/*octopine synthase* (ocs)-element-binding factor (OBF), a class of bZIP proteins bind to the *activation sequence-1* (*as-1*)/*ocs* element and regulates the expression of some stress- responsive genes and are thus, linked to stress responses (Fode et al. 2008a, b). Overexpressions of *AREB2/ABF4* or *ABF3* gene in *Arabidopsis* result in hypersensitivity of ABA, reduced transpiration and elevate drought tolerance (Kang et al. 2002). *OsABF1* gene from rice (Amir et al. 2010) and *SlAREB* gene from tomato (Hsieh et al. 2010) are known to enhance tolerance to drought and salt stress and can be utilized as potential candidates for improving stress tolerance in crops.

MYB and MYC proteins are key factors involved in the response of plants to harsh environmental conditions. *AtMYB30* encodes an activator of the hypersensitive cell death program in response to pathogen attack (Raffaele et al. 2008); *AtMYB33* and *AtMYB101* are involved in ABA-mediated responses to environmental signals (Perez-Clemente et al. 2013); *AtMYB96* regulate water stress and disease resistance by acting through the ABA signaling cascade (Seo and Park 2010); *AtMYB15* is involved in cold stress tolerance (Agarwal et al. 2006). The rice gene *OsMYB4* imparts cold and drought tolerance in transgenic plants of *Arabidopsis* (Vannini et al. 2004), tomato (Vannini et al. 2007) and apple (Pasquali et al. 2008). Yang et al. 2012 reported the regulatory role of *OsMYB2* gene in salt, cold and dehydration tolerance in rice. MYC protein family play a role in the induction of apoptosis and hypersensitive cell death program in response to pathogen attack and also activate the major ABA-dependent stress response (Agarwal and Jha 2010). For instance, *MYC2* is an ABA- and drought-responsive gene and is also capable of activating the expression of the ABA response gene *Responsive to Dessication22* (*RD22*) (Abe et al. 2003).

The NAC family are plant-specific transcriptional factors (TFs) identified in *Arabidopsis* (Ooka et al. 2003), rice (Fang et al. 2008), soybean (Le et al. 2011) and poplar (Hu et al. 2010). These TFs are involved in responses to various biotic and abiotic stresses including drought, cold, salinity, pathogenic bacteria and fungi and low-oxygen stress (Nuruzzaman et al. 2013). In Arabidopsis, the expression of NAC genes viz. *ANAC019*, *ANAC055* and *ANAC072* was induced by drought, salinity and ABA respectively. Moreover, overexpression of these genes also enhances tolerance to drought (Tran et al. 2004). *MlNAC5* gene, when overexpressed, impart hypersensitivity to exogenous ABA and confers enhanced drought and cold tolerance in Arabidopsis (Yang et al. 2015). In transgenic rice, the *Os01g66120/OsNAC2/6* (Nakashima et al. 2009) and *Os11g03300/OsNAC10* genes (Jeong et al. 2010) were reported to enhance drought and salt tolerance, and *Os03g60080/SNAC1* increased grain yield by 21–34% under drought stress condition (Hu et al. 2006).

Recently, the identification and characterization of an assortment of Al resistance genes in plants allow us to understand in depth about the expression of these genes in response to Al stress. Al resistance gene expression is localized to the root tip, where Al toxicity is prevalent. This gene expression is often increased by Al stress. For e.g., Al-induced increase in expression of *ALMT* and *MATE* gene has been documented for *AtALMT*, *BnALMT1*, *SbMATE*, *ScALMT*, *ScFRDL2*, *OsFRDL4*, *VuMATE1* and *ZmMATE1* (Kochian et al. 2015). Transposon-mediated alteration of *TaMATE1B* expression in wheat is known to confer constitutive citrate efflux from root apices (Tovkach et al. 2013). OsART1 in rice (Yamaji et al. 2009) and AtSTOP1 in Arabidopsis (Iuchi et al. 2007) are the transcription factor family involved in the positive regulation of Al-induced expression of Al resistance genes. AtSTOP1 is reported to involve in the Al-induced expression of several *Arabidopsis* Al resistance genes, including *AtALMT1*, *AtMATE1* (Liu et al. 2009) and *AtALS3* (Sawaki et al. 2009).

Heat stress affect plant growth and development, physiological processes, and most importantly, yield (Hasanuzzaman et al. 2013). Heat stress generates excess reactive oxygen species (ROS), which leads to oxidative stress (Hasanuzzaman et al. 2012). At the molecular level, heat stress causes alterations in expression of genes for osmoprotectants, detoxifying enzymes, transporters, and regulatory proteins (Krasensky and Jonak 2012) and lead to modification of physiological and biochemical processes. During heat stress there is up-regulation of several heat inducible genes; commonly referred as “heat shock genes” (HSGs) which encode HSPs (Chang et al. 2007). This is the basis for the development of heat tolerance for acclimatization or adaptation against heat stress (Moreno and Orellana 2011). In tomato, *HSFA1* act as the ‘master regulator’ of the heat shock response and if this gene is suppressed, normal HSP production does not occur and the plant show extremely susceptible to heat stress (Mishra et al. 2002). In rice plants, overexpression of *sHSP17.7* gene confers tolerance to heat stress (Murakami et al. 2004).

The role of Late Embryogenesis Abundant (LEA) proteins in generating drought tolerant plants has been reported by many researchers. Xu et al. (1996) confirmed the role of LEA protein under water stress by overexpressing the *HVA1* gene from barley into rice plants. The *OsLEA3-1* gene in rice, *BnLEA4-1* and *JcLEA* gene in transgenic *Arabidopsis*, *TaLEA* gene in transgenic poplar, *SmLEA* gene in transgenic *Salvia miltiorrhiza* and *SiLEA14* gene in foxtail millet play a big role in activating the plant defense and adaptation under dehydration conditions (dos Reis et al. 2016).

Aquaporins are protein family that regulates the movement of water through intracellular and plasma membranes of plants and animals (dos Reis et al. 2016) and contributes to the maintenance of cell turgidity (Gomes et al. 2009). Several studies reported the role of aquaporins in response to stress in plants. For e.g. *TaTIP2;2* act as a negative regulator of drought and salinity and its response is independent of ABA (Xu et al. 2013). Khan et al. (2015) reported that the overexpression of *JcPIP2;7* gene help in faster water uptake through outer water channels and allow faster imbibition and thus, accelerate germination. Zhou et al. (2012) showed that *TaAQP7* gene enhance drought tolerance in transgenic tobacco by improving the water retention ability, reduce reactive oxygen species accumulation and membrane damage, and increase the antioxidants' activities. In *Arabidopsis thaliana*, MaPIP1;1 increased drought tolerance associated with decreased membrane injury and improved osmotic adjustment (Xu et al. 2014).

Submergence/water-logging is one of the major harmful abiotic stress for low-lying areas and crop losses due to waterlogging are considerably high (Ahmed et al. 2012). The stem elongation in deepwater rice varieties is mainly controlled by three QTLs. Hattori et al. 2009 found out that ethylene response factors *SNORKEL1* and *SNORKEL2* allow rice to adapt to deep water. The *Submergence-1* (*SUB1*) locus on chromosome 9 of rice (Xu and Mackill 1996) contains *SUB1A*, *SUB1B*, and *SUB1C*, all of which encode ethylene response factors and are upregulated under submergence, but only *SUB1A* is responsible for the water- logging tolerance (Xu et al. 2006). *SUB1A* expression is induced by drought and oxidative stress upon desubmergence and positively regulates the expressions of genes involved in ABA-mediated acclimation to drought conditions. Moreover, under oxidative stress, *SUB1A* promotes the genes expression related to the detoxification of reactive oxygen species (ROS) and reduces the accumulation of ROS (Fukao et al. 2011).

A large number of genes and transcription factors are upregulated in response to salinity in different plant species. The SOS gene family (e.g. *AtNHX1* and *H+-ATPase* gene) is believed to play a very intriguing role in ion homeostasis, thereby conferring salt tolerance (Liu et al. 2000). It is also found out that some ROS-scavenging and osmotic-regulating genes are also upregulated by salinity in some plant species. For instance, a continuous exposure to salinity for about 24 h in rice plant resulted in upregulation of glutathione S-transferase and ascorbate peroxidase, both of which play an active role in ROS scavenging (Kawasaki et al. 2001; Gupta and Huang 2014). A rice transcription factor gene *SALT-RESPONSIVE ERF1* (*SERF1*) that showed a root-specific induction upon salt and H_2O_2 treatment were identified by Schmidt et al. (2013).

Although phosphate (Pi) is an essential macronutrient that is necessary for many metabolic processes in plants including photosynthesis and respiration, it is a frequent limiting factor for plant productivity. Pi deficit elicits a complex array of morphological, physiological, and biochemical adaptations, collectively known as the Pi-starvation response (Plaxton 2004). Biochemical adaptations of Phosphate-starved plants include increased efficiency of cellular phosphate uptake through high-affinity Pi transporters, induction of Phosphate scavenging and recycling enzymes like acid phosphatase, secreted nuclease and phosphodiesterase, replacement of membrane phospholipids with non-phosphorus galacto- and sulfonyl-lipids, induction of metabolic phosphate recycling enzymes, enhanced excretion of organic acids like malate and citrate due to PEPCase induction, induction of alternative pathways of cytosolic glycolysis and Respiratory Electron Transport and induction of tonoplast H+-Pumping Pyrophosphatase (Plaxton and Carswell 1999).

In plants, heavy metal toxicity may lead to the over production of ROS, resulting in peroxidation of many vital constituents of the cell (Kumar et al. 2017). Plant defense mechanisms against heavy metal toxicity include Antioxidant defense system and cellular homeostasis. Many enzymatic antioxidants like superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and glutathione-s-transferase (GST) may efficiently convert the superoxide radicals into hydrogen peroxide and further into water and oxygen while low molecular weight non-enzymatic antioxidants like proline, ascorbic acid and glutathione may directly detoxify the ROS (Singh et al. 2015; Singh and Prasad 2014; Yadav et al. 2014; Xu et al. 2009). The exogenous application of proline elevates the level of endogenous proline under heavy metal stress and this helps in maintaining intracellular redox homeostasis potential (Hoque et al. 2008). Proline may enhance the tolerance level of plants by chelating heavy metals in the cytoplasm, regulating the water potential and maintaining osmotic adjustment through cellular homeostasis and reduce metal uptake (Singh et al. 2015).

Molecular Crosstalk and Epigenetic Memory for Stress Response and Adaptation

Recently omics data analysis and functional characterization of individual genes showed a convergence of signaling pathways for biotic and abiotic stress adaptation. Crop resilience to combined biotic and abiotic stress can be enhanced through thorough dissection of physiological and molecular crosstalk (Kissoudis et al. 2014). Whole genome expression meta-analysis experiments under different biotic and abiotic stress treatments revealed that a significant number of genes are commonly regulated under both stress conditions (Shaik and Ramakrishna 2014). These genes include response to ABA, Salicylic acid (SA), Jasmonic acid (JA) and ethylene which are major stress hormone imparting adaptation against both biotic and abiotic stress (Ma and Bohnert 2007). Moreover, several signaling pathways members including mitogen-activated protein kinase (MAPK), Ca^{2+}, ROS, Phospholipids, vesicle trafficking, mitochondrial functions and apoptosis were also induced under both biotic and abiotic stresses (Ma and Bohnert 2007; Kissoudis et al 2014). Transcription factors like WRKY, MYB, ERF, NAC and HSF are also involved in the crosstalk across stress treatment (Shaik and Ramakrishna 2013).

It has been recently shown that plants can remember past stress conditions and can utilize these memories to aid responses when these stresses reappear. The scene behind such memories is the epigenetic mechanisms in the control of gene expression through small RNAs, histone modifications and DNA methylation. Such epigenetic mechanisms are essential for stress memories and adaptation in plants (Kinoshita and Seki 2014). One good example for stress memory is ‘defense priming’ in which the plant displays a more rapid and robust response to pathogen or herbivore in a second attack compared with the first one (Pastor et al. 2013). Priming is also found for abiotic stress like drought (Ding et al. 2012).

Defence Mechanisms of Plants During Environmental Stress

Plants are constantly confronted to various abiotic and biotic stresses that seriously reduce their productivity and growth. Plants have a number of inherent and adaptive mechanisms to cope with stresses in their environment, which include such physical conditions as water (too much as well as drought), temperature (hot and cold), saline soils and oxygen deprivation, as well coping with biotic stresses such as insect pest and pathogens. Exposure of plants to various biotic and abiotic stress induces a disruption and physiological imbalance and thus leading to a reduction in fitness and productivity (Atkinson and Urwin 2012). Abiotic stress is the primary cause of huge loss of cop plants worldwide and resulting growth reduction cane reach >50% in most plant species (Rejeb et al. 2014; Surekha et al. 2015; Wani and Sah 2014). Other than abiotic stress, biotic stresses such as insect pest and pathogens also cause huge loss in term of yield and productivity. The occurrence of simultaneous abiotic and biotic stresses in natural environment presents an added degree of complexity, as the responses to these complex stresses are largely controlled by different hormone signaling pathways that may interact and inhibit one another. Often exposure of plants to long term abiotic stress can weaken the tissue/organs and cause enhanced susceptibility (Goel et al. 2008). Long exposure of biotic stress by insect pest and pathogen also increases the effect of abiotic stress such as water deficit and others (Englishloeb 1990; Khan and Khan 1996). Evidences also suggest that the climate change will also expand the host range of pathogens with increased chances of virulent strain development (Garrett et al. 2006). The ever changing climatic factors has further increases predisposition of complex nature of biotic and abiotic. Therefore, the occurrence of combined biotic and abiotic stress is likely to be higher in future.

Abiotic Stress and Defense Mechanism in Plants

A number of abiotic stresses such as extreme temperature (drought, flooding), high light intensity, osmotic stresses, heavy metals and a number of herbicides and toxins lead to over production of reactive oxygen species (ROS) including H_2O_2 causing extensive cellular damage and inhibition of photosynthesis (Wani and Gosal 2010; Das et al 2016).

Drought

Drought stress has been reported to severely reduce germination and seedling stand (Kaya et al. 2006) in different crop plant of *rainfed* ecology. Drought stress is characterized by reduction of water content, reduced leaf water potential and turgor loss, closure of stomata and decrease in cell growth and enlargement. Acute shortage of water in tissues may result in the arrest of photosynthesis, disturbance of metabolism and finally the death of plant. The adverse effect of drought on plant structure, behavior and function such as xylem embolism, reduced carbohydrate pool size, leaf and fine root production, on the ability of plants to resist pathogen attacks, the impacts on soil microbial dynamics, decomposition and nutrient supply processes, and shifting competitive abilities between plant species could not be underestimated (Ciais et al. 2005).

Mechanism of Drought Tolerance

Plants under drought stress survive by the means of several morphological, biochemical and physiological traits (Wani et al. 2008; Gosal et al. 2009). Drought tolerance of a crop plant has inherent ability to grow, flower and display economic yield under suboptimal water status. Drought stress negatively affects the water relations of plants at cellular, tissue and organ levels, causing specific as well as unspecific reactions, damage and adaptation reactions (Beck et al. 2007). Drought stress for a prolonged period act as a limiting factor at the initial phase of plant growth and establishment.

Morphological Mechanism

Water deficit stress mostly reduced leaf size and growth and in turns the leaf areas in many species of plant like Populus (Wullschleger et al. 2005), soybean (Zhang et al. 2004) and many other species (Farooq et al. 2009). Production of ramified root system under drought is important to above ground dry mass and the plant species or varieties of a species have great differences in the production of roots. The development extensive root system enhances the water uptake and maintains the osmotic pressure through higher proline levels in Phoenix dactylifera (Djibril et al. 2005). Reduced biomass has been recorded under water stressed soybean (Specht et al. 2001), Poncirus trifoliatae seedlings (Wu et al. 2008), common bean and green gram (Webber et al. 2006). Drought stress affects the growth, fresh dry weight and harvestable index in a number of plant species, but the quantitative effect may vary from species to species or varieties to varieties.

Drought Escape

Drought escape occurs when plants develop rapidly and reproduce before drought conditions become severe. Cession of vegetative growth may or may not accompany a drought escape response. Time of flowering is an important aspect related to drought adaptation, where a short life cycle can lead to drought escape (Araus 2002). Drought escape as an adaptive strategy comes from temporally replicated series of observations examining changes in populations following drought events. The genetic base under lying the variation in drought escape has been elucidated in a number of species, although the specific genes/QTLs and their physiological aspects remain to be characterized. Breeding of short-duration varieties has been an effective and viable strategy for minimizing yield loss from reproductive phase drought (Terminal drought).

Drought Avoidance

Drought avoidance consists of a mechanism that maintains relatively high tissue water potential despite a shortage of moisture status in soil. It is performed by maintenance of turgor through deep root system, stomatal control of transpiration and by reduction of water loss by reduced epidermal layers (Kavar et al. 2007). Drought avoidance adaptations can occur through a variety of morphological and physiological traits. Genotypes that adapted to xeric conditions may have lower specific leaf area, greater succulence (Eggli and Nyffeler 2009), increased leaf reflectance (Ehleringer et al. 1976), accentuated leaf lobing (Talbert and Holch 1957) with altered stomatal size and density (Masle et al. 2005) The root character such as root biomass, root length, root angle, root density and root depth are the major drought avoidance traits that contribute to final yield under terminal drought environments. Drought avoidance can evolve a constitute change in water use efficiency or as plastic response under moisture stress or other environment cues. Presently much research has been focused on transpiration efficiency despite the existence of other mechanism of drought avoidance. Therefore it is need to emphasize more on the all possible mechanism for drought avoidance.

Phenotypic Flexibility/Plasticity

Phenotypic plasticity is unique quality of genotype that change their phenotype expressed by a single genotype in different environments. At morphological level, the shoot and root biomass are the most affected and both are the key traits for plant adaptation under drought. Certain morphological traits such as leaf pubescence, root length, trichome and cuticle which help to protect the plants from drought stress. Selection for a deep and extensive root system has been practiced to enhance productivity of food legumes under moisture stress conditions as it can optimize the capacity to acquire water (Subbarao et al. 1995). Intra-specific and inter-specific variation reported in different crop plant for specific morphological traits contributing in drought stress.

Physiological Mechanism

Inadequate water availability in drought prone environment often affects growth and productivity of crops by reducing its water potential and turgor (Kiani et al. 2007; Gosal et al. 2010). Drought tolerant plants have several physiological mechanisms such as Osmotic adjustment, osmo-protection, antioxidation and scavenging defense system which operate at different stage of growth and development. Osmotic adjustment is the main component of plant at cellular level to minimize the damage caused by drought stress in crop plants (Blum 2005). Under drought, excess accumulation of compatible solutes as proline, amino acid and glycine betaine occur (Farooq et al. 2008; Wani et al. 2013b). Drought alters the endogenous growth substances and creates imbalance of hormonal level and ultimately lead to change in metabolic system in stress induced plants.

Antioxidant Defense Mechanism

Under drought stress conditions cell or tissue has exposed for oxidative stress with production of reactive oxygen species. Stress lead to decline in CO_2 influx and stomatal closure. Reactive oxygen species (ROS) such as H_2O_2 (hydrogen peroxide), O_2^- (superoxide) and OH (hydroxyl) radical produced under stress through enhanced leakage of electrons to molecular oxygen (Arora et al. 2002). Oxidative stress damages the plants by oxidizing photosynthetic pigments, membrane lipids, proteins and nucleic acids (Yordanov et al. 2000). To keep the levels of active oxygen species under control, plants have non-enzymatic and enzymatic antioxidant systems to protect cells from oxidative damage (Mittler 2002). Non-enzymatic antioxidants include β-carotenes, ascorbic acid (AA), α-tocopherol. (α-toc), reduced glutathione (GSH) and enzymes including: superoxide dismutase (SOD), guaiacol peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT), polyphenol oxidase (PPO) and glutathione reductase (GR).

Defense Mechanism for Water Logging in Plants

Waterlogging is a serious problem, which affects plant growth and development in low lying rainfed areas in large part of globe (Lone et al. 2016). The prime cause of plant damage under waterlogging is oxygen deprivation, which leads to problem in nutrient and water uptake and ultimately plants die when flooding prevails for longer duration. The reduction of oxygen content below optimal levels, termed hypoxia, is the most common form of stress in wet soils and occurs during short-term flooding when the roots are submerged. Oxygen deficiency generally leads to the substantial decline in net photosynthetic rate (Ashraf et al. 2011). Ethylene accumulates in flooded soils and in submerged plant parts as an adaptive mechanism under hypoxic root and shoot. Ethylene initiates act as regulatory mechanism for many adaptive molecular, chemical and morphological responses that allow the plant to avoid anaerobiosis by enhancing oxygen availability to the roots in a flooded or waterlogged soil conditions (Sairam et al 2008). Aerenchyma formation under submerged or waterlogged condition have been reported in a range of crop species such as, Trifolium subterraneum (Aschi-Smiti et al. 2004), soybean (Bacanamwo and Purcell 1999), wheat (Watkin et al. 1998), rice (Justin and Armstrong 1991), maize (Gunawardena et al. 2001), *Carex* spp. (Visser et al. 2000). Due to shift in energy production metabolism from aerobic to anaerobic mode the energy requirements of tissue is greatly restricted because of very few ATPs generated per molecule of glucose. This necessitates availability of comparatively higher amount of readily metabolizable sugar pool. At the DNA level, low oxygen induced genes have been identified and characterized by the presence of an anaerobic response element (ARE) in the promoter (Walker et al. 1984). Manipulation of gene expression under low oxygen stress in different crop species provides a new insight in order to develop high tolerant crop plants. In future, generation of genetically improved flood tolerant crop plants thought insertion, regulation and manipulation of different genes conferring different metabolic and physiological pathway is one of the most sustainable and viable straggles for low lyaing area or flood prone area.

Defense Mechanism for Heat

Presently global climate change has risen the temperature and it severely affects the growth and production of crops. Heat stress affects plant growth throughout its ontogeny however heat-threshold level varies considerably at different developmental stages. Heat stress also induces indirect injuries that include inactivation of enzymes, inhibition of protein synthesis, protein degradation and loss of membrane integrity (Howrath 2005). Major impact of high temperatures on shoot growth is a severe reduction in the first internode length resulting in premature death of plants (Hall 1992). The most noticeable effect of high temperatures on reproductive processes in tomato is the production of an exerted style (i.e., stigma is elongated beyond the anther cone), which may prevent self-pollination. Poor fruit set at high temperature has also been associated with low levels of carbohydrates and growth regulators released in plant sink tissues. Heat tolerance is the ability of the plants to maintain normal metabolic process and produces economic yields under high temperature. Heat stress causes an abrupt increase in the expression of stress-associated low molecular weight proteins which provide tolerance by stimulating the defense response in plants (Roy et al. 1993). Heat-shock proteins (Hsps) osmoprotectants and antioxidant enzymes are important defence mechanism in encountering heat stress in plants. Hsps play a role in stress signal transduction, protecting and repairing damaged proteins and membranes, protecting photosynthesis as well as regulating cellular redox state (Asthir 2015). Three classes of proteins, as distinguished by molecular weight, account for most HSPs, viz., HSP90, HSP70 and low molecular weight proteins of 15–30 kDa. HSP70 and HSP90 mRNAs can increase ten-fold, while low molecular weight (LMW) HSPs can increase as much as 200-fold under heat stress conditions (Wahid et al. 2007). The major sites of thermal damage are the oxygen-evolving complex (OEC) along with other associated cofactors in photosystem II, carbon fixation by Rubisco and the ATP-generating system. Heat stress also negatively interferes with electron transport and consequently leading to excessive production of reactive oxygen species (ROS) in plant cells. Apart from HSPs, there are a number of other ubiquitin proteins including cytosolic Cu/Zn-SOD and Mn-POD whose expressions are stimulated upon heat stress. The ability of the plant to cope with or adjust to the heat stress varies across and within species as well as at different developmental stages. Traditional breeding has greatly exploited the variability among the species for development of heat tolerance variety. Manipulations of the heat shock protein gene in transgenic plants have the potential to improve common abiotic stress tolerance and this may have a significant impact on the exploitation of the inherent genetic potential of agronomically important plants.

Defense Mechanism for Salinity/Alkalinity

Salinity is a major abiotic stress limiting the increase in the demand for food crops (Gupta and Huang 2014). Salinity stress involves changes in various, morphological, physiological, biochemical and metabolic processes, depending on severity and duration of the stress, and ultimately limits the crop production. Salinity in initial stage induces, osmotic stress in the which causes various physiological changes, such as interruption of membranes, nutrient imbalance, impairs the ability to detoxify reactive oxygen species (ROS), differences in the antioxidant enzymes and decreased photosynthetic activity, and decrease in stomatal aperture (). Under saline conditions, plants accumulate high Na+ concentration which interfere with uptake of K+ ions which is an essential element for plant growth and development (Dang et al. 2010). Under high salt concentration, plant develops different physiological and biochemical mechanisms for survival and growth. Principal mechanism includes ion homeostasis, compartmentalization, ion transport and uptake, biosysyntesis and accumulation of osmoprotectant, activation of antioxidant enzyme, generation of nitric oxide, synthesis of polyamines and hormone modulation (Pathak et al. 2014). Plants have inherent mechanisms to mitigate osmotic stress by reducing water loss while maximizing water uptake. plants generally minimize the adverse effects of Na+ stress by exclusion of Na+ from leaf tissues and by compartmentalization of Na+, mainly into vacuoles. Genetic transformation of plants for enhancing the salinity tolerance can be successfully executed by focusing on genes controlling ion transport, as regulation of Na+ uptake and compartmentalization is a critically important mechanism of plant survival under salinity stress. Several studies on model plant have identified allelic variation for sodium transporters genes viz., HKT1 (High-Affinity K+ Transporter1), SOS1 (Salt Overly Sensitive1) and NHX (Na+/H+ Exchanger) as crucial determinants of cellular Na+ homeostasis.

Defense Mechanism for Metal Toxicity

Heavy metals include the transition-metal elements essential to plant nutrition, iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), nickel (Ni) and molybdenum (Mo), cobalt (Co), which is required for nitrogen fixation in legumes, and the non-essential elements, chromium (Cr), cadmium (Cd), mercury (Hg) and lead (Pb). All these elements if exceed its threshold level cause toxic to crop plants. The presence of toxic heavy metals (HMs), is one important factor that can cause damage to plants by altering metabolic and plant physiological and metabolic processes (Farid et al. 2013). Symptoms associated with metal toxicity includes, reduction in plant growth including leaf chlorosis, necrosis, turgor loss, a decrease in the rate of seed germination, and a dysfunctional photosynthetic apparatus, often correlated with plant death. Some crops plants appear to tolerate metals either by excluding them from the shoot or by accumulating metals in older leaves as similar in case of salinity tolerance. Some plant tolerates high concentration of metals even up to four times than normal termed as hyperaccumulators. Hyperaccumulators are plant are the actual metal tolerant plant and frequently occurs in at least 500 plant species (or about 0.2% of taxa), and accumulate metals that include aluminium, cadmium, copper, lead, nickel and zinc (Hodson 2012). Antioxidant defenses components including SOD, ascorbate peroxidase (APX), peroxiredoxins (PRXs) and catalase (CAT). Glutathione (GSH) and ascorbate have a major role in defense against oxidative damage caused by reactive oxygen species that generated as a result of metal toxicity. Besides ROS and antioxidants, the other mechanisms of metal toxicity tolerance include, complexion, chelation and compartmentation. Classic genetic demonstrated many genes/QTLs are involved in HM uptake, translocation, sequestration, chemical modification, and tolerance. Role of regulatory genes other than structural genes are more important for simultaneously induction of many HM-related genes, in order to enhance heavy metal (HM) phytoremediation capacity (Hossain et al. 2012) Overexpression of single or in combination of genes are a possible strategy for genetic engineering. Genetic engineering would act as an efficient mechanism or tools for phytoremediation of crop plants by engineering of transporter, uptake and regulatory genes.

Biotic Stress

Pathogens

Biotic stresses result from an array of potential pathogens: fungi, bacteria, nematodes and insects intercept the photosynthate produced by plants, and viruses use replication machinery at the host's expense. Plants, in turn, have evolved sophisticated mechanisms to perceive such attacks, and to translate that perception into an adaptive response (Jeffery et al. 2001).

Passive Defenses

Potential plant pathogens must overcome the physical barrier presented by healthy plants to gain access to the nutrients or replication machinery available within the host cell. These barriers may be physical (the cuticle, cell wall, stomatal aperture or lenticel) or chemical (including inhibitory compounds or the absence of stimulatory compounds needed for pathogen development). Saprophytes lack the ability to penetrate these natural barriers (Guest and Brown 1997).

Physical Barriers

The importance of the cuticle as a barrier to penetration has been demonstrated by the dependence of many pathogens on adhesion and the subsequent release of cutin-degrading enzymes at the time of penetration. Although cutin-degrading enzymes are also secreted by many saprophytic fungi and bacteria, their primary activity is to allow access to cellulose in plant cell walls as a nutritional substrate. Different forms of cutin-degrading enzymes are used by pathogens to puncture the cell wall. The activity of this type of cutinolytic enzyme in isolates of *Fusarrum solani*, F. sp. *pisi* is directly related to their aggressiveness on pea stems, indicating that pathogens unable to dissolve the cuticle at the point of penetration are excluded (David and John 1997).

The cuticle provides a physical barrier against water loss and protects against irradiation, xenobiotics, and pathogens. Components of the cuticle are perceived by invading fungi and activate developmental processes during pathogenesis. In addition, cuticle alterations of various types induce a syndrome of reactions that often results in resistance to necrotrophs (Serrano et al. 2014). These are important factors in the resistance of some plants to certain pathogens by making direct penetration fungal pathogens difficult or impossible. Many pathogenic fungi and bacteria enter plants only through stomata. The structure of stomata e.g. a very narrow entrance and broad, elevated guard cells, may confer resistance to some varieties against certain bacterial pathogens (Tom Schultz 2006). Thicker, tougher cell walls could be associated with adult plant resistance. Some pathogens such as *Puccinia graminis* only infect young barberry leaves with thin cuticles and the germ tubes emerging from basidiospores do not penetrate thicker cuticles on mature leaves. Similarly, the ability of *Taphrina dejorrnans* to infect only young, newly unfolded leaves has been attributed to the inability of germ tubes to penetrate the thicker cuticles of older leaves. The presence of secondary cell walls in sclerenchyma, xylem or older plant tissue often retards pathogen development, leading, for example, to angular leaf spots where pathogen spread is restricted by leaf veins. Thick cuticles may physically prevent the eruption of sporophores and release of spores. However, most experimental evidence suggests that toughened cuticles and cell walls are just one of the many factors that contribute to resistance (David and John 1997).

Waxy cuticles and vertically oriented leaves may prevent the formation of moisture films on leaf surfaces. Dry leaf surfaces inhibit infection by pathogens such as bacteria, nematodes and fungal zoospores that require a film of water for motility. Fungal spores might also be inhibited because most require moisture for germination. This must be balanced with the fact that vertically oriented leaves are more prone to impaction by wind-borne pathogen propagules and are likely to face higher inoculum levels compared with those that are horizontally oriented (Guest and Brown 1997).

Pathogens enter the host by direct penetration through natural openings or are introduced by vectors. Bacteria and fungi are capable of triggering stomatal closure through pathogen-associated molecular patterns (PAMPs), which prevents penetration through these pores. Therefore, the stomata can be considered part of the plant innate immune response. Some pathogens have evolved mechanisms to evade stomatal defense. The bacterial pathogen *Xanthomonas campestris* pv. *campestris* (Xcc), which infects plants of the Brassicaceae family mainly through hydathodes, has also been reported to infect plants through stomata. A recent report shows that penetration of Xcc in Arabidopsis leaves through stomata depends on a secreted small molecule whose synthesis is under control of the rpf/diffusible signal factor (DSF) cell-to-cell signaling system, which also controls genes involved in biofilm formation and pathogenesis. The same reports shows that Arabidopsis ROS- and PAMP-activated MAP kinase 3 (MPK3) is essential for stomatal innate response (Gudesblat et al. 2009). The black pod pathogen, *Phytophthora palmiuora*, enters cocoa pods through stomata. Cocoa genotypes that produce pods with few, relatively smaller stomata, allow fewer lesions to establish than genotypes with more numerous, larger stomata. Not surprisingly, as the pathogen enters through stomatal pores, there is no correlation between cuticle thickness or pod case hardness and resistance to black pod. The bacterium that causes citrus canker, *Xanthomonas campestris* pv. *citri*, enters grapefruit through open stomata. Mandarins are resistant because their stomata are too small to allow entry of the bacterium. Similarly, lenticels that suberise rapidly so that their size is reduced may physically exclude pathogens such as Streptomyces scabies, the cause of common scab of potato (David and John 1997).

Lignifications: Lignified cell wall provide effective barrier to hyphal penetration. Lignin is a highly branched heterogeneous polymer found principally in the secondary cell walls of plants, although primary walls can also become lignified. It consists of hundreds or thousands of phenolic monomers and is a primary component of wood. Because it is insoluble, rigid, and virtually indigestible, lignin provides an excellent physical barrier against pathogen attack. Lignin acts as impermeable barrier for free movement of nutrient causing starvation of pathogen. Some examples of lignin containing crops and fungi inhibited include *Peronospora parasitica*, *Alternaria japonica* Potato: *Phytophtora infestans* Wheat: *Septoria nodorum* Cucumber: *Cladosporium cucumerium*, *Colletorichum lagenarium* Carrot: *Botrytis cineria* (Jones and Dangl 2006).

Suberization: In several plants the infected cells are surrounded by suberized cells, thus, isolating them from healthy tissue. Corky layer formation is a part of natural healing system of plants. e.g. common scab of potato and rot of sweet potato are good examples (Van Baarlen et al. 2007).

Abscission layers: It is a gap between host cell layers and devices for dropping–off older leaves and mature fruits. Plant may use this for defence mechanism also. i.e., To drop-off infected or invaded plant tissue or parts, along with pathogen. Shot holes in leaves of fruit trees is a common feature.

Tyloses: The tyloses are formed by protrusion of xylem parachymatous cell walls, through pits, into xylem vessels. The size and number of tyloses physically block the vessel. The tyloses are inductively formed much ahead of infection, thus blocking the spread of pathogen. It suggests biochemical elicitors and movement of tyloses inducing facto (TIF) up the stem. e.g. Sweet potato: Fusarium oxysporum f. sp. Batatas (Jan et al. 2011; Dou and Zhou 2012).

Gum deposition: The gums and vascular gels quickly accumulate and fill the intercellular spaces or within the cell surroundings the infection thread and haustoria, which may starve or die.

Chemical Barriers

The pathogen may not be provided with the required nutrients by the plants and thus resist infection. The compounds in plant cells may stimulate or inhibit the development of pathogens. Some fungal resting spores such as *Spongospora subterranea* (powdery scab of potato), *Urocystis agropyri* (flag or leaf smut of wheat) and *Plasmodiophora brassicae* (club root of crucifers) and eggs of the potato cyst nematode, *Globodera rostochiensis*, require specific substances to stimulate germination or hatching. These are provided in secretions from certain plants, including potential hosts. Plants that fail to secrete these stimulators are resistant by default (David and John 1997).

Plant chemicals can be divided into two major categories: primary metabolites and secondary metabolites. Primary metabolites are substances produced by all plant cells that are directly involved in growth, development, or reproduction. Examples include sugars, proteins, amino acids, and nucleic acids. Secondary metabolites are not directly involved in growth or reproduction but they are often involved with plant defense. These compounds usually belong to one of three large chemical classes: terpenoids, phenolics, and alkaloids (Brian and Gwyn 2008).

The name phytoanticipins was coined by J.W. Mansfield. “Phytoanticipins are low molecular weight, antimicrobial compounds that are present in plants before challenge by microorganisms or are produced after infection solely from preexisting constituents” (VanEtten et al. 1994). Phytoanticipins may be excreted into the external environment (e.g. rhizosphere or phylloplane), accumulate in dead cells or they may be sequestered in vacuoles in an inactive form. The dead cells of brown onion skins contain the quinones, catechol and protocatechuic acid, which inhibit germination of spores of the smudge pathogen, *Colletotrichum circinans*, and the neck rot pathogen, *Botrytis cinerea*; white onions do not produce these compounds and are susceptible to smudge. *Aspergillus niger* is insensitive to these inhibitors and attacks both white and brown onions. Avocado rootstocks resistant to root rot caused by *Phytophthora cinnamomi* secrete borbinol, an antimicrobial phenolic compound, into the rhizosphere. The secretion of nematode-inhibiting substances into the rhizosphere surrounding asparagus and marigold roots has already been mentioned. Symptoms of anthracnose of avocado, caused by *Colletotrichum gloeosporiordes*, only develop on ripe fruit. The peel of unripe avocado fruit contains antifungal lipids called dienes that prevent appressorial germination. As these dienes are gradually metabolised during fruit ripening to less toxic compounds, quiescent appressoria germinate and susceptibility to anthracnose increases. In anthracnose-resistant cultivars, diene breakdown is blocked following infection, so that antifungal levels are sustained for longer periods. The resistance of immature apples and pears to scab, caused by *Venturia inaequalis* and *V. pirina* respectively, correlates with the presence of the phenolic compounds chlorogenic acid, phloridzin, arbutin and iso-chlorogenic acid in the outer layers of the fruit. These compounds also contribute to the bitter taste of unripe apples and pears and, as the fruit ripens and sweetens, it also becomes more susceptible to scab (David and John 1997).

One group of phytoanticipins, the saponins, are plant glycosides with surfactant (wetting agent) properties. Saponins bind sterols in pathogen cell membranes, destroying membrane integrity and function. In this way saponins are toxic to organisms containing sterols in their membranes (e.g. plants and fungi, but not Oomycota). Inactive saponin precursor molecules appear to be stored in vacuoles of intact plant cells, but hydrolase enzymes released following wounding or infection convert these precursors to active, antimicrobial forms. Several lines of evidence suggest that saponins are involved in disease resistance and host range determination. It appears that the ability of some pathogens to detoxiff specific saponins matches their host range. For example, a strain of the take-all pathogen that attacks oats as well as wheat and barley (*Gaeumannomyces graminis* var. *auenae*), releases the enzyme avenacinase. Avenacinase detoxifies the triterpenoid saponin, avenacin, found in epidermal cells of the roots of oat plants. Mutants in which the gene for avenacinase production has been deleted are sensitive to avenacin in vitro and are not pathogenic on oats, but remain pathogenic to wheat and barley. *Gaeumannomyces graminis* var. *tritici* lacks avenacinase and attacks wheat and barley, but not oat species containing avenacin. An oat species that does not produce avenacin, *Avena longiglumisis* susceptible to *Gaeumannomyces graminis* var. *tritici* (Osbourn 1994; David and John 1997). Another saponin, tomatine, contributes to the resistance of tomato leaves to *Botrytis cinerea* (Osbourn 1996). Some plant peptides also inhibit the development of fungi, bacteria, viruses and insects. They act as proteinase and polygalacturonase-inhibitors, as ribosome inhibitors or lectins. These inhibitors interfere with pathogen nutrition and retard their development, thus contributing to disease resistance. Because of their similarity to peptides called defensins found in insects and mammals, they have been termed plant defensins.

The first plant defensins were isolated from wheat *T. aestivum* and barley *Hordeum vulgare* and initially classified as γ-thionins. Plant defensins are small (ca.

5 kDa), basic, cysteine-rich peptides ranging from 45 to 54 amino acids, and are positively charged. Biological activities reported for plant defensins include antifungal, antibacterial, proteinase, and insect amylase inhibitor activities (Wijaya et al. 2000; Stotz et al. 2009). The plant defensins have quite diverse amino acid composition and conserved three-dimensional structure, which comprises a triple-stranded β-sheet with an α-helix in parallel stabilized by four disulfide bridges. Plant defensins are very similar to defense peptides of mammals and insects what suggest their ancient and conserved origin. Generally, plant defensins are composed by one subunit, being found in monomeric forms. On the other hand, the defensins from *Pachyrrhizus erosus* and other from *Vigna unguiculata* showed the ability to dimerism (Pelegrini and Franco 2005). The mode of action of plant defensins is still unclear and not all plant defensins have the same mode of action. Probable defensins used glucosylceramides as receptors for fungi cell membrane insertion. Then, repulsion of defensins into cell membrane by their positive charges leads to membrane disruption, membrane destabilization, and ion efflux (Pelegrini and Franco 2005). Plant defensins can be divided in two groups: (1) plant defensins that inhibit fungal growth through morphological distortions of the fungal hyphae and (2) plant defensins that inhibit fungal growth without morphological distortion (Hegedus and Marx 2013). Most plant defensins were isolated from seeds. In radish, defensin RS-AFPs represents 0.5% of total protein in seeds. Defensins were also isolated from leaves, pods, tubers, fruits, roots, bark, and floral organs of such plants as *Heuchera sanguinea* (Hs-AFp1), *Raphanus sativus* (Rs-AFP1), *Aesculus hippocastanum* (Ah-AMP1), Dahlia merckii (Dm-AMP1), and *Clitoria ternatea* (Ct-AMP1; De Lucca et al. 2005). Defensins are expressed during normal plant growth and development and induced by environmental factors and biotic and abiotic stress (Pestana-Calsa and Calsa 2011). The defensins gene induced upon pathogen infection has been identified in pea, tobacco, Arabidopsis, and spruce (Lay and Anderson 2005). Defensins may constitute up to 10% of the total proteins in cereal, legume and solanaceous seeds. Similar studies have shown defensins are also present in the outer cell layers of other plant organs such as flowers, leaves and tubers. While many defensins accumulate during normal plant development, others are induced, or their accumulation is enhanced, after wounding. Defensins, because of their anti-feeding activity against insects, provide a defence against insect-transmitted viruses (David and John 1997).

Rapid Active Defenses

Plant responses to infection are complex and there is no universal model or sequence of events that accurately describes the dynamics of resistance in the few interactions studied, let alone the vast majority of undescribed interactions. Almost every host-parasite interaction is unique in the details of the activation, localization, timing and magnitude of each component of the defense response. As previously stated, resistance is rarely absolute and whether a plant ends up being resistant or susceptible depends on the sum of many individual responses.

Changes in Membrane Function

Most studies on the earliest stages of the host-parasite interaction conclude that the host membrane is involved in pathogen recognition and signal transduction. Electrolyte leakage accompanies plant response to stresses, such as salinity, pathogen attack, drought, heavy metals, hyperthermia, and hypothermia; however, the mechanism and physiological role of this phenomenon have only recently been clarified. Accumulating evidence shows that electrolyte leakage is mainly related to K^+ efflux from plant cells, which is mediated by plasma membrane cation conductances (Demidchik et al. 2014). Membrane permeability changes rapidly following the exposure of plant cell suspension cultures to fungal and bacterial elicitors, usually leading to a loss of cellular electrolytes such as K+ and an uptake of H+. Most probably they are encoded by GORK, SKOR, and annexin genes. Hypothetically, cyclic nucleotide-gated channels and ionotropic glutamate receptors can also be involved. The stress-induced electrolyte leakage is usually accompanied by accumulation of reactive oxygen species (ROS) and often results in programmed cell death (PCD). Recent data strongly suggest that these reactions are linked to each other. ROS have been shown to activate GORK, SKOR, and annexins. ROS activated K+ efflux through GORK channels results in dramatic K+ loss from plant cells, which stimulates proteases and endonucleases, and promotes PCD. This mechanism is likely to trigger plant PCD under severe stress. However, in moderate stress conditions, K+ efflux could play an essential role as a 'metabolic switch' in anabolic reactions, stimulating catabolic processes and saving 'metabolic' energy for adaptation and repair needs. At the same time, there is often an influx of Ca^{2+}, a key intracellular signal in plants that is involved in the activation of enzymes and gene expression. Ca^{2+} signaling in plants functions in local and systemic communication. Rapid systemic signaling is activated in response to different stimuli including mechanical force, pathogen infection, and abiotic stresses and results in systemic propagation of Ca^{2+} and reactive oxygen species (ROS) waves. This allows plant cells to transmit long-distance signals via cell-to-cell communication (Steinhorst and Kudla 2013; Gilroy et al. 2014). The experimental blocking of Ca^{2+} transport across membranes in inoculated bean cells also inhibits gene activation and subsequent defence responses.

The Oxidative Burst

Rapid generation of superoxide and accumulation of H_2O_2 is a characteristic early feature of the hypersensitive response following perception of pathogen avirulence signals. Emerging data indicate that the oxidative burst reflects activation of a membrane-bound NADPH oxidase closely resembling that operating in activated neutrophils. The oxidants are not only direct protective agents, but H_2O_2 also functions as a substrate for oxidative cross-linking in the cell wall, as a threshold trigger for hypersensitive cell death, and as a diffusible signal for induction of cellular protectant genes in surrounding cells. Activation of the oxidative burst is a central component of a highly amplified and integrated signal system, also involving salicylic acid and perturbations of cytosolic Ca^{2+}, which underlies the expression of disease-resistance mechanisms (Lamb and Dixon 1997).

Reactive oxygen species (ROS) are produced as a normal product of plant cellular metabolism. Various environmental stresses lead to excessive production of ROS causing progressive oxidative damage and ultimately cell death. Despite their destructive activity, they are well-described second messengers in a variety of cellular processes, including conferment of tolerance to various environmental stresses. Whether ROS would serve as signaling molecules or could cause oxidative damage to the tissues depends on the delicate equilibrium between ROS production, and their scavenging. Efficient scavenging of ROS produced during various environmental stresses requires the action of several nonenzymatic as well as enzymatic antioxidants present in the tissues (Sharma et al. 2012).

Doke a plant pathologist at Nagoya University (Nagoya, Japan), first reported on the involvement of ROS in the plant–pathogen interaction, after observing that infection by *Phytophthora infestans* in potato tubers causes the generation of O_2^- at the host cells' plasma membrane (PM), only in the incompatible interactions. *P. infestans* is a typical pseudofungal species which is now classified within the class of Oomycetes (Subclass, Peronosporomycetidae; Order, Pythiales) that causes the serious potato disease known as late blight or potato blight. A series of Doke's works demonstrated for the first time that ROS generation occurs in plants upon attacks by a pathogenic microorganism and that the members of ROS possibly function as the chemical signals required for induction of hypersensitive response (HR) as typified by host cell death, now often referred to as plant apoptosis. Doke also demonstrated that the treatment of potato tuber protoplasts with the cell wall preparation from *P. infestans* effectively induces the oxidative burst, suggesting that chemical components derived from pathogenic microorganisms (elicitors) trigger the burst of ROS production in order to stimulate the plant defense mechanisms (Kawano and Bouteau 2013).

The work of Doke and his colleagues undergo a two-step oxidative burst. The first burst rapidly follows wounding and inoculation, while a much larger burst in incompatible interactions immediately precedes hypersensitive cell death. Since then, an oxidative burst has been described in a range of plant-fungal and plant bacterial interactions. The rapid oxidative burst generates levels of reactive oxygen species that initiate membrane lipid peroxidation and cell death. The oxidative burst in plants is associated with the release of local and systemic signals that trigger gene expression and the oxidative cross-linking of host cell wall components. Levels of reactive oxygen species accumulate at the infection courts that are sufficient to kill micro-organisms in vitro. Experimental suppression of the oxidative burst shows that it is involved in initiating later defence responses. On the other hand, colonisation of avocado fruit by the necrotroph, *Botrytis cinerea*, apparently exploits the oxidative burst to kill host cells in advance of invasion (David and John 1997).

Cell Wall Reinforcement

Prospective plant pathogens must overcome the physical barrier presented by the plant cell wall. In addition to being a preformed, passive barrier limiting access of pathogens to plant cells, the cell wall is actively remodeled and reinforced specifically at discrete sites of interaction with potentially pathogenic microbes. Active reinforcement of the cell wall through the deposition of cell wall appositions, referred to as papillae, is an early response to perception of numerous categories of pathogens including fungi and bacteria. Rapid deposition of papillae is generally correlated with resistance to fungal pathogens that attempt to penetrate plant cell walls for the establishment of feeding structures. Despite the ubiquity and apparent importance of this early defense response, relatively little is known about the underlying molecular mechanisms and cellular processes involved in the targeting and assembly of papillae (Underwood 2012). The rapid deposition of papillae is a common response of cereals to attempted penetration of epidermal cells by the powdery mildew fungus (*Blumeria graminis*). Papillae in resistant cultivars form more rapidly and are more difficult to penetrate, than those formed by susceptible cultivars. As a result, haustorial development is prevented. Lignitubers are lignified callose deposits that ensheath invading hyphal tips. Lignitubers have been observed in both resistant and susceptible cereals following challenge by the take-all pathogen, *Gaeumannomyces graminis*, demonstrating again the importance of timing-the more rapid the response, the more likely it is to succeed (David and John 1997).

The most abundant structural proteins in plant cell walls are the hydroxyproline rich glycoproteins (HRGPs) which involved in the organization of secondary cell wall thickening. They are induced as defense responses, specifically in incompatible plant-pathogen interactions (Davis et al. 1997). Functionally, there is evidence that HRGPs act as impenetrable physical barriers against pathogen ingress and that they immobilize the pathogens by binding to their negatively charged surfaces, making cell walls tougher (Leach et al. 1982; Mazau et al. 1987; Cassab and Varner 1988). HRGPs include extensins, arabinogalactan proteins (AGPs), proline/hydroxyproline-rich proteins (P/HRGPs), and solanaceous lectins (Sommer-Knudsen et al. 1998). Among these, the P/HRGPs and extensins are known to be insoluble proteins whereas AGPs are soluble. Most of the earlier studies on infection-induced accumulation of HRGPs were carried out in dicotyledons, e.g. French bean infected with *Colletotrichum lindemuthianum* (Templeton et al. 1990; Millar et al. 1992; Bindschedler et al. 2006), lettuce infected with *Pseudomonas syringae* (Bestwick et al. 1995), French bean infected with *Xanthomonas campestris* (Brown et al. 1998) and tobacco leaves infected by *Erysiphe cichoracearum* (Raggi 2000). The HRGPs accumulation in monocotyledons as a response to pathogens has also been reported, e.g. in pearl millet with *Sclerospora graminicola* (Shailasree et al. 2004), in wheat infected by *Fusarium culmorum* (Kang and Buchenauer 2003) and in maize treated with a *Fusarium monilifore*-derived elicitor (García Muniz et al. 1998).

The plant cell wall constitutes one of the first lines of defense against pathogen invasion, and peroxidases are key enzymes in the wall-building processes. These processes include peroxidase-mediated oxidation of hydroxycinnamyl alcohols into free radical intermediates (Gross 1980), phenol oxidation (Schmid and Feucht 1980), polysaccharide cross-linking (Fry 1986), crosslinking of extensin monomers (Everdeen et al. 1988), lignification (Grisebach 1981; Walter 1992), and suberization (Espelie and Kolattukudy 1985; Espelie et al. 1986). The rapid deposition of lignin and suberin following infection is associated with resistance to non-pathogens and to avirulent pathogens in many plants, including cereals, Solanaceae, brassicas, melons and carrots. Hydrogen peroxide, released during the oxidative burst following pathogen challenge, causes extensive cross-linking between hydroxyproline-rich glycoproteins and other cell wall components. The oxidative cross-linking of cell wall structural proteins is thought to be a rapid defense response to strengthen the cell wall against the invading pathogen prior to the activation of other post-transcription dependent defense responses such as accumulation of pathogenesis-related proteins (PR-proteins) and defense genes expression (Bradley et al. 1992; Brisson et al. 1994). This oxidative cross-linking at the cell surfaces following fungal infection is known to be driven by peroxidases and H_2O_2, which rapidly accumulates from an oxidative burst (Brisson et al. 1994; Ribeiro et al. 2006). HRGPs cause cell wall strengthening by the formation of intra and inter-molecular cross-links resulting in their rapid insolubilization in cell walls. The cross-linking process is reported to involve isodityrosine (IDT) links (Cooper and Varner 1984; Epstein and Lamport 1984; Fry 1986; Smallhood et al. 1995). The whole process strongly decreases solubility of HRGPs meaning that they lose their extractability by salts and SDS (Bradley et al. 1992). Finally, this leads to the formation of a cross-linked mesh of defined porosity interpenetrated by a cellulose microfibrilar wrap (Epstein and Lamport 1984).

Lignification renders the cell wall more resistant to mechanical pressure applied during penetration by fungal appressoria as well as more water resistant and thus less accessible to cell wall-degrading enzymes. Lignin also binds to hyphal tips and bacterial cells, preventing further growth and movement and restricting the diffusion of pathogen enzymes and toxins and the uptake of water and nutrients by the pathogen. For examples resistance of cucumber to *Cladosporium cucumerinum* and *Colletotrichum lagenarium* is due to deposition of lignin in cell wall (Hammerschmidt and Kuc 1995). It is mentioned previously that Trichoderma GT3-2, Fusarium GF18-3 and Phoma GS8-2 were able to induce resistance in melon plants against *Colletotrichum orbiculare*, the causal agent of cucumber anthracnose and found lignin deposition at the point of pathogen infection in the epidermal tissues of cucumber hypocotyls (Koike et al. 2001).

Furthermore, precursor molecules and free radicals produced during lignin biosynthesis are toxic to pathogens and inactivate pathogen enzymes, toxins, elicitors or suppressors. The effect of lignin can be further enhanced by the release of reactive oxygen species and the activation of phenol oxidase enzymes that convert phenolic compounds to more toxic complex polymerised phenolics and quinones during the defence response. The evidence that cell wall reinforcements are important components of plant disease resistance can be summarised as follows:

- Their deposition often coincides with failed penetration and sometimes precedes the cessation of pathogen growth.
- Reinforcements in resistant hosts are larger, form more quickly (often before penetration) and are more dense than those formed by susceptible hosts.
- Experimental attempts to re-penetrate induced reinforcements usually fail.
- Inhibition of lignin or callose biosynthesis enhances penetration efficiency.

However, the deposition of cell wall reinforcements is not always associated with disease resistance. Clearly, cell wall reinforcements contribute to resistance and cell repair but are not always sufficient on their own to prevent infection (David and John 1997).

Hypersensitive Cell Death

Cell death has a central role in innate immune responses in both plants and animals. Besides sharing striking convergences and similarities in the overall evolutionary organization of their innate immune systems, both plants and animals can respond to infection and pathogen recognition with programmed cell death. The fact that plant and animal pathogens have evolved strategies to subvert specific cell death modalities emphasizes the essential role of cell death during immune responses. The hypersensitive response (HR) cell death in plants displays morphological features, molecular architectures and mechanisms reminiscent of different inflammatory cell death types in animals (pyroptosis and necroptosis) (Coll et al. 2011). The term hypersensitivity indicates that the host cells are 'over- (hyper-) sensitive' to the presence of the pathogen. Host cells suicide in the presence of the pathogen, preventing further spread of the infection. The first observations of HR date back to 1902 in the wheat-*Puccinia glumarum* pathosystem (Ward 1902), and the counter-intuitive term 'hypersensitiveness' was coined in 1915 (Stakman 1915), to describe a pathogen-triggered cell death reaction that correlated with disease resistance in wheat infected with *Puccinia graminis*. Morphologically, HR is a specific and unique type of cell death. Its hallmarks, some of which are typical for different forms of animal cell death, include cytoplasmic shrinkage, chromatin condensation, mitochondrial swelling, combined with other characteristics that are plant specific, such as vacuolization and chloroplast disruption during the final stages (Mur et al. 2008).

The chloroplast has a central role in defense responses and HR in plants. First, it constitutes a very important source of defense signaling molecules such as reactive oxygen species (ROS), reactive nitrogen oxide intermediates (NOI) and the defense hormones salicylic acid (SA) and jasmonic acid (JA). Second, in many cases, light is required for HR development. Third, several pathogen effectors have chloroplast localization signals (Guttman et al. 2002), and in some cases they have been shown to suppress immunity (Fu et al. 2007; Jelenska et al. 2007).

In plants, the molecular events that lead to HR during effector-triggered immunity (ETI) are partly overlapping with those associated with (Microbial-associated molecular patterns) MAMP-triggered immunity (MTI), including accumulation of SA, ROS and NOI, activation of mitogen-activated protein kinase(MAPK) cascades, changes in intracellular calcium levels, transcriptional reprogramming and synthesis of antimicrobial compounds (Mur et al. 2008). Compared with MTI, ETI is typically an accelerated and amplified response, suggesting that quantitative rather than qualitative differences account for HR induction (Jones and Dangl 2006).

Increased ROS levels are a hallmark of necroptosis and may be one of the main causes of necroptotic cell death. Enhanced ROS production during necroptosis can be mediated by mitochondria, due to a RIP3-dependent increase in energy metabolism (Zhang et al. 2009), and/or by the NADPH oxidase NOX1, which is recruited to the plasma membrane by RIP1 (Kim et al. 2007). Hypersensitive cell death in plant cells shares many features in common with apoptosis, or programmed cell death, observed during development of defense against disease in animals. In plants, apoplastic ROS (superoxide) generated by the plasma membrane NADPH oxidases are essential for HR development and activation of systemic immunity (Torres and Dangl 2005), drawing a possible mechanistic connection between these two types of cell death. ROS produced in other plant organelles as the chloroplast, mitochondria and peroxisomes also contribute to the HR response and, in fact, compartmentalization might be essential for ROS signaling functions during defense (Torres 2010).

Necroptosis has a pivotal role in inflammation and immunity. Similar to pyroptotic cells, necroptotic cells secrete a broad array of pro-inflammatory molecules that signal through PRRs (Zitvogel et al. 2010). Necroptosis has been reported to occur in response to infection by certain viruses that block apoptosis in the host cell as a colonization strategy (Challa and Chan 2010). Because of the pro-inflammatory nature of necroptosis, it may constitute not only a backup mechanism for virus clearance when apoptosis is inhibited, but also a way to engage the immune system leading to a systemic response.

The emerging similarities between hypersensitive cell death in plants and apoptosis in animal cells suggest that cell suicide is an ancient defense response. It is not always easy to conclude from research data whether host cell death is a consequence of murder or suicide. Recent experiments have shown that in many host-parasite interactions hypersensitive cell death precedes pathogen death, regardless of whether biotrophic or necrotrophic pathogens were involved. In some interactions however, disease resistance does not depend on hypersensitive cell death. The success of hypersensitive cell death as a resistance mechanism in individual host-parasite interactions depends on the nutritional requirements of the pathogen and on the timing, location and magnitude of the host response in relation to pathogen development. In some interactions the rapid suicide of challenged host cells undoubtedly restricts pathogen development, contributing to the overall defence response (David and John 1997).

Phytoalexins

Phytoalexins are low molecular weight antimicrobial compounds that are produced by plants as a response to biotic and abiotic stresses. As such they take part in an intricate defense system which enables plants to control invading microorganisms. In the 1950s, research on phytoalexins started with progress in their biochemistry and bio-organic chemistry, resulting in the determination of their structure, their biological activity, as well as mechanisms of their synthesis and catabolism by microorganisms. Elucidation of the biosynthesis of numerous phytoalexins also permitted the use of molecular biology tools for the exploration of the genes encoding enzymes of their synthesis pathways and their regulators. This has led to potential applications for increasing plant resistance to diseases. Phytoalexins display an enormous diversity belonging to various chemical families such as for instance, phenolics, terpenoids, furanoacetylenes, steroid glycoalkaloids, sulfur-containing compounds and indoles (Jeandet 2015). An astonishing range of structures may be formed, as many as 25 substances in a single interaction. They are formed consistently within a given plant group; in a survey of 60 species of the legume tribe Vicieae, every member tested responded. Although phytoalexins have been most widely studied in the Leguminosae, they have been recorded in over 20 other families. These plants range from trees, through shrubs to herbs and from monocots to dicots. There may be limits to the distribution of this defense mechanism and results of exploring this response in further families will be presented (Harborne 1986). Between 1986, when the first cruciferous phytoalexins were reported, and 2011, the chemical structures, syntheses and antimicrobial activities of 44 cruciferous phytoalexins were reported (Pedras et al. 2011).

Phytoalexins are synthesized by either the cells adjacent to the infection site, the infected host cells or by the invading pathogen. It is thought that such infected cells produce some sort of signals which induces the adjacent cells to produce the phytoalexins, which are packaged in lipid vesicles and exported to the infected cell (Mogensen 2009). Consequently, the infected cell becomes a toxic microenvironment for the invading pathogen. Phytoalexin accumulation is often associated with hypersensitive cell death. Examples include medicarpin (alfalfa, Medicago sativa), rishitin (tomatoes and potatoes (the Solanaceae family), and camalexin, (*Arabidopsis thaliana*) (Jones and Dangl 2006; Cai et al. 2009).

Some plants, such as soybean and chickpea, synthesize phytoalexins upon infection, but convert a proportion into inactive sugar conjugates held in reserve in vacuoles. If the initial defense response fails to check pathogen growth, enzymes that cleave the sugar molecule are activated and the phytoalexin reserves are rapidly releases. Like other active defense responses, the success of phytoalexin accumulation depends on the speed, location and magnitude of the response. There is a good experimental correlation between resistance and rapid, localized phytoalexin accumulation in many host-parasite interactions. There is evidence that:

- Phytoalexins accumulate faster and to higher concentrations in resistant cultivars. In resistant plants, gene transcription begins within one hour of recognition, phytoalexins appear within four hours and concentrations peak to fungitoxic levels about 18–24 h after challenge. These events are delayed and more diffuse in susceptible plants.
- Phytoalexin biosynthesis is localized in cells immediately surrounding the infection court. There is no evidence that they disperse in the plant. Experiments using laser microprobe analysis, radioimmunoassay, hybridisation histochemistry and immunocytochemistry of the phytoalexin biosynthesis pathway have confirmed this in several host pathogen interactions.
- In a number of interactions, resistance is lost if phytoalexin biosynthesis is blocked by inhibitors of enzymes involved in the process of phytoalexin biosynthesis and is reduced in mutants that are slow to accumulate phytoalexins.
- Resistance is increased in plants transformed to express novel phytoalexins or if exogenous phytoalexins are applied. For example, although the biochemical precursor of resveratrol is widely distributed in the plant kingdom, only grapevine and peanut have the en4rme required to complete its synthesis. When the genes encoding this enzyme are transformed into tobacco, resveratrol is synthesized in response to infection.
- Phytoalexin synthesis is not universal among plants. Wheat and cucumber apparently do not produce phytoalexins, yet effectively resist most pathogenic fungi and bacteria. Nevertheless, in many interactions the rapid accumulation of toxic concentrations of phytoalexins at the infection court plays a decisive role in the expression of resistance (David and John 1997).

Delayed Active Defences

Pathogen containment and wound repair

While earlier responses retard the development of pathogens, later responses restrict their spread and contain the damage to host tissues. The ability of a plant to repair tissue damage may contribute to its ability to fight off-secondary infections by opportunistic pathogens. Infected areas of fleshy tissues, roots, fruits and bark are sealed by layers of cork cells with thick, suberised walls. Wound cork is produced by a secondary meristem, the cork cambium, formed from mature parenchyma tissue in response to the damage caused by infection. In some cases, such as in the response of potato tuber tissue to the powdery scab pathogen (*Spongospora subterranea*), cork barriers appear to seal the infected area and prevent further colonization by the pathogen. However in other interactions, including the response of brassicas to the leaf spot pathogen, *Alternaria brassicae*, cork layers do not restrict infection. Some pathogens induce plants to form abscission layers in which cork cambium develops around the infected area and extends from the upper to lower surface of the infected leaf. The infected areas fall out, leaving the classical 'shothole' symptom. Such pathogens include *Stigmina carpophila* and *Pseudomonas syringae* pv. *morsprunorum* on plum and Cercospora *beticola* on silverbeet. Wounded tree trunks often secrete gums that effectively seal the wound from opportunistic pathogens (David and John 1997).

Tyloses are outgrowths of vessel-associated parenchyma cells which protrude into the xylem vessel through pits and block the spread of pathogens (Beckman 1964; Talboys 1972; Grimault et al. 1994; Agrios 2005). They are formed during both compatible and incompatible interactions between the host and vascular wilt pathogens, although the time and extent of tylose formation significantly differs. Tyloses form much faster and more extensively in resistant plants when compared to susceptible plants (Grimault et al. 1994; Fradin and Thomma 2006). The formation of tyloses involves a cost to the plant, as they not only block the spread of the pathogen, but reduce the translocation of water, possibly causing wilt symptoms.

Pathogenesis-Related Proteins

During the massive shift in cellular metabolism and gene expression referred to earlier, plants synthesize many novel proteins following infection. Some of these novel proteins may be enzymes involved in phytoalexin biosynthesis and some may have no role in disease resistance at all. However, the 'pathogenesis-related proteins have β-glucanase, chitinase or lysozyme activity. Some are related to plant defensins while others are proteinase inhibitors that disrupt pathogen nutrition. Pathogenesis-related proteins are sometimes present in low levels before infection and are induced following stress, wounding or flowering, indicating that they may have a wider function in plant growth and development than just disease resistance (David and John 1997).

PR proteins are categorised into structurally homologous families. Some of these PR-protein families have direct antimicrobial activities, whereas for others, no intrinsic antimicrobial effects have been found yet, suggesting that those without intrinsic antimicrobial effects might have different functions. An important common feature of most antimicrobial PR proteins is their antifungal activity, although some of them also have antibacterial, insecticidal and antiviral properties. Originally, five main groups of PR proteins (PR-1 to PR-5) were characterised in tobacco. Since then, the number of PR protein groups has increased up to PR-17 across many plant species (Table 1) (Buonaurio et al. 2009). It has also been reported that several genes could be induced after AMF colonisation in host plants and are involved in plant defence against pathogens. These genes encode pathogenesis related proteins

Table 1 Families of pathogenesis related proteins (PRPs)

S. No.	Protein family	Protein activity	Targeted pathogen sites
1.	PR-1	Unknown	Active against oomycetes
2.	PR-2	1,3 β-glucanase	Cell wall glucan of fungi
3.	PR-3	Chitinases	Cell wall chitin of fungi
4.	PR-4	Chitinase type I, II	Active against oomycetes
5.	PR-5	Thaumatin	
6.	PR-6	Proteinase inhibitor	Active on nematodes and insects
7.	PR-7	endoproteinase	Microbial cell wall dissolution
8.	PR-8	Endochitinase with lysozyme activity	Cell wall chitin of fungi and mucopeptide cell wall of bacteria
9.	PR-9	Peroxidase	Strengthening of plant cell wall
10.	PR-10		
11.	PR-11	Endochitinase	Cell wall chitin of fungi
12.	PR-12	Defensin	Antifungal and antibacterial activity
13.	PR-13	Thionin	Antifungal and antibacterial activity
14.	PR-14	Lipid transfer proteins	Antifungal and antibacterial activity
15.	PR-15	Oxalate-oxidase	Produce H_2O_2 that inhibits microbes and also stimulates host defence
16.	PR-16	Oxalate-oxidase-like with super dismutase activity	Produce H_2O_2
17.	PR-17	Uncharacterized	Unknown

Source Niderman et al. (1995), Van Loon et al. (1998), Sels et al. (2008), Okushima et al. (2000)

such as PR-1a, β-1,3 glucanase and PR-10 in tomato, pea and parsley (Haneef Khan et al. 2010).

Indeed, defence-related genes that are activated by AMF are key players in the defence against several root pathogens. Previous studies have identified a large number of defence-related genes in both compatible and incompatible plant–pathogen interactions (Liu et al. 2007; Lehtonen et al. 2008). However, in potato plants challenged with *Rhizoctonia solani*, 24 induced genes related to cell defence were identified by microarray analysis (Lehtonen et al. 2008). These genes encode chitin-hydrolysing enzymes such as acidic chitinases of classes II, III and IV, members of the pathogenesis-related (PR) protein groups (including 1,3-bglucanase and lignin-catalysing peroxidases), osmotin-like proteins, defence-associated signalling kinases, host protein protecting substances and enzymes leading to phytoalexin accumulation (Van Loon et al. 1998; Lehtonen et al. 2008). In tobacco roots, AMF-induced defence genes encode isozymes catalysing to peroxidation and production of phytoalexins and phenolic compounds such as phenylalanine ammonia lyase (PAL) and peroxidase (Blilou et al. 2000). Recently, Liang et al. (2005) reported that foliar applied Si only produced physical barrier and osmotic effect, but root applied Si leaded to systemic acquired resistance when *Cucumis sativus* plants were infected by powdery mildew pathogen, *Podosphaera xanthii* (syn. *Sphaerotheca fuliginea*) and the production of pathogenesis-related proteins (PRs) (Ismail and Hijri 2012).

These novel proteins are accumulated maximally 7–10 days after infection, and indicate the attainment of the systemic acquired resistance by the plant. The PRPs accumulate in the intercellular spaces and in the vacuole. PRPs are recognized as markers of the systemic acquired resistance (SAR), and PR genes are involved in the list of the so-called SAR-genes. The term "SAR-genes" is used to collectively designate this family of nine genes whose expression is correlated with the onset of SAR. Seventeen families of PRPs have been officially recognized (Van Loon et al. 1998), most of which have enzymatic activities.

Systemic Acquired Resistance

Systemic acquired resistance (SAR) refers to a distinct signal transduction pathway that plays an important role in the ability of plants to defend themselves against pathogens. After the formation of a necrotic lesion, either as a part of the hypersensitive response (HR) or as a symptom of disease, the SAR pathway is activated. SAR activation results in the development of a broad-spectrum, systemic resistance (Hunt and Ryals 1996; Neuenschwander et al. 1996). Although SAR is interesting as a paradigm for signal transduction, it may have practical value as well. An understanding of the biochemical changes leading to the resistance state could enable the development of either genetically engineered plants with enhanced disease resistance or novel mode-of-action plant protection chemicals that act by stimulating the plant's inherent disease resistance mechanisms.

There are three steps involved in the development of systemic acquired resistance:

- The induction of systemic acquired resistance usually requires the development of a slowly expanding necrotic lesion. Induction of systemic resistance may be associated with other localised responses such as hypersensitive cell death, phytoalexin accumulation, papilla deposition and lignification.
- Two or three days after the inducing lesion first appears, a signal is released that is systemically translocated in the phloem. This signal is graft transmissible and is not cultivar, species or genus specific, but is not active once plants have begun flowering. All the signals originate from the induction site.
- The systemic signal primes the rest of the plant against further pathogen challenge. Defence responses such as the rapid release of reactive oxygen species, hypersensitive cell death, phytoalexin accumulation, and enhanced levels of pathogenesis-related proteins are expressed more rapidly and intensely than in uninduced plants.

The identity of the signal that triggers systemic acquired resistance is the subject of intense study, but remains unresolved. There are several molecules that can induce features characteristic of systemic acquired resistance, including salicylic acid, β-ionone and jasmonic acid. The entire response is, however, apparently mediated by a complex signal transduction pathway regulated by a number of stress signals.

Salicylic acid, a precursor of aspirin widely distributed in the plant kingdom, plays a key role in systemic acquired resistance. Salicylic acid binds to at least two proteins found in plant cell membranes. One salicylic acid-binding protein has catalase activity that is inhibited upon binding, causing a localised build-up of hydrogen peroxide. This form of reactive o4ygen, as previously mentioned, causes a number of changes in plant cells that increase their resistance to pathogens. A second, high affinity, salicylic acid-binding protein appears to directly activate gene expression. Levels of salicylic acid rise rapidly around necrotic lesions in plants and remain high in plants that have acquired resistance. However, a series of experiments show that it is a local, rather than a systemically translocated, signal. Although it must be present for systemic acquired resistance to be expressed, salicylic acid is not translocated over long distances in plants and presumably interacts with another systemic signal. Synthetic analogues of salicylic acid, such as dichloroisonicotinic acid (INA) and the benzothiazoles, induce similar responses to those induced by salicylic acid and have potential use as practical disease-protectants. Although INA induces resistance in field and glasshouse trials, the effective dose is sometimes phytotoxic and this risk will probably prevent its commercialization. A more promising benzothiazole, benzo(1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH), is similarly effective but less phytotoxic (David and John 1997).

Insect Pests

Any organism that consumes plant tissue is an herbivore. Insect and plants have coevolved for millions of years. Plants respond to herbivory through various morphological, biochemicals, and molecular mechanisms to counter the effects of herbivore attack. These defence strategies against herbivores are wide-ranging, highly dynamic, and could be direct and/or indirect. Host plant defence against herbivores is a complex array of structural, chemical, and physiological traits intended to perceive the attacking organisms, and restrain them before they are able to cause extensive damage. Since plants lack the physical mobility, they have evolved a number of strategies which enable them to withstand insect pressure. Plant defence against insect pests is mediated through morphological (toughness, thorns, thickness, and hairiness) and biochemical (nutritional composition of the plant tissue, and the nature and amounts of secondary metabolites) factors. The defensive compounds are produced constitutively or in response to plant damage, and affect feeding, growth, and survival of herbivores. In addition, plants also release volatile organic compounds that attract the natural enemies of the herbivores. These strategies either act independently or in conjunction with each other. Direct defences are mediated by plant characteristics that affect the herbivore's biology such as mechanical protection on the surface of the plants (e.g., hairs, trichrome, thorns, spines, and thicker leaves) or production of toxic chemicals such as terpenoids, alkaloids, anthocyanins, phenols, and quinones) that either kill or retard the development of the herbivores. Indirect defences against insects are mediated by the release of a blend of volatiles that specifically attract natural enemies of the herbivores and/or by providing food (e.g., extra floral nectar) and housing to enhance the effectiveness of the natural enemies. Plants defence against herbivory or host-plant resistance (HPR) describes a range of adaptations evolved by plants which improve their survival and reproduction by reducing the impact of herbivores. Host plant resistance is one of the most effective tools for reducing insect damage. Phytophagous insects are often suppressed by plant defence mechanisms. Their mating, oviposition feeding, feeding ingestion, fertility and growth are interrupted.

Host plant resistance is the result of interactions between two biological entities, the plant and the insect under influence of various environmental factors (Dhaliwal and Singh 2004). Plants use several strategies to defend against damage caused by herbivores. Painter (1951) grouped mechanism of resistance into three main categories, Viz. Antixenosis (also called as non-preference), antibiosis and tolerance. Antixenosis is employed by the plant to deter or reduce colonization by insects. Plants that exhibit antixenotic resistance should have a reduced initial number of colonies early in the season. Antibiosis operates after the insect have colonized and have started utilizing the plant. Tolerance of the plant does not affect the rate of population increase of the target pest but does raise the threshold level.

Mechanism of Resistance

Antixenosis (Non Preference)

Antixenosis refers to the resistance mechanism employed by host plant to deter or reduce colonization by insects. It refers to plant characteristics that lead insects away from a particular host; it includes activities of both plant and insect. It is mainly the response of insect to the characteristics of host plant which make it unattractive to the insect for feeding, oviposition or shelter. It denotes the presence of morphological or allelochemicals in host plant factor that adversely alter insect behaviour resulting in poor establishment of the insect. Antixenosis may represent one or more breaks in the chain of responses leading to oviposition or feeding. These breaks may be due to (i) the absence of an arrestant or attractant, (ii) the presence of a repellent, or (iii) an unfavourable balance between an attractant and a repellent (Panda and Khush 1995).

Antixenosis is of 2 types: Morphological antixenosis and Allelochemic antixenosis.

Morphological antixenosis

Morphological characters of plant interfere with insect behaviour activities such as mating, oviposition, feeding and feeding ingestion. Various morphological characters are

(a) Trichomes
(b) Pubescence
(c) Surface waxes
(d) Thickness of cell wall and proliferation of plant tissues
(e) Silica content
(f) Solidness and other stem characters
(g) Anatomical adoptions of organs
(h) Colour and intensity of light.

Allelochemic nonpreference

Several chemical present in the plant affect the olfactory and gustatory stimuli in insects. These chemicals may attract or repel the insects and also inhibit feeding by the insects. Ex: In cucurbits, a compound called cucurbitacin acts as an attractant and feeding incitant to spotted cucumber beetles.

Antibiosis

Antibiosis refers to the adverse effect of the host plant on the biology (survival, development or reproduction) of the insects and their progeny infesting it. All these adverse physiological effects of permanent or temporary nature following ingestion of a plant by an insect are attributed to antibiosis. The insects feeding on resistant plants may manifest antibiotic symptoms varying from acute or lethal to sub chronic or very mild. The most commonly observed symptoms in insects include adverse effects on the nutritional physiology of the insect including consumption, assimilation, utilization and subsequent allocation for reproduction (Ananthakrishnan 1994). This is manifested by larval death in first few instars, abnormal growth rates, disruption in conversion of ingested food, decline in size and weight of larvae or nymphs, prolongation of the larval period, failure to pupate, failure of adults to emergence from pupae, abnormal adults, inability to concentrate food reserves followed by failure to hibernate, decreased fecundity, reduction in fertility, restlessness and abnormal behaviour (Panda and Khush 1995). These symptoms may appear due to various physiological processes such as presence of toxic substances, absence or insufficient amount of essential nutrients, nutrient imbalance or improper utilization of nutrients. This mechanism impairs an insect's metabolic processes and involves consumption of plant metabolites. Both insect and plant factors are involved in the antibiosis mechanism. The physiological explanations for antibiosis are

a. Presence of toxins
b. Absence/insufficiency of essential nutrients
c. Unbalanced proportion of nutrients
d. Presence of antibodies.

Tolerance

Tolerance refers to the ability of the host plant to withstand an insect population sufficient to damage severely the susceptible plants. It is generally attributable to plant vigour, regrowth of damaged tissues, resistance to lodging, ability to produce additional branches, utilization of non-vital parts by insects and compensation by growth of neighbouring plants. However, tolerance has no adverse effect on the insect infestation for longer periods without loss in yield or quality than the susceptible varieties and enables them to frequently escape insect damage through compensation by the plants. Tolerance is a plant response to an insect pest. Whereas, antibiosis, antixenosis, resistance cause an insect response when the insect attempts to use the resistant plant for food, oviposition or shelter. Tolerance is useful in pest management programmes due to certain distinct advantages (Panda and Khush 1995):

(a) Tolerant varieties have a higher economic threshold level than the susceptible varieties and hence require less insecticide application and promote biocontrol.
(b) Tolerant varieties do not depress insect populations nor do they provide any selection pressure on the insect and thus are useful in preventing the development of insect biotypes.
(c) In varieties with a combination of thee mechanisms of resistance, tolerance increases yield stability by providing at least a moderate level of resistance, when vertical genes providing a high level of resistance through antixenosis and antibiosis succumb to the new biotype.

Conclusion

Plants are challenged by different types of environmental stresses during their life cycle. In order to avoid such stresses, plants develop various adaptation strategies and defense mechanisms at morphological, anatomical, physiological, biochemical and molecular levels. Having deep knowledge about the various mechanisms by which plants tolerate environmental stresses is necessary to improve the crop performance under stress conditions. The study of plant response to stress will let us know about the metabolism, various pathways and cascades involved during the stress condition. Further, the understanding about anatomical and molecular mechanisms of plant response to stress will help in development of genetically engineered plants possessing high tolerance to biotic and abiotic stresses. This will also provide better insights of the highly complex anatomical, molecular and biochemical strategies adopted by the plants to adapt themselves under several environmental stresses (dos Reis et al. 2016).

Breeding for resistance to stress is challenging. Various novel techniques like genomics, proteomics and metabolomics can aid in identifying the genetics behind plant stress responses, enabling a direct and unbiased monitoring of the factors affecting crop growth, development and productivity. They also provide data that can be directly used to investigate the complex interplay between the plants, its metabolism and the environmental stress, both biotic and abiotic (Perez-Clemente et al. 2013). Moreover, the genome analysis of plants enable identification of DNA markers linked to stress tolerant/resistant genes, facilitating the crop improvement under climate change scenario.

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Biotechnological Approaches for In Vitro Conservation of *Picrorhiza kurroa* Royle ex Benth

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Abstract Medicinal plants have been used by humans for the treatment of various diseases since ancient times. The plants being rich in secondary metabolites are exploited for their probable utility due to the presence of phytochemical properties. The pure compound can easily be obtained from the plants resulting in safer drugs. One of such important medicinal plant is *Picrorhiza kurroa* which is a principle source of iridoid glycosides, with a wide range of biological activities. Today the plant has become endangered due to restricted distribution and unskilled harvesting and is demanding to be conserved for its posterity. In order to safeguard and preserve such medicinally important plant species, certain biotechnological approaches are found to be very valuable for their conservation and further manipulation so as to augment their yield and quality traits.

Keywords *Picrorhiza kurroa* · In vitro · Conservation · Micropropagation Endangered · Secondary metabolites

Medicinal plants have been used by humans globally as a valuable source of drugs. The hunt for the medicines based on plants has always played a significant role for improving the quality and longetivity of life. The therapeutic utility of plants is traced from more than thousand years. The earliest known medical document is a 4000-year-old Sumerian clay tablet that recorded the use of plants as remedy for many popular diseases (Pan et al. 2014). In India, the earliest reference of use of plants in medicine was seen in Rig-Veda, and later the properties and therapeutic uses of medicinal plants was studied in detail by the ancient physicians which is a primary foundation of ancient medical science today itself (Hosseinzadeh et al. 2015).

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This increased interest in natural medication has resulted in great challenge of meeting the demand of growing markets for plant-based medicines and at the same time protecting the medicinal biodiversity. Apart from this, these plant species have provided an important contribution to local economies and cultural integrity of both the rural poor and fragile social group (Padulosi et al. 2002). The development of effective cultivation practices cannot cope up with the ever increasing demand for the plant yield in terms of both biomass and active phytochemicals. Thus, it is enormously important for the long-term conservation of medicinal plants and also their sustainable use.

The increase in the demand of medicinal plants for the expansion of drug industry has threatened their natural habitats and put them in the brink of extinction (Bodeker et al. 2014). To manage up with this alarming situation, the use of modern biotechnology tools has come as a boon where they select and conserve the critical genotypes of medicinal plants using different approaches. The use of plant tissue culture has resulted in the aspects of protection, multiplication, conservation and preservation of the medicinal plants especially those that are in the verge of extinction (Chen et al. 2016). Similarly, elicitation and genetic transformation are employed as potent tools for enhanced production of secondary metabolites from these plants (Srivastava et al. 2016). Recently, different functional genomic strategies have been developed for differential gene expression analysis in plant cell culture towards production of designer secondary metabolites (Ochoa-Villarreal et al. 2016).

Importance of *P. Kurroa*

One of such highly valued herb is *Picrorhiza kurroa* Royle ex. Benth, that belongs to family Scrophulariaceae and is prevalent in the higher altitudes of Himalayan region. It grows from Kashmir to Sikkim, between altitudes of 3000–4500 m above mean sea level (Thakur et al. 1989; Arya et al. 2013). The rhizomes of the plant are the principle source of iridoid glycosides out of which the major is Picrosides and kutkosides (Patil et al. 2013).

These iridoids have abundant therapeutic uses like antihepatotoxic, choleretic, hypolipidemic, antiinflammatory, antispasmodic, antiallergic and antiasthamatic (Tiwari et al. 2012; Sultan et al. 2016). *P. kurroa* has been mentioned as a well known herb in the ancient Ayurvedic system of medicine (Kashyap 1970). The plant is also called as kutki which is derived from Sanskrit name ‘Katuka’ meaning bitter taste. The roots of this plant possess much bitterness and are used in purgative preparation. The dried extracts from the plant has a cure for liver disorders, fever, jaundice and allergies (Baruah et al. 1988). The herbal preparation of the plant plays a major role in antidiabetic and anticancerous activity (Pradhan 2011; Kumar and Ramesh 2014; Mallick et al. 2015).

The over exploitation of the rhizomes along with the poor cultivation and small population size of *P. kurroa* has depleted this species from its natural habitat, for the extraction of bioactive constituents (Verma et al. 2012; Chand et al. 2016). This has resulted in the plant to be enlisted in red data book by International Union for Conservation of Nature (IUCN). The conventional method of propagation using rhizomes and seeds restricts multiplication as seed germination is very poor in *P. kurroa*, so use of in vitro approaches are essential for its conservation (Rawat et al. 2013).

Conservation Using Biotechnological Techniques

In today's scenario, biotechnological techniques play an important role in conserving the plant biodiversity for rare and endangered plant species. In vitro cell culture techniques ensure the rapid production and multiplication of plant material without any seasonal constraint. Even for the long-term conservation of plant material, cryopreservation (i.e. storing in liquid nitrogen at −196 °C) allows the storing of plant material without modification, contamination or alteration in the genome for unlimited periods (Benson 2008; Vasava 2016). The major advantages of in vitro culture are the production of disease free plantlets throughout the year for germplasm exchange and storage and also rapid mass multiplication of genotypes which cannot be multiplied by conventional propagation methods.

Seed Germination

Germination from seeds is the most common method of propagation in most plant species (Khanna et al. 2013). The germination rate of *P. kurroa* seeds is poor (Chandra et al. 2006) as the seeds need a chilling treatment below subzero temperatures during winters to break their dormancy. Chandra et al. (2006) observed poor seed germination after presoaking treatment of seeds with different PGRs followed by subsequent incubation at 10 °C. However, addition of gibberellic acid (GA_3) increased the rate of seed germination as well as reduced the time taken for their germination. This is possibly due to dormancy, exhibited by many alpine seeds so as to overcome the harsh climatic conditions prevalent at high altitudes. However, Nautiyal (1988) observed up to 60% germination in *P. kurroa* seeds incubated at 20 °C in light. Even, in polyhouses use of moss covering the sandy loam soil without any chemical treatment resulted in 58% seed germination (Nautiyal et al. 2001). Thus, the use of GA_3 alone or in combination with BAP doubles germination as well as the time taken for germination in other alpine herbs (Nadeem et al. 2000).

Jan et al. (2009) observed highest rate of seed germination in *P. kurroa* by soaking seeds in sterile water for 10 days at 4 °C and then keeping them under continuous dark conditions at 25 ± 2 °C on moist absorbent cotton placed in the Petri plates till germination occurred.

Shoot Initiation and Multiplication

Lal et al. (1988) developed the first successful protocol for the clonal propagation of *P. kurroa* through shoot tip culture. He observed multiple shoots from the explants on MS (Murashige and Skoog 1962) medium supplemented with kinetin in different concentrations from 3.0 to 5.0 mg/l. Also, the addition of 1.0 mg/l Indole acetic acid (IAA) along with Kinetin (Kn) showed marked improvement in the growth of regenerated shoots while addition of auxin IAA in the medium did not vary the frequency of shoot multiplication.

Patial et al. (2012) observed shoot multiplication in the in vitro regenerated microshoots growing in MS medium containing Kn and Benzyl Amino Purine (BAP) in different concentrations. A three fold increase in shoot length and leaf size was observed when the explants were cultured in liquid medium but prolonged culturing resulted in vitrification. In 2016, Patial et al. (2016) studied the potential of thidiazuron (TDZ) on shoot multiplication after treating the nodal segments with 0.5 μM TDZ for 15 days before transferring them to basal MS medium. They also observed that pretreatment with TDZ beyond 15 days resulted in browning and necrosis of the plants.

Sharma et al. (2010) multiplied shoot cultures on MS medium containing 0.2 mg/l BAP for shoot multiplication while Bhat et al. (2012) observed multiple shoots in Gamborg's B5 medium containing 3.0 mg/l Kn and 1.0 mg/l IBA. A 90% plant regeneration from leaf explants was observed.

In vitro shoot multiplication from nodal explants on MS medium without cytokinin was observed by Lal et al. (1988). They studied that the addition of NAA in the culture medium favours both shoots and root formation. They also compared the average number of shoots per explant on MS and B_5 media, which was highest on MS medium supplemented with 0.6 mg/l NAA while the maximum shoot length was observed in MS with 0.4 mg/l NAA.

Mahajan et al. (2016) tried ten different combinations of BAP and Kn in MS medium (Fig. 1). They observed 85.63% of shoots MS medium containing 1.0 mg/l BAP, 0.5 mg/l Kn and 1.0 mg/l GA_3. Also, the increase in concentration of kinetin to 1 mg/l, resulted in the decreased the shoot growth to 80.21%. It was observed that the MS medium supplemented with BAP only resulted in healthy shoots with long and thick leaves with less number of shoots was less while the number of shoots was more but thin in MS medium containing kinetin only.

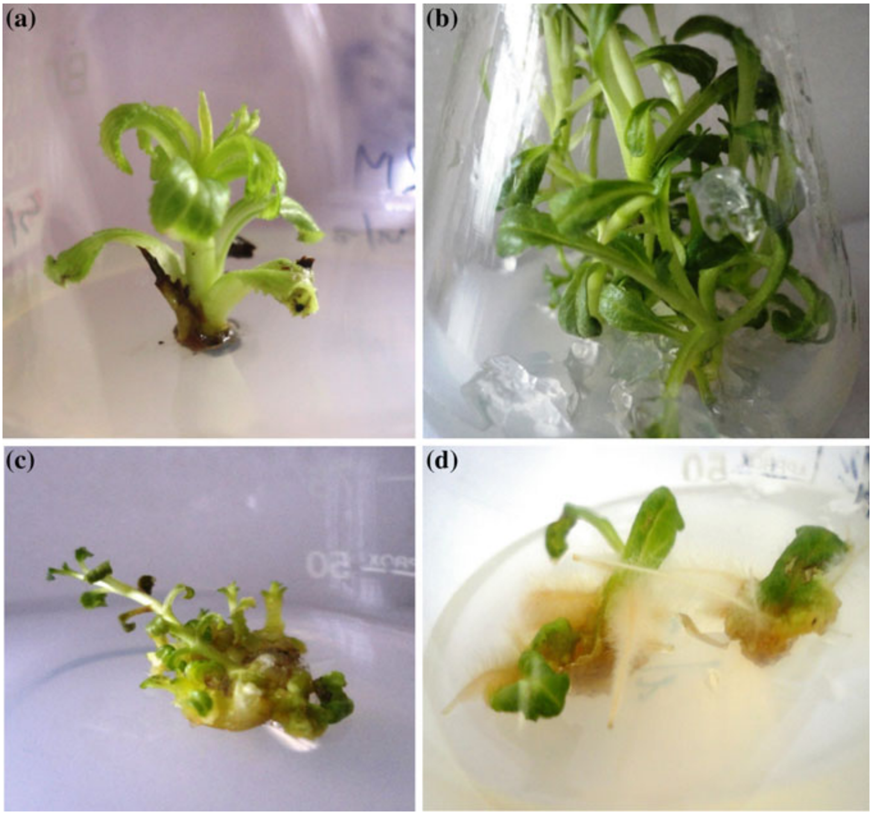

Fig. 1 **a** Leaf proliferation from auxillary buds. **b** Shoot multiplication. **c** Shoot induction from the callus. **d** Root induction from the callus

Rooting and Hardening

MS basal medium without PGRs resulted in in vitro rooting in *P. kurroa* (Patial et al. 2012). They observed rooting on MS medium supplemented with kinetin while Helena et al. (2015) observed roots on ½ strength MS basal medium supplemented with different concentrations of 2,4-D, IAA, IBA and NAA. Root initiation was observed within 12–13 days of transferring the microshoots in rooting medium. However, profused rooting occurred on medium containing NAA at the concentration of 0.4 mg/l and 0.5 mg/l (2,4-Dichlorophenoxyacetic acid) 2-4,D while Lal et al. (1988) observed rooting on MS medium containing 1.0 mg/l of NAA.

Patial et al. (2012) observed that the rooted plantlets kept at 5 °C prior to hardening turned brown while those kept at 15 °C have thickened leaves where the leaf tissue was differentiated into distinct palisade layer and thick cuticle. Also the shoots treated with TDZ showed profused rooting and transfer of these rooted microshoots in sand under polyhouse conditions after incubating at 15 °C for 10 days resulted in 100% survival rate, as it improved the cellular differentiation and vascularization (Patial et al. 2016). Since stomatal regulation of transpiration and water loss is crucial for the survival of plants the stomata and chlorophyll content of the tissue culture-raised plantlets treated at 15 °C so was compared with the plants growing in the field conditions (Patial et al. 2016). They observed that the number of stomata and chlorophyll content was low in the plants that were not treated at 15 °C. The elongated shoots rooted within 15–25 days either on MS alone or supplemented with NAA (0.4 mg/l), IAA and IBA (0.1–0.5 mg/l).

Lal et al. (1988) also observed rooting from microshoots on MS with a combination of three auxins IBA, IAA and NAA. They achieved 81.5% of survival rate on transfer of in vitro grown plants to the greenhouse which when matured showed normal development as that of the mother plants while Mahajan et al. (2016) used a single growth regulator (IBA) in MS medium at different concentrations and observed direct, healthy and long roots with numerous root hairs but best rooting was observed on MS containing 2.5 mg/l IBA.

Biological hardening of in vitro grown *P. kurroa* plants was well studied by Trivedi and Pandey (2007). They inoculated micropropagated plants with three plant growth-promoting rhizobacteria viz. *Bacillus megaterium*, *B. subtilis* and Pseudomonas before transferring them to field conditions and this resulted in up to 94.5% survival rate when the plants were inoculated with *B. megaterium* with respect to the survival rate of 38.5% in control plants.

Callus Multiplication and Organogenesis

Lal and Ahuja (1996) observed callus from leaf and shoot explants in MS medium supplemented with 0.5–2.0 mg/l 2,4-D. The callus proliferation was studied in MS containing 4.0 mg/l NAA and 1.0 mg/l Kn and shoot organogenesis was observed in MS supplemented with 0.25 mg/l BAP. Rooting was observed in MS containing 0.2 mg/l NAA.

Sood and Chauhan (2009) used different explants of *P. kurroa* such as leaf discs, nodal and root segments for callus establishment. A high frequency of callus induction was observed in root segments (70%), followed by leaf discs (56.3%) and least in nodal segments (38.3%) when cultured on the MS medium supplemented with 0.2 mg/l and 0.5 mg/l IBA. The occurrence of high frequency callusing from root segments of *P. kurroa* is highly desirable as most of the phytopharmaceuticals accumulate in roots. Also the callus derived from root segments can be readily used for cell suspension cultures for producing secondary metabolites on large scale. Multiple shoots in the callus cultures derived from different explants on MS medium containing different concentrations and combinations of BA, Kn and IBA were observed. The calli derived from root segments and leaf discs resulted in high frequency regeneration on MS supplemented with BA and Kn. From their observations they concluded that that root segments are the best explant for in vitro studies in *P. kurroa*. Sood and Chauhan (2010) determined picroside content from the different stages of the callus cultures and concluded that the biosynthesis and accumulation of Picroside-I started in callus cultures that differentiate into shoot primordia. They observed that the concentration of Picroside-I in the shoots formed from root-derived callus cultures was low as compared to shoots derived from callus cultures of leaf and stem segments. Their study concluded that the biosynthesis and accumulation of Picroside-I is developmentally regulated in different morphogenetic stages of *P. kurroa* tissue cultures. Even, Gupta et al. (2016) analyzed in vitro grown callus, leaf and roots for the presence of Picroside-I (P-I) using HPLC. They observed maximum P-I content (0.9/100 g) in leaves followed by roots (0.06/100 g) whereas no P-I content was detected in the callus.

Helena et al. (2015) cultured leaf explants and shoot explants on MS medium supplemented with different combinations and concentrations of 2,4-D, IAA, IBA and NAA and cytokinin TDZ to initiate callus. They observed best growth of callus on MS medium from both leaf and shoot explants medium containing two growth regulators viz. TDZ and IBA. The callus obtained from leaf explants were large in size as compared to shoot. This could be due to differential triggering of the cells in presence of different growth regulators.

A comparative study was made by Patial et al. (2012) using ex vitro and in vitro grown leaves of *P. kurroa*. They observed callus induction from ex vitro leaf explants in MS medium supplemented with TDZ which further resulted in shoot bud formation after transferring the calli to MS medium containing Kn. The in vitro grown leaves resulted in direct shoot formation in MS medium without PGRs, though the shoot number was low. They observed that the exposure of cultures to TDZ for 15 days interval enhanced the rate of shoot formation.

Mahajan et al. (2016) observed creamy white and friable callus from leaf explants on MS medium containing 2.0 and 3.0 mg/l 2,4-D. Increase in the concentration of 2,4-D to 4.0 mg/l resulted in the browning of explants with no callus formation. Similarly Gupta et al. (2016) reported 100% callus induction frequency from leaf and stem segments on MS medium supplemented with 0.5 mg/l TDZ and 0.3 mg/l IBA. Frequent subculturing was done to minimize the accumulation of phenolic compounds and by adding anti-phenolic substances, such as 0.5 mg/l ascorbic acid and 0.5 mg/l PVP. The callus derived from leaf explants resulted in 77.7% shoot formation when transferred on to the MS medium supplemented with 1.0 mg/l BA and 0.75 mg/l Kn. About 70% roots were observed on rooting media containing NAA.

Somatic Embryogenesis

There was no published report of somatic embryogenesis in *P. kurroa* till 2013 (Rawat et al. 2013). However, Mahajan et al. (2016) used cytokinins as promoters for the induction of somatic embryos in *P. kurroa*. They observed that the use of 2,4-D along with BAP and GA_3 in MS medium affected the number of embryo production. The callus when subcultured on MS medium containing 2,4-D resulted in the formation of different stages of somatic embryos. Mature somatic embryos when transferred to medium containing BAP and GA_3 turned green and differentiated into shoots on MS medium supplemented with 1.0 mg/l BAP and 1.0 mg/l GA_3.

Cryopreservation

Sharma and Sharma (2003) made the first attempt to cryopreserve the vitro grown shoot apices of *P. kurroa* using vitrification technique. They took about 1 mm long shoot tips and precultured them on MS medium supplemented with various osmotic which is an essential step for successful cryopreservation. They also observed that preculture at 4 °C for five days exhibited better growth of shoots as compared to those cultures without preculture treatment. Also, cold hardening at 4 °C enhanced the survival of explants prior to dehydration in PVS2. The dehydrated shoot tips were directly immersed in LN2 followed by rapid rewarming at 45 °C. The shoot tips turned green after 10 days without callus formation and produced multiple shoots on transfer to MS medium. The morphology of cryopreserved shoots of *P. kurroa* was comparable to that of non-frozen (control) in vitro grown shoots was also observed.

Mishra et al. (2011) reported the encapsulation of microshoots of *P. kurroa* in a 3% sodium alginate solution. The encapsulated microshoots were maintained by spraying with sterile distilled water in a flask for a period of 3 months at 25 ± 2 °C moist conditions (Rawat et al. 2016). These encapsulated microshoots on half strength MS basal medium grew into plantlets with a growth rate of 90%. These shoots further formed roots on half-strength MS medium supplemented with 1.0 M NAA.

Genetic Transformation

The use of in vitro techniques for generation of hairy root can prevent the indiscriminate up-rooting and exploitation of this commercially important plant by producing the desired bioactive molecules under in vitro conditions. Verma et al. (2012)

developed the first successful establishment protocol for induction, establishment and growth of hairy root cultures of *P. kurroa* using *Agrobacterium rhizogenes* (LBA9402 strain) with the option of producing desired phyto-molecule. Use of appropriate explant and bacterial strain resulted in 66.7% relative transformation frequency. They studied nine independent, opine and TL-positive hairy root clones for their growth and production of specific glycoside at different growth phases. They observed the expression of active constituents were expressed by all the hairy root clones tested by them, even though different inter-clonal variations in terms of quantity could be noted. A yield potential in terms of biomass as well as individual glycoside contents with one of the clones tested was also observed. Thus the use of in vitro techniques for generation of hairy root can prevent the indiscriminate up-rooting and exploitation of this commercially important plant by producing the desired bioactive molecules under in vitro conditions.

Similarly, Bhat et al. (2012) observed efficient plant regeneration and genetic transformation using *Agrobacterium tumefaciens*. They used A. tumefaciens strain GV3101, harboring binary vector pCAMBIA1302 that contains a green fluorescent protein and hygromycin phosphotransferase genes. A protocol was generated genetic transformation using leaf explants and the putative transformants were selected using 15 mg/l hygromycin. They verified the transformation both by using fluorescence microscopy and polymerase chain reaction. They observed a transformation frequency of 56% with an average of 3.4 ± 0.4 transgenic plantlets per explant. This efficient developed transformation protocol can serve as a boon in the metabolic engineering of *P. kurroa*.

Conclusions and Future Prospects

Biotechnological tools are necessary for the conservation and improvement of the medicinally important plant species that have become endangered due to human intervention. Loss of genetic diversity of such plant species from their natural habitats has resulted in the decrease in their survival rate and also their ability to cope with changing environment conditions. Thus it is necessary to use the various biotechnological tools that could result in the conservation and improvement of such plant species. This further requires the integration of biotechnological approaches with the conventional methods to develop effective ways for conserving natural plant population. Use of hairy root cultures can be used as an efficient means of producing secondary metabolites. Also the use of biotic and abiotic elicitors can be used as a potential source for enhancing the production of secondary metabolites that can cope up the pressure from the various herbal industries. Further, the use of metabolic engineering can offer the over expression of single genes which can results in the accumulation of the target products.

Physiological Aspects of Heat Stress

In response to heat stress, certain physiological changes results in the enhanced level of metabolites like amino acids, sugars, peroxidases. This results in an increased antioxidant activity and resistance to biotic and abiotic stresses which is beneficial for the plants but at the same time also reduces the photosynthetic activity.

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In the original version of the book, a new chapter "Biotechnological Approaches for In Vitro Conservation of *Picrorhiza kurroa* Royle ex Benth" has been included at the end. The erratum book has been updated with the change.

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