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Environmental Challenges and Medicinal Plants

Sustainable Production Solutions under Adverse Conditions

Springer

Environmental Challenges and Solutions

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Tariq Aftab
Editor

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Sustainable Production Solutions under Adverse Conditions

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This book is dedicated to

Sir Syed Ahmad Khan
(October 17, 1817–March 27, 1898)

Sir Syed Ahmad Khan, one of the architects of modern India, was born on October 17, 1817, in Delhi and started his career as a civil servant.
The 1857 revolt was one of the turning points in Syed Ahmed’s life. He clearly foresaw the imperative need for the Muslims to acquire proficiency in the English language and modern sciences, if the community were to maintain its social and political clout, particularly in Northern India.

He was one of those early pioneers who recognized the critical role of education in the empowerment of the poor and backward Muslim community. In more than one way, Sir Syed was one of the greatest social reformers and a great national builder of modern India. He began to prepare the road map for the formation of a Muslim University by starting various schools. He instituted Scientific Society in 1863 to instil a scientific temperament into the Muslims and to make the Western knowledge available to Indians in their own language.

The Aligarh Institute Gazette, an organ of the Scientific Society, was launched in March 1866 and succeeded in agitating the minds in the traditional Muslim society. Anyone with a poor level of commitment would have backed off in the face of strong opposition but Sir Syed responded by bringing out another journal, Tehzibul Akhlaq which was rightly named in English as "Mohammedan Social Reformer."

In 1875, Sir Syed founded the Madarsatul Uloom in Aligarh and patterned the MAO College after Oxford and Cambridge universities that he went on a trip to London. His objective was to build a college in line with the British education system but without compromising its Islamic values. He wanted this College to act as a bridge between the old and the new, the East and the West. While he fully appreciated the need and urgency of imparting instruction based on Western learning, he was not oblivious to the value of oriental learning and wanted to preserve and transmit to posterity the rich legacy of the past. Dr. Sir Mohammad Iqbal observes:

“The real greatness of Sir Syed consists in the fact that he was the first Indian Muslim who felt the need of a fresh orientation of Islam and worked for it—his sensitive nature was the first to react to modern age.”

The aim of Sir Syed was not merely restricted to establishing a college at Aligarh but at spreading a network of Muslim Managed educational institutions throughout the length and breadth of the country keeping in view this end, he instituted All India Muslim Educational Conference that revived the spirit of Muslims at national level. The Aligarh Movement motivated the Muslims to help open a number of educational institutions. It was the first of its kind of such Muslim NGO in India, which awakened the Muslims from their deep slumber and infused social and political sensibility into them.

Sir Syed contributed many essential elements to the development of the modern society of the subcontinent. During Sir Syed’s own lifetime, The Englishman, a renowned British magazine of the nineteenth century, remarked in a commentary on November 17, 1885: ‘Sir Syed’s life “strikingly illustrated one of the best phases of modern history.” He died on March 27, 1898, and lies buried next to the main mosque at Aligarh Muslim University.

Preface

Plants have evolved an incredible arrangement of metabolic pathways leading to molecules/compounds capable of responding promptly and effectively to challenging situations imposed by biotic and abiotic factors. Medicinal plants supply the ever-growing needs of humankind for natural chemicals, such as pharmaceuticals, nutraceuticals, agrochemicals, and chemical additives. Medicinal plants are used in traditional medicine to cure various ailments, and several studies have highlighted the therapeutic properties and biological activities of medicinal plants. These plants contain bioactive secondary metabolites which possess antimalarial, anthelminthic, anti-inflammatory, analgesic, antimicrobial, antiarthritic, antioxidant, antidiabetic, antihypertensive, anticancer, antifungal, antispasmodic, cardioprotective, antithyroid, and antihistaminic properties. Secondary metabolites play a major role in the adaptation of plants to the changing environment and stress conditions as they are affected by both biotic and abiotic stress.

Humans rely on medicinal plants for various needs since ancient times, and their population still seems enough for fulfilling our demands. But in the foreseeable future we will be forced to think about the accessibility of resources for the generations to come. For these reasons, we must look for alternative sustainable options of resources which can protect these immensely important medicinal plants from various stresses induced by the challenging environment. Moreover, we need to understand current advancements of molecular mechanisms of cross talk in relation to plant abiotic stress in order to create climate resilient medicinal plants which can survive under stress combinations. Evolving eco-friendly methodologies and mechanisms to improve these plants' responses to unfavorable environmental circumstances is important in creating significant tools for a better understanding of plant adaptations to various abiotic stresses and sustaining the supply of pharmaceuticals as global climate change intensifies.

One of the great challenges in the near future will be the sustainable production of medicinal plants in growing climate changes. A combination of adverse demographic factors and climatological perturbations is expected to impact food and pharmaceutical production globally. Despite the induction of several tolerance mechanisms, medicinal plants often fail to survive under environmental extremes.

To ensure their sustainable production under adverse conditions, multidisciplinary approaches are needed, and useful leads are likely to emerge. However, improving plants' performance under restrictive growth conditions requires a deep understanding of the molecular processes that underlie their extraordinary physiological plasticity. Therefore, this book aims to review and analyze the studies that investigate impacts of environmental challenges on medicinal plants and the possibilities for increased sustainable production. This book reviews the emerging importance of medicinal plants and how their production and sustainability is affected by environmental factors and provides eco-friendly solutions for the production of medicinal plants under challenging environmental conditions.

This comprehensive volume emphasizes the recent updates about the current research on the medicinal plants covering different aspects related to challenges and opportunities in the concerned field. This book is an attempt to bring together global researchers who have been engaged in the area of stress signaling, cross talk, and mechanisms of medicinal plants. The book will provide a direction toward the implementation of programs and practices that will enable sustainable production of medicinal plants, resilient to challenging environmental conditions. I believe that this book will instigate and commence readers to state-of-the-art developments and trends in this field. Moreover, I hope to have disseminated the chapters of this book in a way that will be novel for the readers and can be readily adopted as references for newer and further research.

I am highly grateful to all our contributors for accepting our invitation for not only sharing their knowledge and research but for venerably integrating their expertise in dispersed information from diverse fields in composing the chapters and enduring editorial suggestions to finally produce this venture. I also thank the Springer Nature team for their generous cooperation at every stage of the book production.

Lastly, thanks are also due to well-wishers, research students, and editor's family members for their moral support, blessings, and inspiration in the compilation of this book.

Aligarh, India Tariq Aftab

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About the Editor

Tariq Aftab received his Ph.D. in the Department of Botany at Aligarh Muslim University, India, and is currently Assistant Professor there. He is the recipient of a prestigious Leibniz-DAAD fellowship from Germany, Raman Fellowship from the Government of India, and Young Scientist Awards from the State Government of Uttar Pradesh (India) and Government of India. After completing his doctorate, he has worked as Research Fellow at the National Bureau of Plant Genetic Resources, New Delhi, and as Postdoctorate Fellow at Jamia Hamdard, New Delhi, India. Dr. Aftab also worked as Visiting Scientist at Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany, and in the Department of Plant Biology, Michigan State University, USA. He is a member of various scientific associations from India and abroad.

He has edited 12 books with international publishers, including Elsevier Inc., Springer Nature, and CRC Press (Taylor & Francis Group), coauthored several book chapters, and published over 70 research papers in peer-reviewed international journals. His research interests include physiological, proteomic, and molecular studies on medicinal and crop plants.

Chapter 1

Current Status of Medicinal Plants in Perspective of Environmental Challenges and Global Climate Changes

Mohammad Javad Ahmadi-Lahijani and Saeed Moori

Abstract The elevation in [CO_2] since the industrialization era has become a severe problem in plant physiology and human life. The level of CO_2 emission has drastically increased during the past 40 years mainly due to anthropogenic activities, which created a significant environmental challenge for plants. The expected behavior of plants is influenced by the climatic changing factors, which finally impact the morphophysiological traits and secondary metabolites (SMs) of pharmaceuticals. Medicinal plant SMs have been utilized to discover new drugs in the alleviation of many diseases over the past two decades. Medicinal plants possibly are able to adapt to their changing environment; hence, metabolic elasticity may impact metabolite production, which is the basis for their medicinal values. Primary metabolites such as the SMs are also impacted by climatic change. Medicinal plant growth, biomass production, and SMs are influenced by climatic fluctuations, e.g., temperature and [CO_2], due to alterations in the metabolic pathways, which regulate plant signaling, physiology, biochemistry, and defense mechanisms. The population of plant species including medicinal plants may be threatened by the elevated [CO_2], extreme temperatures, changing precipitation regimes, increases in pests and pathogens, and anthropogenic habitat fragmentation. Nevertheless, the potential effects of the abrupt climate change on medicinal plants have not been elucidated in-depth yet. The current status of medicinal plants under climate change, emphasizing its consequences, i.e., elevated [CO_2], drought, and extreme temperatures, is discussed in this chapter.

Keywords Biomass · Cold stress · Drought · Elevated [CO_2] · Global warming · Heat stress · Secondary metabolites

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1.1 Introduction

Medicinal and aromatic plant usage have dramatically been increased in recent years (Mishra 2016; Anand et al. 2019). The demand for medicinal plants has been increasing worldwide as the natural products have fewer or perhaps no side effects, and their accessibility and affordable costs might impact their demand for cultivation. Medicinal and aromatic plants are cultivated for their essential oils and cut flower marketing. Pharmacy, cosmetology, perfumes, and the food industry utilize their products. There are about half a million medicinal plant species worldwide with a promising future since most of their pharmaceutical effects have not been discovered yet and would be in demand of future studies.

Medicinal plants, particularly endemic medicinal plants, are precious for human life (Dewick 2002). According to the World Health Organization (WHO), approximately 21,000 plant species are being used for medicinal purposes (WHO 2013). Around 80% of the developing countries' population and 60% of the world's population depend on traditional medicines derived from plants (WHO 2013). However, the worldwide anthropogenic climate changes have adversely influenced medicinal plants. Further increases in the temperature from 1.4 to 5.8 °C are expected by 2100. There would be extreme and unpredictable weather incidents by 2033, for instance, warmer summers, stronger and more frequent storms, high winds, and more frequent and heavier rainfall (Cleland et al. 2012; Field et al. 2014).

Climate change has various adverse effects to not only melting polar ices but also changing in seasons and overall weather scenario, new plant disease occurrence, and frequent occurrence of floods. Climate change adversely affects every day human life, agriculture, forestry, biodiversity, and whole ecosystem functions (Lepetz et al. 2009). The increasing global human population, rapid industrialization, and vast amounts of chemical fertilizers and pesticide utilization in the agricultural section are some important factors causing climate change. Carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), sulfur dioxide (SO_2), nitrogen oxides (NO_x), and secondary pollutants like ozone (O_3) are among the greenhouse gases leading to the global warming. Simultaneously with the warming up of the climate, other climatic and environmental factors, i.e., the temperatures, [CO_2], drought, and rainfall patterns, are also changing. Figure 1.1 summarizes some of the major consequences of climate change.

Plant species are threatening by climate change. The endemic plant species are considered more vulnerable to climate change and facing a high risk of extinction as their narrow edaphic niches limit their possibilities to adapt through migration (Panchen et al. 2012). As for other species, medicinal species are also threatened by changing temperature and precipitation regimes, disruption of commensal relationships, pest and pathogen increases, and anthropogenic habitat fragmentation. Additionally, medicinal species are often harvested unsustainably, and the combination of those pressures may push many plant species to extinction. Besides, some species may respond to environmental stresses not only through a decline in biomass

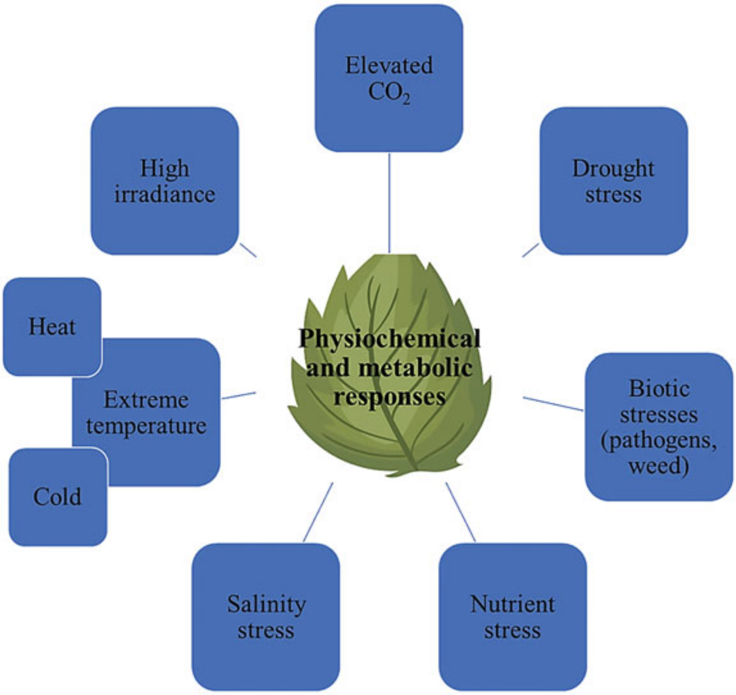

Fig. 1.1 Potential environmental stressors affecting the plant physiochemical and metabolic traits under climate change

production but also with changes in biochemical content and composition, potentially affecting the quality or even safety of medicinal products.

It is important to study the climate change effects on medicinal plants due to their dual use as medicine and food. High temperatures have been reported to reducing the oil content and unsaturated fatty acids of some oil-bearing crops, leading to declining their nutritional quality and ability to ameliorate chronic diseases (Canvin 1965; Mozaffarian et al. 2010; Dawczynski et al. 2015). Climatic changes are expected to make plant species more sensitive to pests and pathogens such as mycotoxin-producing fungi leading to reducing their quality and long-term food security (Chakraborty and Newton 2011; Magan et al. 2011; Bebber et al. 2013; Van der Fels-Klerx et al. 2016). Undoubtedly, anthropologically environmental changes will affect medicinal plants like other plant species, especially in higher altitude ecosystems, where endemic medicinal plants mainly grow (Applequist et al. 2020). Nevertheless, studies to evaluate the effects of climate change and its consequences, i.e., elevated [CO_2], higher or lower temperatures, and water stress, on medicinal plants and their physiology, biochemistry, and SMs need to be conducted in-depth. Limited and sporadic perceptions on the impacts of climate change and global warming on plant growth and development, physiology, biochemistry, and primary and secondary metabolites exist. In the present chapter, the impacts of changing climatic

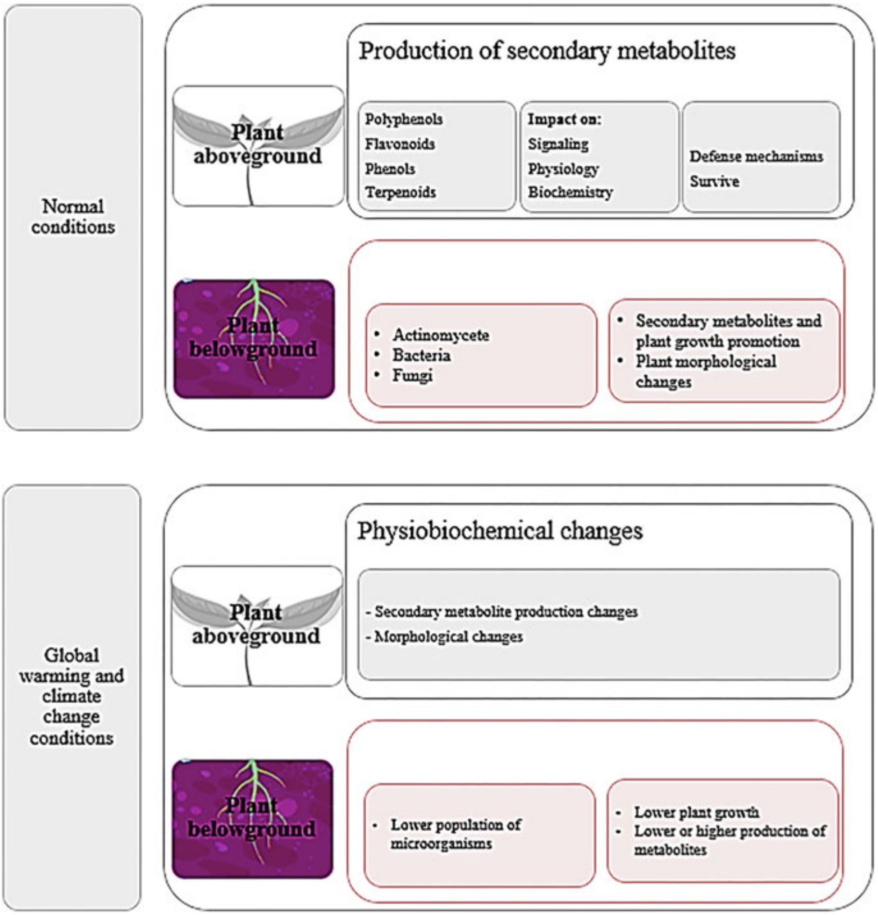

Fig. 1.2 An overview of medicinal plant status under climate change conditions

conditions on medicinal plants, with an emphasis on the main consequences of climate change, i.e., elevated [CO_2], high and low temperatures, and drought, and the current status of medicinal plants under a changing climate are discussed (Fig. 1.2).

1.2 Medicinal Plants' Availability and Population Extinction Under a Changing Climate

It seems many plant species are expected to be locally or globally extinct in the near future (Applequist et al. 2020). Almost 600 plant species have been extinct during the last hundred years (Humphreys et al. 2019). Research indicated that the world species of wild plants is threatened mainly by human activities such as habitat destruction (Skole and Tucker 1993; Riitters et al. 2002; Harper et al. 2007; Haddad et al. 2015). On the other hand, unsustainable and uncontrolled medicinal plant harvesting has made the situation even worse. The American ginseng (*Panax quinquefolius* L.), an important nourishing herb, for instance, has been harvested in large and uncontrolled quantities for commercial purposes, and illegal harvesting due to the great demand has become a serious problem in which their distribution and abundance have dramatically decreased (McGraw 2001; Case et al. 2007; Souther and McGraw 2014; Applequist et al. 2020). Similar situations have also been reported for the slow-growing medicinal herbs, goldenseal (*Hydrastis canadensis* L.) and snow lotus (*Saussurea laniceps* Hand. -Mazz.) (Mulligan 2003; Zedan 2004; Law and Salick 2005). In worse cases, habitat destruction and irregular and commercial harvesting may result in species distinction, as happened to the North African herb, *silphium Ferula* sp., which was extinct a thousand years ago (Parejko 2003; Kiehn 2007).

Even without climate changes, their isolated and small populations are endangered locally. However, environmental conditions change due to climate change will alter plant habitats, making them no longer survivable or optimum for plants. Due to global warming and a rise in the air temperature, many plant distributions have shifted toward higher latitudes, hence increasing competition between the species, leading to vanishing some of the species (Applequist et al. 2020). Nevertheless, some medicinal species, such as arnica (*Arnica montana* L.), chamomile (*Matricaria chamomilla* L.), and bush tea (*Athrixia phylicoides* DC.), are more adaptable and potent when grown at higher altitudes (Ganzera et al. 2008; Spitaler et al. 2008; Turner et al. 2011; Nchabeleng et al. 2012). This habitat fragmentation and phenological changes may also lead to disruption in the plant-pollinator relationship. Although human activities have reduced the pollinator populations, climate change will worsen these situations (Applequist et al. 2020). Medicinal plants are not exempted from these situations. Predictions showed that the ecological distribution of some medicinal herbs, e.g., *Rhodiola quadrifida*, will become smaller; however, it would be varied depending on the species (You et al. 2018).

1.3 Medicinal Plant Physiology, Biochemistry, and SMs in a Changing Climate

In addition to affecting their distribution, climate change may influence, either negatively or positively, the productivity and quality of medicinal plants and their chemical compositions. The climate change consequences, i.e., weather extremes, disturb the growth and development of unadapted plants to such conditions, resulting in reduced sustainable harvest and productivity (IPCC 2014). Nevertheless, the responses will be inconsistent within plant species and their metabolisms. Mild drought stress often stimulates bioactive compound production by enhancing the actual metabolite production or decreasing plant biomass. The effects of climate change factors, solely or in combination with other environmental stimuli, on the morphology, physiology, and biochemistry of some medicinal plant species are summarized in Table 1.1.

The SMs are the bioactive compounds producing autogenously or by endophytic symbionts; however, SMs might be altered by environmental factors. Those alterations may also impact human health since the medicinal plants are mainly consumed to derive health benefits from their bioactivities. The changes in chemical compositions of SMs might be unnoticed by the new-generation customers without chemical testing, leading to the loss of the effectiveness of medicinal plants. Although plant SMs are increased in some species to compensate for the biomass reduction under stressful conditions, this is not always desired and safe. The locally used medicinal plants may contain toxic levels of the compounds, which may be used by susceptible individuals or harmful with excessive consumption. For example, toxic metabolites such as pyrrolizidine alkaloids in *Senecio* species have been reported to increase under drought stress (Briske and Camp 1982; Kirk et al. 2010). Therefore, the geographical shifting and phenological changes due to climate change force may result in undesired alteration in the quality and composition of the SMs, leading to the medicinal plants' toxicity or unusability as a medicine.

Al-Gabbiesh et al. (2015) and Selmar and Kleinwächter (2013) reported increases in the bioactive compound concentrations, including essential oils, simple and complex phenolics, alkaloids, terpenes, and glucosinolates, in a variety of species exposed to drought stress. For instance, *Vitellaria paradoxa* Gaertn. active metabolites were increased under a drier region (Maranz and Wiesman 2004). Drought stress, therefore, may increase the potency of some medicinal plants. A decrease in plant biomass, however, due to severe drought stress (Ahmadi-Lahijani and Emam 2016) would outweigh any increases in the secondary metabolite concentrations. On the other hand, although drought may enhance the SMs' concentrations, higher temperature due to stomatal closure and lower transpiration rate reduces the concentration of SMs, as it was observed in *Rehmannia glutinosa* (Chung et al. 2006). Due to reduced biomass production, high temperatures like water stress could enhance the concentration of SMs (Jochum et al. 2007). However, it should be considered that lower biomass production would ultimately lead to enhanced harvest level unsustainability and severe economic harm.

Water scarcity decreases the CO_2 entrance into leaves by reducing the stomatal aperture, which in turn lessen the energetic molecules, i.e., ATP and NADPH, consumed by the Calvin cycle, resulting in more ATP and NADPH provided to the production of SMs. Elevated levels of [CO_2], despite a reduction in the stomatal aperture due to a greater [CO_2] availability to the plant (Ahmadi-Lahijani et al. 2018, 2021), reduce ATP and NADPH redirected toward the SMs' production pathways (Applequist et al. 2020). For instance, Nowak et al. (2010) found that the reduction capacity arising from drought stress pushed metabolic activity toward the biosynthesis of the SMs in sage (*Salvia officinalis* L.) monoterpenes here, but elevated [CO_2] decreased the monoterpene concentration. However, those observations were not found in all tested species. The CO_2 concentration and duration of exposure to

Table 1.1 Impact of climate change on the morphology, physiology, biochemistry, and secondary metabolites on a number of medicinal plant species

Medicinal plant species	Environmental stimuli	Level of treatment	Environmental conditions	Morpho-physiochemical and metabolic changes	References
Labisia pumila	Enriched [CO_2] × light intensity	(400, 800, and 1200 µmol/mol) (225, 500, 625, and 900 µmol/m^2/s)	Glasshouse	↑Flavonoids ↑Phenolics	Ibrahim et al. (2014)
Isatis indigotica Fort	Enriched [CO_2]	Ambient and 550 ± 19 µmol/mol	Free-air carbon dioxide enrichment (FACE)	↑Net photosynthetic rate ↑Water use efficiency ↑Maximum rate of electron transport ($J_{\max}$) ↓Stomatal conductance ↓Transpiration ratio ↓Maximum velocity of carboxylation ($Vc_{\max}$) ↑Efficiency of PSII (F_v'/F_m') ↑Quantum yield of PSII (Φ_{PSII}) ↓Non-photochemical quenching (NPQ) ↑Starch grains ↑Yield	Hao et al. (2013)
Catharanthus roseus	Enriched [CO_2]	600 and 900 ppm	Open top chambers	↑Phenolics ↑Flavonoids ↑Tannins ↑Alkaloids	Saravanan and Karthi (2014)
Zingiber officinale	Enriched [CO_2]	400 to 800 µmol/mol	Growth chamber	↑Flavonoids ↑Phenolics ↑DPPH ↑Photosynthesis ↑Plant biomass ↓Stomatal conductance ↑Water use efficiency ↑Soluble carbohydrates and starch ↑Antioxidant activities	Ghasemzadeh and Jaafar (2011) Ghasemzadeh et al. (2010)

(continued)

Table 1.1 (continued)

Medicinal plant species	Environmental stimuli	Level of treatment	Environmental conditions	Morpho-physiochemical and metabolic changes	References
Catharanthus roseus	Enriched [CO_2] × nitrogen supply	375 ± 30 ppm and 560 ± 25 ppm	Open top chambers	↑Phenolics ↑Flavonoids ↑Tannins ↑Alkaloids	Singh and Agrawal (2015)
Artemisia annua	Enriched [CO_2]	378–374 (ambient) and 570–577 (elevated) μmol/mol	Free-air [CO2] enrichment (FACE)	↑Artemisinin ↑C:N	Zhu et al. (2015)
Ginkgo biloba	Enriched [CO_2] × [O_3]	Ambient and doubled	Open top chambers	↓Tannins ↑Quercetin aglycon ↓Kaempferol aglycone ↓Isorhamnetin ↓Bilobalide	Huang et al. (2010)
Ginkgo biloba	Enriched [CO_2] × [O_3]	40 and 80 nmol/mol	Open top chambers	↓Tannins ↑Quercetin aglycon ↓Kaempferol aglycon ↓Isorhamnetin ↓Bilobalide	Xingyuan et al. (2009)
Papaver setigerum	Enriched [CO_2]	(300, 400, 500, and 600 μmol/mol)	Controlled environment chambers	↑Morphine ↑Codeine ↑Papaverine ↑Noscapine ↑Leaf area ↑Above-ground biomass ↑Capsules ↑Capsule weight ↑Latex	Ziska et al. (2008)

Brassica oleracea	Enriched [CO_2]	[430–480] ppm [685–820] ppm	Greenhouse	↑Glucoiberin ↑Methylsulfinylalkyl glucosinolates Glucoraphanin ↓Indole glucosinolates ↓Glucobrassicin ↓4-Methoxyglucobrassicin ↓N/S ratio ↑qN ↑qP	Schonhof et al. (2007)
Hypericum perforatum L.	Enriched [CO_2] × light intensity	100, 300, and 600 μmol/m^2/s (PPF) 500, 1000, and 1500 μmol/mol [CO_2]	Controlled environment chambers	↑Hypericin ↑Pseudohypericin ↑Hyperforin	Mosaleeyanon et al. (2005)
Quercus ilicifolia	Enriched [CO_2]	Ambient and 350 ppm above ambient	Open top chambers	↑Tannins ↑Phenolics ↓Herbivore abundance ↑Relative consumption rates ↑Development time ↑Total consumption ↓Relative growth rate ↓Conversion efficiency ↓Pupal weight ↑Biomass ↑C/N ratio ↓Nitrogen	Stiling and Cornelissen (2007)
Hypericum perforatum L.	Enriched [CO_2]	Ambient and 950–1050 μmol/mol	Closed controlled environment system (CCES)	↑Hypericin ↑Pseudohypericin ↑Hyperforin ↑Growth parameters ↑Biomass	Zobayed and Saxena (2004)

(continued)

Table 1.1 (continued)

Medicinal plant species	Environmental stimuli	Level of treatment	Environmental conditions	Morpho-physiochemical and metabolic changes	References
Panax ginseng	Enriched [CO_2]	(1%, 2.5%, and 5%)	Bioreactor	↑Phenolics ↑Flavonoids ↑DPPH ↑Fresh weight ↑Dry weight ↑Growth ratio	Ali et al. (2005)
Pseudotsuga menziesii	Enriched [CO_2] × temperature	Ambient and + 179 μmol/mol [CO_2] Ambient and +0.3.5 °C	Controlled environment chambers	↓Monoterpenes	Snow et al. (2003)
Digitalis lanata	Enriched [CO_2]	Ambient and 1000 ppm	Greenhouse	↑Cardenolide ↑Digoxin	Stuhlfauth and Fock (1990)
Hymenocallis littoralis	Enriched [CO_2]	400 and 700 ppm	Open top enclosures	↑7-Deoxy-trans-dihydronarciclasin ↑Pancratistatin ↑7-Deoxynarciclasine	Idso et al. (2000)
Digitalis lanata	Enriched [CO_2]	Ambient and 1000 ppm	Greenhouse	↑Cardenolide ↑Digoxin	Stuhlfauth et al. (1987)
Salvia officinalis	Enriched [CO_2] × drought	Normal and 70% of the optimal water supply 385 ppm or 700 ppm		↑Monoterpenes (cineole, camphor, and α-β-thujone) under drought ↓Monoterpenes exposed to enriched [CO_2]	Nowak et al. (2010)
Labisia pumila	Drought	Evapotranspiration replacement (100%, 75%, 50%, and 25%)	Glasshouse	↑Phenolics ↑Flavonoids ↑Anthocyanin ↑Phenylalanine ammonia-lyase ↓Net photosynthesis ↓Quantum yield ↓f_v/f_m ↓Dark respiration	Jaafar et al. (2012)

Petroselinum crispum	Drought	Soil water potentials (0–10%, 30–45%, and 45–60% of field capacity)	Greenhouse and outdoors	↑Monoterpenes ↓Foliage and root weight ↓Leaf number ↑Essential oil	Petropoulos et al. (2008)
Hypericum brasiliense	Drought × temperature		Greenhouse	↑Phenolic compounds ↑Reallocation of carbon ↓Growth	de Abreu and Mazzafera (2005)
Arnica montana L. *cv. ARBO*	Low temperature and ultraviolet (UV)-B radiation	5 °C	Climate chamber	↑Ortho-diphenolics	Albert et al. (2009)
Salvia sclarea	Low temperatures	Altitudes, 305, 1730, and 3505 msl	Field	↑Essential oils ↑Linalool ↑Sclareol ↑Antioxidants	Kaur et al. (2015)
Origanum dictamnus L.	Low temperatures	Natural conditions	Field	↑Proline ↑Soluble sugar ↑The antioxidative enzyme ↓Stomata and peltate hairs ↓Sclerenchymatous fibers ↓Vacuoles with phenolics ↓Chloroplasts ↓Grana and starch grains ↑Plastoglobuli ↓Net photosynthetic rate ↓Chlorophyll content ↑p-Cymene ↑Carvacrol ↑γ-Terpinene ↑Borneol ↑Antioxidant essential oils secreted ↑Glandular hairs	Lianopoulou and Bosabalidis (2014)

(continued)

Table 1.1 (continued)

Medicinal plant species	Environmental stimuli	Level of treatment	Environmental conditions	Morpho-physiochemical and metabolic changes	References
Teucrium polium	Low temperatures	Natural conditions	Field	↑Linalool ↑Terpinene-4-ol ↑Germacrene D ↑Spathulenol	Lianopoulou et al. (2014)
Withania somnifera	Low temperatures	4 °C	Controlled environment	↑Anolide (steroidal lactones) ↓Withanone ↑Superoxide anion and MDA ↑Enzymatic activities	Mir et al. (2015)
Withania somnifera	Low temperatures	25 °C and 8 °C	Growth chamber	↑Anolide ↑Withanolide A ↑Withanone	Kumar et al. (2012)
Picea abies (L.) Karst.	High temperatures × elevated UVB × fertilization	Natural conditions	Outdoor experiment	↑Piperidine alkaloids Catechins and acetophenones and bark flavonoids	Virjamo et al. (2014)
Aquilaria sinensis	High temperatures	50 °C for 30 min	Controlled environment	↑Jasmonic acid ↑Agarwood sesquiterpene	Xu et al. (2016)
Ribes nigrum L.	High temperatures	Natural conditions	Field	↑Delphinidin-3-O-glucoside ↑Delphinidin-3-O-rutinoside ↑Myricetin-3-O-glucoside	Zheng et al. (2012)
Cyanea acuminata	High temperatures	40 °C	Controlled environment	↑10-Hydroxycamptothecin	Zu et al. (2003)
Panax quinquefolius	High temperatures	25/20 or 30/25 °C (day/night)	Greenhouse	↑Ginsenoside ↓Photosynthesis ↑Leaf senescence ↓Carbon accumulation ↓Stomatal conductance ↓Root and total biomass	Jochum et al. (2007)

Catharanthus roseus	High temperatures	16 °C to 40 °C	Controlled environment	↓Alkaloids ↓Serpentine ↓Ajmalicine	Morris (1986)
Catharanthus roseus	High temperatures	Short-term heat shock at 30 °C and 40 °C Long-term heat at 20 °C, 25 °C, and 35 °C	Controlled environment	↑Vindoline ↑Catharanthine ↑Vinblastine	Guo et al. (2007)

CO_2 levels, which both either increased or decreased SMs' production, have been proposed depending on the species (Table 1.1). Many studies have also reported an enhanced biosynthesis of the SMs under elevated levels of [CO_2]. For instance, elevated [CO_2] levels increased the concentration of artemisinin in Sweet Annie (*Artemisia annua* L.) and phenolic and flavonoid compounds in ginger (*Zingiber officinale* Roscoe) rhizome under controlled conditions (Ghasemzadeh et al. 2010; Zhu et al. 2015).

1.4 The Climate Change Consequences on Medicinal Plants

Abiotic stresses reduce crop performance and yield. However, mild stresses may positively affect the quality of plant products, e.g., through the activation of the phenylpropanoid pathway and the accumulation of bioactive compounds (Imai et al. 2006). These can improve postharvest performance and enhance the nutritional quality of the products, which is particularly important for their consumers. Abiotic stresses must be continuously studied with multidisciplinary approaches, from the basic science to understand crop responses and their adaptation to the identification of practical agronomic solutions for alleviating the stressful effects and preserving crop productivity (Mariani and Ferrante 2017; Ferrante and Mariani 2018).

The interaction of plants with the biotic and abiotic environmental stimuli influences metabolite biosynthesis (Akula and Ravishankar 2011). The synthesis of metabolites is regulated by and restricted to specific vegetal tissues or development stages in response to environmental stimulation (van der Plas et al. 1995; Gargallo-Garriga et al. 2014). Plant SMs, besides participating in the pharmaceutical industry, play a significant role in plant survival, and their synthesis is induced by the plant-environment interaction (Radušienė et al. 2012). The plant primary and secondary metabolisms are closely related to each other (Kumar et al. 2017); the primary metabolites are utilized as substrates to plant SMs' biosynthesis. When a plant is affected by adverse environmental conditions, e.g., climate change, plant growth and the production of primary metabolites are influenced, which in turn affect the SMs' production (Table 1.1). As a survival strategy and to make diversity at the organism level, plant species are variable in their potentials of synthesizing SMs. There are even variations in the content of the chemical compounds within a species. These characteristics are possibly associated with genetic variability and the differences in the growth conditions (Radušienė et al. 2012; Mishra 2016).

Environmental parameters have direct effects on crop performance in different seasons and nutrient availability. Cultivation of two cultivars lettuce (*Lactuca sativa*) in different seasons with various nutrient availabilities showed that suboptimal growing conditions limited nutrient utilization and had adverse effects on biomass accumulation. Secondary metabolites involving the antioxidant capacity of lettuce were affected by the seasons through effects on the compositions and total concentrations of different flavonoids (Toscano et al. 1982; Sublett et al. 2018). Under stressful conditions, plants tend to come up with reactive oxygen species (ROS) like superoxide (O_2^-), H_2O_2, and hydroxyl radical ($OH^•$), which might promote cellular damage by triggering off an oxidative chain reaction (Imlay 2003). Plants eliminate the ROS by producing defense compounds through enzymatic and non-enzymatic mechanisms, as some secondary metabolism compounds. For a balanced ROS level and not being harmful to cells, the combination of enzymatic and non-enzymatic mechanisms is fundamental (Shohael et al. 2006; Moori et al. 2012). Generally, under stressful conditions, plants tend to increase their enzymatic activity and synthesize secondary metabolite compounds. This accumulation is because of a rise in the enzymes such as phenylalanine ammonia-lyase and chalcone synthase activities (Heldt and Piechulla 2011), which are vital enzymes in the flavonoid synthesis pathway and might be affected by environmental stresses. The phenylalanine ammonia-lyase, by producing phenols and lignin, is the main enzyme in plant stress defense (Dixon et al. 1992). Medicinal plants may accumulate terpenes in the type of essential oils under stressful conditions. Terpenoids are the main constituents of the essential oils; however, phenylpropanoids would also contribute to the essential oil composition (Sangwan et al. 2001; Jaafar et al. 2012).

Glucosinolate’s function may also be affected by climate change. Glucosinolates are a class of SMs that their biological activity, mainly in preventing cancer, has attracted attention (Schonhof et al. 2007). Studies have shown that the synthesis of glucosinolate compounds in *Brassicaceae* beyond the biotic factors was influenced by abiotic factors such as salinity, drought, extreme temperatures, nutrient deficiency, and soil acidity (low pH) (Steinbrenner et al. 2012). Aromatic amino acids are likely the main precursors of SMs contributing to plant stress defense. For instance, tryptophan is the precursor of alkaloids, phytoalexins, and indole glucosinolates. Phenylalanine is the main precursor of phenolic compounds such as flavonoids, tannins, and phenylpropanoids, and tyrosine is the precursor of isoquinoline alkaloids and quinones (Cheynier et al. 2013). The metabolic pathway of synthesis for these compounds is performed in three phases, where the chain elongation is affected by the stress (Ruelland et al. 2009; Holopainen and Gershenzon 2010; Khan et al. 2011).

1.5 Effects of Elevated [CO_2] on Medicinal Plants

The plant photosynthetic gas exchange is directly affected by elevated [CO_2], where it indirectly contributes to the global warming. The photosynthesis of many plant species is not fully saturated under the present [CO_2]; hence, its enrichment enhances the photosynthetic rate and stimulates crop growth and productivity (Reddy et al. 2010; Fleisher et al. 2014; Ahmadi-Lahijani et al. 2021). Accordingly, a higher photosynthetic rate was observed in the elevated [CO_2] compared with an ambient [CO_2] (Ainsworth and Long 2005; Hao et al. 2013; Ahmadi-Lahijani et al. 2018). Studies revealed that elevated [CO_2] enhanced photosynthetic carbon assimilation rates in some plant species (Ainsworth and Long 2005; Ahmadi-Lahijani et al. 2018, 2019, 2021). They also observed that the elevated [CO_2] increased the above-ground biomass and dry matter partitioning to the underground parts of the plants. The higher carboxylation rate and inhibition of the Rubisco oxygenation are responsible for the improvement in photosynthesis at elevated [CO_2], although its effects might be varied depending on the plant species, [CO_2], developmental stage, and environmental conditions (Hao et al. 2013). Higher photosynthesis of plants at elevated [CO_2] could also be due to adjustment of the photosynthetic apparatus, such as cellular fine structures, i.e., chloroplast and mitochondria number and size, to such conditions (Ainsworth and Long 2005; Hao et al. 2013; Ahmadi-Lahijani et al. 2018).

Nevertheless, long-term exposure to elevated [CO_2] might result in photosynthetic acclimation and a decrease in photosynthesis (Lawson et al. 2001; Katny et al. 2005). For instance, some research found a photosynthetic acclimation at elevated [CO_2] (Huang et al. 2003; Aranjuelo et al. 2011; Hao et al. 2012). However, Hao et al. (2013) did not observe a photosynthetic acclimation in *Isatis indigotica*, which is used for the clinical treatment of virus infection, tumor, and inflammation in Chinese traditional medicine, at elevated [CO_2] due to developing new carbon sinks. Elevated [CO_2] increased net photosynthetic rate, water use efficiency, and maximum rate of electron transport ($J_{\max}$) of *Isatis indigotica* leaves, although stomatal conductance, transpiration ratio, and maximum velocity of carboxylation ($Vc_{\max}$) were not altered. In addition, the efficiency (F_v'/F_m') and quantum yield (Φ_{PSII}) of PSII were significantly increased at elevated [CO_2], but leaf non-photochemical quenching (NPQ) was decreased. While *Isatis indigotica* yield was higher due to the improved photosynthesis at elevated [CO_2], the content of adenosine was not affected.

Medicinal plants are sources of SMs and show a wide range of plasticity to adapt to changing environments. The SMs may affect other metabolites, which are usually the basis for their medicinal properties (Stuhlfauth et al. 1987; Mishra 2016). It is predicted that an increase in the [CO_2] may increase plant carbon/nutrient ratio, leading to produce non-structural carbohydrates (NSCs) that incorporate in C-based SMs (Heyworth et al. 1998). For instance, elevated [CO_2] increased digoxin, a cardenolide glycoside that is used in heart diseases, by 3.5-fold in *Digitalis lanata* plants (Rahimtoola 2004). Another experiment indicated that although digoxin was enhanced under the elevated [CO_2], digitoxin, digitoxigenin, and digoxin-monodigitoxoside were declined (Stuhlfauth et al. 1987; Stuhlfauth and Fock 1990). The SMs may also be affected by the time of exposure to the elevated [CO_2]. For instance, the alkaloids (pancratistatin, 7-deoxynarciclasine, and 7-deoxy-trans-dihydronarciclasin) of the medicinal plant *Hymenocallis littoralis*, whose bulbs are used for their antineoplastic and antiviral effects, were increased by the first year exposure to the elevated [CO_2]; however, they were decreased in the subsequent year (Idso et al. 2000). Similarly, elevated [CO_2] and [O_3] increased quercetin aglycon up to 15% and decreased kaempferol aglycon by 10% in *Ginkgo biloba*, a traditional Chinese medicinal plant used in Alzheimer's disease (Huang et al. 2010; Weinmann et al. 2010).

It has been shown that elevated [CO_2] enhanced *Hypericum perforatum* phenolic compounds, hypericin, pseudohypericin, and hyperforin (Zobayed and Saxena

2004). Ezuruike and Prieto (2014) also reported increases in *Catharanthus roseus* alkaloids, flavonoids, phenolics, and tannins when treated with elevated [CO_2] (Saravanan and Karthi 2014). Ziska et al. (2008) found that raising the [CO_2] from 300 to 600 μmol/mol enhanced the alkaloid concentrations, i.e., morphine, codeine, papaverine, and noscapine, in *Papaver setigerum*. It has been observed that elevated [CO_2] increased the tannins and phenolic content of *Quercus ilicifolia* (Saravanan and Karthi 2014). A rise in phenols and flavonoids was also observed due to increased primary metabolite phenylalanine, a precursor of various SMs (Ibrahim and Jaafar 2012). The phenols, alkaloids, tannins, and flavonoids of *Catharanthus roseus*, which are known for their antiviral, anticancer, and diuretic properties, were increased by the elevated [CO_2]. It has also been reported that the concentrations of phenolic and flavonoid compounds in *Zingiber officinale* were enhanced with increased [CO_2] (Ghasemzadeh et al. 2010; Ezuruike and Prieto 2014; Saravanan and Karthi 2014).

Further, working on *Labisia pumila*, Ibrahim et al. (2014) found that flavonoids and phenolics were increased in response to increased atmospheric [CO_2]. The findings were more inclined toward the increase in the SMs' concentrations as a response to elevated [CO_2] compared to the present ambient [CO_2]. However, it was observed that *Pseudotsuga menziesii* terpenes, specifically monoterpenes, significantly decreased under elevated [CO_2] (Snow et al. 2003). By reviewing the trend in such findings, although the effects of stimuli such as temperature, nutrient availability, seasonal variation, time duration, etc. were evaluated, it is essential to concentrate on the entire SMs of medicinal plants. Other parameters, solely or in combination with other factors, may also alter the metabolic plasticity of medicinal plants.

1.6 Medicinal Plants' Growth in Drought Stress Conditions

Arid and semi-arid regions are more prone to drought stress, which negatively impacts medicinal plants and is presently more prevalent due to global warming. Drought stress induces species-dependent biochemical, physiological, and genetic alterations (Eisvand et al. 2016; Zhou et al. 2017). The synthesis and accumulation of the plant SMs are strongly influenced by environmental conditions, such as temperature, light regimes, and water and nutrient supply (Siddiqui and Bansal 2017). It has been elucidated that drought stress consistently enhances the plant SMs' concentration (Kleinwächter and Selmar 2014; Nasrollahi et al. 2014; Selmar et al. 2017), which could be a common feature and comprises all classes of natural products. For instance, increases in either the simple or complex phenols and a variety of terpenes were reported. Similarly, drought stress positively impacted nitrogen-containing substances such as glucosinolates, alkaloids, and cyanogenic glucosides (Petropoulos et al. 2008; Albert et al. 2009; Chen et al. 2011; Jaafar et al. 2012). A secondary metabolism called oxidative stress is also compelled by drought stress, leading to reducing the photosynthetic rate and inducing phenolic compound production. These phenolic compounds contribute to the plant's defense mechanism against stressful conditions (Jaafar et al. 2012). Studies manifested higher SMs' accumulation in drought-stressed plants than those in well-watered conditions.

Although drought adversely affects plant growth and development, the concentration of natural products increases under stressful conditions. Theoretically, some possibilities are proposed to clarify this enhancement. It may be the consequence of reduced biomass in the stressed plants, while the rate of natural product biosynthesis is not changed or slightly reduced; hence, their concentration on a dry or fresh weight basis is increased (Kleinwächter and Selmar 2014). Therefore, the SMs' content in drought-stressed and drought-unstressed plants is quite similar (Mishra 2016). Alternatively, drought stress enhances the actual rate of biosynthesis possibly due to either a passive shift or a functional upregulation of the enzymes involved in the natural product biosynthesis (Selmar et al. 2017).

The overall content of essential oils in Greek oregano (*Origanum vulgare*) plants remained constant, although their concentrations were significantly increased (Ninou et al. 2017). However, Nowak et al. (2010) found that the monoterpenes in the drought-stressed sage (*Salvia officinalis*) were higher than the unstressed plants and the SMs' enhancement was over the corresponding biomass reduction. Consequently, the actual biosynthesis of monoterpenes was increased in response to drought stress. Phenolic compounds also follow the same trend. In *Hypericum brasiliense*, the total phenols showed an increase under drought stress, and plants grown under drought-stressed conditions were generally smaller than the unstressed plants due to the massive increase in phenolic content (de Abreu and Mazzafera 2005). The concentration and overall production of total phenols and flavonoids were also enhanced in *Labisia pumila* grown under water-deficient conditions (Jaafar et al. 2012). However, it has been observed that the increase in the coptisine concentration was compensated by a drastically reduced biomass in the drought-stressed plants, which in turn reduced the overall alkaloid content of the stressed plants (Kleinwächter and Selmar 2015).

Genotypes behave differently under drought stress conditions, in which the essential oil content per plant in the *Origanum vulgare* (Greek oregano) subspecies remained constant, while the metabolites' concentrations were increased (Ninou et al. 2017). It indicated that the essential oil content, compositions, and gene expression patterns of the subspecies were different under drought stress conditions (Morshedloo et al. 2017). It seems that the monoterpene synthesis rate remained constant, while the same monoterpene content was obtained from less biomass. Those results were used by Paulsen and Selmar (2016) to explain the increase in thyme (*Thymus vulgaris*) plants' monoterpene concentration. They observed no changes in the total monoterpenes' content, but in a dry weight basis, the rate of monoterpene synthesis was found different in drought-stressed plants compared to the drought-unstressed plants (Paulsen and Selmar 2016).

1.7 Extreme Temperatures and Medicinal Plants

The high rate of greenhouse gas emission from different sources has gradually raised the global ambient temperature, leading to the global warming phenomenon. Climate change is accompanied by extreme temperatures, which will expose the plants to harsher and unpredictable weather conditions. Almost 23% of the earth's surface is subjected to an annual temperature of ~40 °C or higher. High-temperature stress has become a concern for crop production since it truly influences the overall growth, development, and productivity of plants. Therefore, the response of plants and the mechanisms underlying their adaptation to elevated temperatures need to be better understood for important medicinal plants. Furthermore, metabolic adjustments to stressful conditions are vital for acquiring stress tolerance (Hasanuzzaman et al. 2013). Plants exposed to extreme temperatures frequently show a typical reaction as oxidative stress that damages lipids, proteins, and nucleic acids. Although researchers have focused on the plant reactions to heat stress, to date, a complete comprehension of the plant thermoresistance systems stays inconspicuous.

Increasing worldwide demand for food provoked plant breeders to introduce high-yielding plants resistant to environmental stress. High-temperature stress leads to physiological, biochemical, and molecular changes and damages the proteins, lipids, and cell membrane integrity of plants, which instigates the biosynthesis of SMs in plant tissues (Zobayed and Saxena 2004; Kumar et al. 2012). High temperature, on the other hand, diminishes photosynthesis due to a reduction of soluble and Rubisco binding proteins (Hasanuzzaman et al. 2013). Heat stress negatively impacts leaf water potential and leaf area and accelerates premature leaf senescence, adversely affecting the overall photosynthetic performance of plants. The activity of the enzymes, ADP-glucose pyrophosphorylase, sucrose phosphate synthase, and invertase, is reduced under high temperatures, which in turn affects the synthesis of sucrose and starch (Rodríguez et al. 2005). Rubisco catalyzes the first step in the net photosynthetic assimilation and photorespiratory carbon oxidation pathways through the carboxylation and oxygenation of ribulose-1,5-bisphosphate (RuBP). The carboxylation activity of the Rubisco enzyme is reduced in high temperatures, due to competitive inhibition of the O_2 to binding the RuBP. High temperature, in addition, quickly shifts de novo protein synthesis from Rubisco's large and small subunits' expression to the synthesizing heat shock proteins (HSP) (Law et al. 2001).

The temperature has a great influence on the SMs' production in plants (Akula and Ravishankar 2011). One of the plant's approaches to resist high temperatures is the accumulation of osmolytes, the water-soluble metabolites with a low molecular weight such as amino acids and sugars (Wani et al. 2017; Mohammadi et al. 2020). Furthermore, an increase in the biosynthesis of compounds such as glutathione and ascorbate has also been reported to improve plant cellular resistance (Hatami et al. 2017). The aromatic amino acid compounds such as tryptophan, triazine, and phenylalanine, producing in the shikimic acid pathway, are responsible for producing secondary metabolism under temperature stress conditions (Suguiyama et al.

2014). To confront heat stress, a part of plant tolerance mechanisms is the SMs' production for cell homeostasis (Bokszczanin et al. 2013).

Studies have been carried out to investigate the effects of various temperature regimes on medicinal plants. For instance, high temperatures enhanced the morphine content of poppy (*Papaver* spp.) plants (McAlister et al. 2016) and increased SOD, CAT, and POD enzyme activities to scavenge the reactive oxygen species (ROS) (Ncube et al. 2012). Zobayed et al. (2005) found that St. John's wort (*Hypericum perforatum*) peroxidase and hypericin activities and pseudohypericin and hyperforin contents were increased in high temperature (35 °C). The organic and volatile compounds' increases have also been observed in medicinal plants grown in high temperatures (Wani et al. 2017; Mohammadi et al. 2020). A reduction in photosynthesis and enhancement in ginsenoside were observed in *Panax quinquefolius* when the temperature was raised to 5 °C (Jochum et al. 2007). While the pigment production was optimal at 25 °C in suspension-cultured *Perilla frutescens*, a remarkable reduction was observed in the anthocyanins at 28 °C (Zhong and Yoshida 1993).

Low temperature is also one of the most harmful abiotic stresses for plants as it impacts different plant developmental processes and influences the geographic distribution and productivity of plants. Low temperatures, in addition, directly affect plant growth and development processes at the physiological, metabolic, and genetic levels and slow down plant metabolic reactions such as sucrose synthesis (Sage and Kubien 2007; Ruelland et al. 2009; Mir et al. 2015). Furthermore, the contents of cryoprotective proteins and metabolites like soluble solids and amino acids are induced at low temperatures. Plants also develop cellular enzymatic and non-enzymatic mechanisms to cope with cold stress and survive under low temperatures, which are mainly mediated by phytohormones (Rahman 2013). Some medicinal species, such as *Thymus sibthorpii*, *Satureja thymbra*, *Cistus incanus*, *Phlomis fruticosa*, and *Teucrium polium*, show a seasonal dimorphism and develop mechanical and chemical defensive compounds to cope with cold (Lianopoulou and Bosabalidis 2014). Low temperatures also instigate the anatomical, morphological, physiological, biochemical, and cytological changes, which may also be mediated by phytohormones.

In *Origanum* (*Origanum vulgare*), structural and functional changes affect leaf shape, size, and distribution pattern as an adaptation mechanism at low temperatures. The medicinal herb *Salvia sclarea*, commonly known as clary sage, showed a decrease in leaf area but increases in spikes' length and number and inflorescence length with higher essential oil content in low temperatures (Kaur et al. 2015). Chilling stress structurally affected the mountain germander (*Teucrium polium*) and *Thymus sibthorpii* and led to producing smaller and thicker leaves with greater stomata and glandular hairs (Lianopoulou et al. 2014). Apart from structural changes, it has been observed that low temperatures led to a subsequent accumulation of antioxidant compounds contributing to their medical or nutritional values in numerous medicinal and aromatic plants (Nourimand et al. 2012; Mir et al. 2015; Saema et al. 2016). The biomass production and chlorophyll and b-carotene content of fennel (*Foeniculum vulgare*) seedlings grown at 2 °C were impacted, whereas the total antioxidant activity was increased in response to such conditions (Nourimand et al. 2012). Cold stress is responsible for increasing the cell wall-related and phenol compounds to enhance cold tolerance (Christie et al. 1994). Plants grown under cold stress showed an increase in anthocyanin biosynthesis, which was related to acquiring cold tolerance (Christie et al. 1994; Pennycooke et al. 2005).

The biosynthesis and accumulation of withanolide compounds in Indian ginseng or ashwagandha (*Withania somnifera*) synthesized in leaves and roots are directly influenced by environmental factors. Seasonal low temperatures increase the withanolides' accumulation in Indian ginseng (Kumar et al. 2012). *Withania somnifera* plants' exposure to cold stress increased the leaves' withanolide (steroidal lactones) accumulation (Kumar et al. 2012), which was associated with an enhanced plant recovery after the cold exposure (Saema et al. 2016). Low temperatures also concomitantly enhanced the antioxidant enzymatic activities in *Withania somnifera* and *Thymus sibthorpii* leaves (Wang et al. 2013; Lianopoulou et al. 2014), which is often a protection mechanism against cold damages (Kaur et al. 2015).

1.8 Conclusion

Medicinal plant exposure to abiotic stress impacts their physiological, biochemical, phytochemical, and molecular responses to cope with stressful conditions. They need to fortify their defense system in response to extreme environmental conditions resulting from climate change. The availability of water, nutrients, and overall optimal growing conditions of plants are influenced by the changes in climatic conditions, which directly impact the productivity of medicinal plants. Their secondary metabolites protect the plant from stressful conditions and consequently provide a unique source of flavors and pharmaceutical properties for human health. Various studies have revealed that environmental factors, like water scarcity, elevated [CO_2], and extreme temperatures altered the physiological and biochemical mechanisms, quality and quantity of SMs, productivity, and the distribution of medicinal plants. It seems there is a need to understand the response of medicinal plants to climate change on a molecular level toward the improvement of their growth and productivity.

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Chapter 2
Environmental Challenges for Himalayan Medicinal Plants

Jitendra Pandey, Lav Singh, Gauri Saxena, and Hari Prasad Devkota

Abstract The Himalayan region is home to diverse ecological systems covering many important flora and fauna that are indispensable for human livelihood. Himalayan medicinal plants have great value in the traditional systems of medicine such as Ayurveda, Chinese traditional medicine, and Tibetan traditional medicine systems and other indigenous medicine systems. Due to extreme environmental conditions, climate change, and other anthropogenic factors, there is huge biodiversity loss, and various medicinal plants are endangered. Similarly, these environmental challenges also affect the content of bioactive chemical constituents in medicinal plants. This chapter summarizes some of these aspects of Himalayan medicinal plants and their conservation and environmental challenges with a focus on the bioactive chemical constituents.

Keywords Himalaya · Medicinal plants · Environmental challenges · Bioactive chemical constituents

2.1 Introduction

The majestic crescent-shaped mountain range of the Himalayas stretches over 2500 km from the Indus Valley's south end, far beyond Nanga Parbat in the west, to Namcha Barwa in the east. It fringes the entire length of the Indian subcontinent's

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northern boundary. The Himalayas are home to the world's most famous snow-capped mountains, including 10 of the world's 14 peaks over 8000 m including Mt. Everest (Roy and Purohit 2018). These are one of the youngest fold mountain systems running through India, Pakistan, Afghanistan, China, Bhutan, and Nepal. The Himalayas comprise three parallel ranges, the Greater Himalayas, the Lesser Himalayas, and the Outer Himalayas. The Himalayan region is home to diverse ecological system covering many important flora and fauna that are indispensable for human livelihood. A total of 10,503 plant species are reported from the Himalayan region representing 240 families and 2322 genera with around 1206 alien species. With 783 species (7.5 percent of all species), Orchidaceae is the most common family. The Himalayan ranges in Bhutan and Sikkim are the richest and with the highest number of species (6283) among the six sub-regions, followed by Nepal (5516), Uttarakhand (4990), Arunachal Pradesh (4503), Himachal Pradesh (3324), and Jammu and Kashmir (2118) (Rana and Rawat 2017).

Himalayan medicinal plants have great value in the traditional systems of medicines such as Ayurveda, Chinese traditional medicine, and Tibetan traditional medicine systems and other indigenous medicine systems (Table 2.1). Various phytochemicals obtained from these medicinal plants are also the sources for the discovery and development of modern drugs (Atanasov et al. 2015). Many of these medicinal plants are also used as food and spices (Khanal et al. 2021). Their traditional harvesting practices have altered from time to time due to many reasons, including advent of market forces that have resulted in the decline or even existence of many plant populations. The evaluation of secondary metabolites obtained from these plants with broad medicinal potential has sparked a sudden interest in learning more about their chemistry, analytical techniques, biosynthetic pathways, and pharmacological effects (Malhotra and Singh 2021).

The Himalayas may seem eternal, but they are among the most fragile ecosystems on the planet earth. Steep slopes, heavy rainfall, and poor soil quality make it vulnerable to erosion. Human presence and related activities, such as agropastoralism and natural product extraction, have affected a major fraction of high-altitude landscapes in the Himalayas over a long period of time. The grand Himalayas are home to a plethora of medicinal plants with great ethnobotanical significance. They are one of the most important sources of natural resources and have long been exploited for both local and global trade. Despite the fact that medicinal plants have thrived in highland areas with low human and animal populations, two recent events have raised conservation and management concerns. The first is a significant increase in livestock density; the second is significant growth in medicinal plants' demand in international trade (Ghimire et al. 2006). Himalayan biodiversity and ecosystems face many challenges, especially that of injudicious use of its natural resources. This may be due to the lack of understanding and sufficient training and the method and time of collection being unscientific and unwise, causing damage to medicinal plants as well as a reduction in material quality. Lack of a grazing management system is another threat as animals are allowed to graze to the point of over-grazing, causing serious damage to the flora. This calls for

Table 2.1 Some of the important medicinal plants of the Himalayan region (Badola and Aitken 2011; Chhetri 2014)

Scientific name	Family	Common name
Aconitum ferox	Ranunculaceae	Vatsnabh
Aconitum palmatum	Ranunculaceae	Prativisa
Acorus calamus	Araceae	Sweet flag
Aloe vera	Asphodelaceae	Ghritkumari
Alstonia scholaris	Apocynaceae	Satvin
Arnebia benthamii	Boraginaceae	Himalayan arnebia
Aquilaria agallocha	Thymelaeaceae	Agar
Artemisia annua	Asteraceae	Sweet worm wood
Asparagus racemosus	Asparagaceae	Shatavari
Atropa belladonna	Solanaceae	Belladonna
Berberis aristata	Berberidaceae	Daruhaldi
Berberis asiatica	Berberidaceae	Tree turmeric
Centella asiatica	Apiaceae	Mandookparni
Cinchona officinalis	Rubiaceae	Cinchona
Cinnamomum tamala	Lauraceae	Indian bay leaf
Coleus barbatus	Lamiaceae	Patherchur
Convolvulus microphyllus	Convolvulaceae	Shankhpushpi
Crocus sativus	Iridaceae	Kesar
Dactylorhiza hatagirea	Orchidaceae	Hatta haddi
Digitalis purpurea	Plantaginaceae	Lady’s glove
Ephedra gerardiana	Ephedraceae	Soma
Ferula foetida	Apiaceae	Hing
Ginkgo biloba	Ginkgoaceae	Ginkgo
Gloriosa superba	Liliaceae	Flame lily
Gmelina arborea	Lamiaceae	Gambhari
Hedychium spicatum	Zingiberaceae	Kapurkachari
Holarrhena antidysenterica	Apocynaceae	Kurchi
Hyoscyamus niger	Solanaceae	Henbane
Inula racemosa	Asteraceae	Kasmira
Jurinea dolomiaea	Asteraceae	Dhoop lakkad
Mesua ferrea	Calophyllaceae	Nagakeshar
Orchis latifolia	Orchidaceae	Salam panja
Phyllanthus amarus	Phyllanthaceae	Bhumyamalaki
Picrorhiza kurroa	Plantaginaceae	Kutki
Plantago ovata	Plantaginaceae	Isabgol
Podophyllum hexandrum	Berberidaceae	May apple
Rauwolfia serpentina	Apocynaceae	Sarpagandha
Rheum australe	Polygonaceae	Himalayan rhubarb
Rhododendron campanulatum	Ericaceae	Pink brass
Selinum tenuifolium	Apiaceae	Wallich milk parsley
Solanum nigrum	Solanaceae	Makoy
Swertia chirata	Gentianaceae	Chirata

(continued)

Table 2.1 (continued)

Scientific name	Family	Common name
Taxus baccata	Taxaceae	English yew
Tinospora cordifolia	Menispermaceae	Giloe
Valeriana wallichii	Caprifoliaceae	Tagar-Ganthoda
Withania somnifera	Solanaceae	Ashwagandha

a comprehensive training of local residents and skill development for the collection, preservation, and processing of medicinal and other economically important plants.

Various environmental challenges have affected the biodiversity of medicinal plants in the Himalayan region. Climate change and associated risk factors affect not only the biodiversity and availability of the medicinal plants but also the bioactive chemical constituents present in them (Applequist et al. 2020; Karimi et al. 2021). As the nature and content of bioactive chemical constituents are responsible for the pharmacological activity of the plants used in traditional medicines (Devkota et al. 2017), these environmental challenges may also affect the desired pharmacological activity and therapeutic efficacy.

2.2 Current Status of High-Altitude Medicinal Plants of Himalaya and Their Conservation

Conservation of high-altitude medicinal plants in the Himalayan region is a major challenge faced by traditional healthcare systems and plant-based pharma industries. There have been various studies to understand the current status of distribution and conservation of medicinal plants in Himalaya. For example, according to a study by Islam et al. (2021), internationally documented nine threatened species were found in the Palas Valley, Pakistan. Of these, *Aconitum heterophyllum*, *Taxus wallichiana*, and *Berberis pseudumbellata* have been categorized as Endangered, while *Ephedra intermedia*, *Acer caesium*, *Punica granatum*, *Juniperus communis*, *Populus ciliata*, and *Quercus alba* as Least concern species. The major causes of species acquiring threatened status were due to overexploitation, fragmentation, and habitat loss.

Tali et al. (2019) evaluated 881 medicinal plant species for priority conservation in Jammu and Kashmir (J&K) region, a Himalayan biodiversity hotspot. Ten of these species *Arnebia benthamii* (Critically Endangered), *Angelica glauca* (Endangered), *Dactylorhiza hatagirea* (Endangered), *Fritillaria roylei* (Critically Endangered), *Meconopsis aculeata* (Critically Endangered in the Himalayan ranges of Pakistan and Kashmir), *Picrorhiza kurroa* (Endangered in the Indo-China Himalayan region), *Podophyllum hexandrum* (Endangered high-elevated), *Saussurea costus* (Critically Endangered), and *Thymus linearis* (Least concern) have previously been selected for conservation in Uttarakhand, a neighboring Indian Himalayan state. On the other hand, *Thymus linearis* is distributed in Afghanistan, India, Iran, Nepal, Pakistan, Tadzhikistan, West Himalaya, and Xinjiang.

Many medicinal plants which not only are important for medicinal value but also provide economic support to the local inhabitants are also being threatened for their natural habitats. For example, *Aconitum heterophyllum* (Ranunculaceae), commonly known as "Atis," is a biennial herb native to northwest and east Himalayan regions of the Indian subcontinent. Its nontoxic tuberous roots are often used to treat dyspepsia, stomach pain, diabetes, and diarrhea in the traditional Indian and Chinese medicinal systems. The aconites, which include atisine, are important ingredients and marker compound of *A. heterophyllum*. According to the IUCN Red List, this plant species is categorized as an endangered medicinal plant because of habitat destruction and extensive exploitation (Malhotra and Sharma 2021). Successful cultivation and conservation of *A. heterophyllum* will require an improved agro-technology and genotype strategies that will lead to higher crop yields (Beigh et al. 2008).

In the last few years, identification and management of medicinal plants with special emphasis on protected area have become one of the top conservation priorities. Several initiatives have been taken to implement in situ as well as ex situ management strategies. However, it is important to understand that each species requires a specific set of habitat for its survival; thus, emphasis has been given to the protection of viable population along with management of its natural habitat. Various forums have been created for the categorization as well as prioritization of taxa based upon the degree of threat they are facing to strategize conservation initiatives. However, desired success is yet to be achieved due to complex geological composition and diverse local climatic conditions of the Himalayas (Badola and Aitken 2011). Dhar (2002) recommended the necessity of attempting more studies and assessments to prioritize the distribution and significance of the species important for their medicinal values covering different agro-ecological as well as agro-climatic zones in the Himalayan regions for the management and conservation of such species.

These conservation statuses and the biodiversity of medicinal plants will be greatly affected by the different environmental challenges such as climate change, global warming, and other various anthropogenic factors. Better understanding of the effects of these factors in relation to medicinal plants is necessary for the conservation of biodiversity and sustainable utilization of these natural resources in Himalaya. According to Hoffmann et al. (2007), it is a novel way to entice farmers to participate in medicinal plant cultivation and long-term conservation of their natural ecosystem. It is based on the understanding that local people and scientists have distinct types of information and talents that may be best utilized by collaborating farmers and other stakeholders for plant cultivation and conservation and their long-term use.

2.3 Effects of Environmental Challenges on Himalayan Medicinal Plants and Their Bioactive Chemical Constituents

Climate change, global warming, natural disasters, and various other factors are greatly affecting medicinal plants' biodiversity and habitat. Between 2018 and 2100, an increase of 3.7 °C is expected in mean surface temperatures in the Himalayas (relative to 1986–2005) which will greatly affect many areas of biodiversity and water cycle (Karimi et al. 2021). Such environmental stress will also affect the chemical constituents of the medicinal plants in the Himalayas and will possibly affect their pharmacological activities. Some of these factors are explained in detail in the following sections.

2.3.1 Climate Change

In the present scenario, unprecedented change in the weather condition of earth is manifested clearly. Anthropogenic activities such as uncontrolled emission of greenhouse gases (CO_2, methane, CO, O_3, and chlorofluorocarbon) on the atmospheric surface are the major cause of dramatic climate changes. Major unfavorable alternations in the environment, due to climate change, are most frequent and hotter summer days, most often and heavier rainfall, infrequent and less dry season winter rainfall, and heavy and more frequent storms with high wind. The temperature of the earth is rising spontaneously by 5 °C or more, in a couple of decades, and these adversarial shifts in climate have created environmental stress on the plants and grievously affect plant yield and growth. The amount of secondary metabolites in plants and their normal growth rate are varied by hike in atmospheric temperature, as it can fluctuate metabolic pathway which governs plant physiology, signaling, and defense mechanism. Apart from that, syntheses of primary metabolic products, such as sugars, Krebs cycle's products, and amino acids, are also influenced to a great extent (Gupta et al. 2019; Mishra 2016). This situation is more ubiquitous in the Himalayan range, which comprises diverse classes of ecosystem, broad altitudinal area, assorted climatic expanse, slopes, soil nutrition accessibility, and various habitat groups and is delineated to be warming at an uncommon rate. The Himalayan region encompasses high floral heterogeneity (Pandey et al. 2019). Most Himalayan plants possess valuable medicinal characteristics as they contain pharmacologically active chemical constituents abundantly, which are the ingredients of various life-protecting medicines. Nevertheless, the environmental stresses, created due to climate change, are exhausting their obtainability in the environment, altering their utilization in modern and traditional medicine systems, and depleting the quality of bioactive molecules. Major impacts of environmental stress on the plants are aberration in physio-morphological, molecular, and biochemical traits, reduction on gross plant yield, changing life cycle, impact on species ranges, and ecological balance and obtrude high threat on their biodiversity (Gairola et al. 2010; Mishra 2016). Overall, most noticeable adverse changes on Himalayan medicinal plants due to unprecedented change of climate are discussed below, and some examples of Himalayan medicinal plants and effect of the climatic changes on their secondary metabolites are given in Table 2.2.

Climate change can induce the shifting of plants toward a new range for their appropriate growth and development. Normally, these shifts are oriented toward higher elevations and/or the poles. In this situation, loss of habitat and migratory challenges correlated to climate change may cause the disappearance of various indigenous species all over the world (Keutgen et al. 1997).

The seasonal cue is always a governing factor for plant life cycles and the worldwide climate change is exerting influence on ecosystems and species. These phonological changes have put numerous rare species of medicinal plant species at remarkable risk of extinction. Prominent phenological incidents for medicinal plants to be adapted toward climate alteration can be regarded as (i) unfolding of leaf and bud burst, (ii) fruit setting and flowering, (iii) dry weather leaf fall or autumn, and (iv) the associated phenomenon of winter hardening and breaking. When global warming increases, it may alter the appearance of the spring season and the duration of the plant developing season (Bidart-Bouzat and Imeh-Nathaniel 2008).

2.3.2 Elevated CO_2 Effect

Scientifically, it is proven that in controlled environment, elevation of CO_2 level directly influences the quality and productivity of various plant products as well as amount of bioactive constituents in medicinal plants. Experiments have proven that increased concentration of CO_2 (3000 μl CO_2/l of air) proportionally increased leaf and root numbers along with fresh weight in cultures of many medicinal herbs such as oregano (*Origanum vulgare* L.), lemon basil (*Ocimum basilicum* L.), spearmint (*Mentha spicata* L.), peppermint (*Mentha piperita*), and thyme (*Thymus vulgaris* L.) in comparison to cultures cultivated on the same media under normal air condition (Tisserat 2002). To give another clear example, concentration of digoxin (used to treat cardiac failure) from *Digitalis lanata* is increased by 3.5-fold, when the plant is grown at a high level of CO_2, whereas the amount of other potent bioactive glycosides, such as digitoxin, digoxin, and digoxin-mono-digitoxoside, was found to be reduced remarkably. In fact, besides CO_2 concentration, the extent of its exposure also plays a key role to alter the secondary metabolite concentration in medicinal plants. For example, in high level of CO_2, the concentration of three potent anti-viral and anti-neoplastic alkaloids, pancratistatin, 7-deoxynarciclasine, and 7-deoxy-trans-dihydronarciclasin (present in the bulb of *Hymenocallis littoralis*), was increased over the first year of investigation, but these compounds were reported to be reduced on further exposure (Gupta et al. 2019).

Table 2.2 Effect of changed climatic factors on the biosynthesis of metabolites of Himalayan medicinal plants

Plant name	Family	Traditional uses	Climatic changes	Effect of changes	References
Betula pendula	Betulaceae	Effective against blood pressure, high cholesterol, high blood pressure, gout, kidney stones, and nephritis	Ozone elevation	Elevation in the content of hyperoside, whereas the amount of papyriferic acid and betuloside is reduced	Lavola et al. (1994)
			High UV radiation	Increment in the biosynthesis of flavonoids	Lavola et al. (2000)
Capsicum baccatum	Solanaceae	Digestive complications and asthma	Ozone elevation	50% reduction in dihydrocapsaicin and capsaicin; in seeds, there is no alteration in dihydrocapsaicin content, but reduction of capsaicin is significant	Bortolin et al. (2016)
Ginkgo biloba	Ginkgoaceae	Mixed or vascular dementia, Alzheimer's disease	Increased CO_2	Fluctuated terpenoid content, 10% decrease in kaempferol aglycone, 15% increase in quercetin aglycone, and 15% decrease in isorhamnetin	Weinmann et al. (2010), Huang et al. (2010)
Hypericum brasiliense	Hypericaceae	Diuretic, antiseptic, expectorant, digestive, antidepressant	Drought condition	Enhancement in the total amount and concentration of phenolic compounds	De Abreu and Mazzafera (2005)
Hypericum perforatum	Hypericaceae	Alleviate anxiety and depression	Increased CO_2	Increased production of phenolic compounds such as of hyperforin, hypericin, and pseudohypericin	Zobayed and Saxena (2004)
Lolium perenne	Poaceae	To treat diarrhea, cancer, hemorrhages, and malaria	High UV radiation	Amount of lignin is decreased, whereas alkaloid content remains unchanged	McLeod et al. (2001)
Matricaria chamomilla	Asteraceae	Rheumatoid disease and skin complication	Salt stress	Significant elevation of phenolic compounds like chlorogenic, caffeic, and protocatechuic acids	Kováčik et al. (2009)
Panax quinquefolius	Araliaceae	Increase energy; reduce stress, blood cholesterol, and blood sugar; improve sexual dysfunction	Heat stress	Diminution of photosynthesis and increment of ginsenoside content	Jochum et al. (2007)

Papaver setigerum	Papaveraceae	Lung and eye inflammation	Increased CO_2	Increased synthesis of alkaloids, namely, codeine, morphine, noscapine, and papaverine	Ziska et al. (2008)
Perilla frutescens	Lamiaceae	Useful to treat cough and asthma	Heat stress	Decrease in the biosynthesis of anthocyanins	Zhong and Yoshida (1993)
Pinus sylvestris	Pinaceae	For respiratory complications	Heat stress	Increase in the content of terpenes	Sallas et al. (2003)
Pinus taeda	Pinaceae	Effective for kidney and urinary bladder complications	Ozone elevation	Amount of condensed tannins is increased, but the amount of total phenols remains the same	Jordan et al. (1991)
Salvia officinalis	Lamiaceae	For the treatment of malaria and plaque	Drought condition	Augmentation of monoterpene synthesis	Petropoulos et al. (2008)
Salvia sclarea	Lamiaceae	Insomnia, kidney problems, and digestive complications	Cold temperature	Depletion of leaf surface area, increment in spikes' number and length, along with higher concentration of essential oils	Kaur et al. (2015)
Withania somnifera	Solanaceae	To treat bronchitis, asthma, ulcers, hypertension, and intestinal worms	Cold temperature	Increment of bioactive compound anolide (steroidal lactones)	Mir et al. (2015), Kumar et al. (2012)

2.3.3 Effect of Elevated Ozone Levels

Reckless human activities on earth have dramatically resulted in an increased level of greenhouse gas O_3 in the lower atmospheric layer. Overproduction of O_3 on the tropospheric level of the earth is chiefly due to fossil fuel combustion. This combustion generates different volatile organic molecules and nitrogen oxide that interact with oxygen molecules to produce O_3. In contrast to CO_2, O_3 induces oxidative stress in plant cells and decreases the rate of plant respiration, photosynthesis, and plant growth and also brings about an alteration in nutrient allocation and senescence. Also, fluctuation in O_3 concentration can produce alteration in the production of secondary metabolites (Kanoun et al. 2001; Holton et al. 2003; Kopper and Lindroth 2003). In some cases, physiological stress in the plant foisted by raised O_3 levels can trigger many metabolic pathways (e.g., jasmonic acid and salicylic acid pathways) (Bidart-Bouzat and Imeh-Nathaniel 2008).

Many studies have suggested that susceptibility toward the O_3 level varied among different species. Relatively, gymnosperms have a diverse arrangement of defense mechanisms against this stress, whereas angiosperms are more susceptible toward increased O_3 (Valkama et al. 2007). For instance, augmented atmospheric concentration of O_3 stimulates lignin and catechin biosynthesis in *Picea abies*, catechin production in *Pinus sylvestris*, and phytoalexin synthesis in *Pinus ponderosa* (Grimmig et al. 1997). According to a previous study, variation in phytochemicals' biosynthesis in plants is directly influenced by acute and chronic stress of O_3. According to Kanoun et al. (2001), there was temporal variation in the amount and constitution of phenolic compounds in *Phaseolus vulgaris* leaves which were exposed to moderate O_3 levels. Initially, the amount of hydroxycinnamic acid was decreased followed by de novo synthesis of other diverse phenolic compounds (isoflavonoids) after long-term enrichment of O_3. Another investigation revealed that the synthesis of three quercetin derivatives avicularin, isoquercitrin, and quercitrin was particularly enhanced by elevated O_3 concentration, whereas the amount of other phenolic derivatives remained unchanged. Therefore, phenolic compounds induced by ozone have been considered as significant bioindicators of natural ecosystem pollution (Kanoun et al. 2001; Sager et al. 2005).

2.3.4 Enhanced Ultraviolet Radiation Effect

The supply of ultraviolet light is crucial for various biological phenomena and chemical reactions. However, a high concentration of this radiation may produce an unwanted effect on living organisms, especially for medicinal plants (Roberts and Paul 2006). The UV radiation may result in cellular and molecular damage; for instance, it can impair the integrity of DNA, proteins, hormones, RNA, and other biomolecules (Stratmann 2003). Besides that, this radiation may significantly impair the growth and development of plants and induce alteration in height, flowering time, leaf characteristics, and reproductive or vegetative biomass (Bornman and Teramura 1993). Influence of UV light on plant-derived secondary metabolite biosynthesis is usually associated with the alterations in the formation of phenylpropanoid- or shikimic acid-derived phenolic compounds (Bassman 2004). Apart from some deviations, most of the investigation have reported an elevation in phenolic compounds' synthesis (flavonol, isoflavonoids, anthocyanins, flavoproteins, flavonol glycosides, coumarins, and phenolic acids) under augmented concentration of UV radiation especially UV-B light (Bidart-Bouzat and Imeh-Nathaniel 2008).

2.3.5 Effect of Drought Condition

According to several types of research and investigations, during drought or water stress conditions, plants can store a higher amount of secondary metabolites, like phenolic derivatives, terpenoids, glucosinolates, alkaloids, and cyanogenic glucosides. But, on the other hand, the growth rate of plants is diminished. The main reason for increased secondary metabolites' production is due to retardation in plant biomass formation. However, the rate of secondary metabolite synthesis is always constant. Thus, the increased amount of metabolites is based on the total weight of the dry or fresh plant (Selmar and Kleinwächter 2013). Another study reported that the concentration of monoterpenes in *Salvia officinalis* and *Petroselinum crispum* was extremely high in drought conditions. Also, the rate of biosynthesis was much greater than biomass reduction in comparison to controls, which were cultivated on an environment with sufficient availability of water (Petropoulos et al. 2008). Similarly, in drought conditions, the total amount of essential oil per plant of *Origanum vulgare* was constant, whereas the metabolite concentration was high. It indicates that the extent of monoterpene production in plants remained unchanged. However, the same quantity of monoterpene was obtained, even using a lesser amount of plant biomass (Paulsen and Selmar 2016).

2.3.6 Effect of Cold Environment

Overall, the low temperature can result in severe stress on most plants. Mainly, it can retard the whole development phenomenon, diversity, productivity, and distribution, to a great extent (Chinnusamy et al. 2007). Low temperature has a direct effect on the physiology of plants and can change the cell membrane fluidity by altering its concentration (Ruelland et al. 2009; Sevillano et al. 2009; Upchurch 2008). To cope with a cold environment, plants start to alter their surviving strategies. For this, they can lower the growth rate and redistribute essential resources (Eremina et al. 2016). Due to low temperature, generation of stress-producing free oxygen radicals increases, and plants have to scavenge these radicals by activating the synthesis of antioxidants (Ruelland et al. 2009; Sevillano et al. 2009). Also, it is crucial to raise the concentration of soluble solids, cryoprotective proteins, and amino acids to keep up cellular solidarity. To survive in a harsh cold environment, plant cells switch on numerous metabolic and enzymatic pathways (Ruelland et al. 2009; Eremina et al. 2016).

Several medicinal plants, like *Cistus incanus*, *Thymus sibthorpii*, *Teucrium polium*, *Satureja thymbra*, and *Phlomis fruticosa*, exhibit seasonal dimorphism by developing different defense mechanisms (Lianopoulou and Bosabalidis 2014). Hormones play a vital role to activate their defense system. For example, *Origanum dictamnus* develops various anatomical (rigid and thick cuticle, wax layer formation over the epidermal surface of leaves, and development of dense non-glandular trichomes) and structural variations (size, shape, and leaves distribution) to combat against cold weather (Lianopoulou and Bosabalidis 2014).

Low-temperature stresses modify the structure and anatomy of the plants as well as their results in the upraising of their antioxidant components, which ultimately leads to an increase in their nutritional and medicinal significance (Khan et al. 2015; Lianopoulou & Bosabalidis, 2014; Saema et al. 2016). In a previous investigation, when ashwagandha (*Withania somnifera*) was grown at low temperature, it exhibited deposition of bioactive compound "withanolide" inside leaves (Khan et al. 2015; Kumar et al. 2012). Contradictorily, the amount of the same compound in root tissues was found to be reduced at low-temperature stress (Khan et al. 2015), suggesting that the effect of cold conditions is varied on different plant tissues and organs.

2.3.7 Global Warming

Due to the incautious anthropogenic release of greenhouse gases into the atmosphere, the mean temperature of the earth has been increased since the twentieth century. Generally, the physiological processes of insects and plants are directly connected with atmospheric temperature. Alteration or elevation of this parameter may result in prominent effects for natural interaction between these two classes of living organisms. For instance, a rise in global temperature may cause mismatches in morphogenesis between insects and plants. Some investigations have suggested the consequence of raised temperature on the biosynthesis of plant secondary metabolites (Table 2.2); the result seems to be dependent on the nature of the chemicals and type of the plant species (Mishra 2016). For example, many types of research have studied the effect of elevated temperature on total phenolic content (Kuokkanen et al. 2001; Williams et al. 2003; Hansen et al. 2006), volatile organic compounds (VOCs) like hexenal and terpenes (Sallas et al. 2003; Snow et al. 2003; Loreto et al. 2006), and glucosinolates (Matusheski et al. 2004; Valente Pereira et al. 2002; Velasco et al. 2007). High temperature can result in the release of VOCs and may cause pollution on the tropospheric surface (Loreto et al. 2006). The high temperature leads to an elevation in the amount of some hydrolyzed products of glucosinolates like isothiocyanates. On the other hand, the number of nitrile products gets decreased. Furthermore, alteration in secondary metabolites caused by high temperature can have an inauspicious effect on the insect life cycle. In a study, Dury et al. (1998) reported that increased content of condensed tannin (due to raised temperature) in *Quercus robur* exerted a harmful effect on the development phase of larva and fertility of phytophagous insects, whose life is linked with this plant species.

2.3.8 Anthropogenic Factors

In the present scenario, the vast majority of the medicinal plants, growing wildly, are at the risk due to their irrational utilization and overexploitation. Therefore, these precious medicinal species are declining abruptly. Expanding human population, habitat fractionation, the elevated reliance of enormous inhabitants of the world within restricted natural resources of the large population of the world on the limited natural resources, and urbanization are driving factors for this detrimental situation. Because of these incautious human activities, it is approximated that the rate of medicinal plants' annihilation has been seriously expedited. According to the data given by IUCN 2018, out of 96,951 investigated plants, 26,840 species are at the risk of extinction.

The Himalayan regions are a hotspot area for universal biodiversity, as these lands nurture a prosperous repository of remedial flora. Also, it is a territory of large numbers of endemic medicinal plants. Despite that, in the present situation, natural floras of these medicinal plants are suffering through extreme risk from numerous anthropogenic activities, like unrestrained deforestation, habitat destruction, illegal trade of plant-derived medicinal product, over-grazing, chaotic tourism influx, non-scientific development, broadening of road, shifting agriculture, harvesting before the maturation of seed or flower of plant, unrestricted and non-selective harvesting of target medicinal plant without leaving possible germinating parts (like roots, rhizome), logging, over a collection of forest resources, industrialization, overexploitation for local use, and outspread of intrusive alien plant species. Also, these human activities can result in calamitous climatic changes, such as landslides, avalanches, soil erosion, and forest fire, which have a direct effect on the availability of medicinal plants (Ganie et al. 2019; Paul et al. 2015). Ganie et al. (2019) conducted a research to investigate the overall threat impact on medicinal plants native to Kashmir Himalaya. Some of the medicinal plants, which are on high threat of extinction due to anthropogenic factors, are depicted on Table 2.3.

Table 2.3 Medicinal plants that are on high threat of extinction due to anthropogenic factors

S. N.	Plants	Family	Cause of threat	Severity	Overall threat impact
1	*Achillea millefolium*	Compositae	Overharvesting, construction project on native place, utilization for fodder	Modest	Low
2	*Aconitum heterophyllum*	Ranunculaceae	Soil erosion/landslides, overutilization for folk use, illegal trade	Critical	Very high
3	*Adiantum capillus-veneris*	Pteridaceae	Overharvesting, construction project on native place, landslide	Moderate	Low
4	*Corydalis cashmeriana*	Papaveraceae	Landslide, over-grazing by cattles	Slight	High
5	*Corydalis thyrsiflora*	Papaveraceae	Landslide, over-grazing by cattle	Slight	Low
6	*Digitalis lanata*	Plantaginaceae	Urbanization, transformation of grassland and forest for agriculture	Large	High
7	*Fritillaria cirrhosa*	Liliaceae	Building construction, unmanaged tourism, overharvesting, over-grazing by cattle, illegal trade	Moderate	Very
8.	*Gentiana carinata*	Gentianaceae	Overharvesting, over-grazing by cattle, forest exploitation	Moderate	Low
9	*Hyoscyamus niger*	Solanaceae	Over-grazing by cattle, overharvesting, urbanization, unmanaged tourism	Moderate	Moderate
10.	*Lavatera cachemiriana*	Malvaceae	Forest overexploitation, over-grazing by cattle, overharvesting, urbanization	Slight	Medium

2.4 Conclusions and Future Recommendations

Himalayan medicinal plants are an integral part of livelihood of the local communities living in rural area. They are not only used for the treatment of various diseases as traditional medicines, but many are also used as food and spices and also collected, processed, and sold in the market for generating economy. Various environmental challenges such as climate change, global warming, and various other factors are threatening their biodiversity and natural abundance along with various changes in physiology and chemical constituents (Fig. 2.1).

Community awareness and involvement is necessary for protecting the threatened medicinal plants species in Himalaya. Although there have been many studies related to the effects of environmental challenges in biodiversity, relatively few studies are carried out regarding the physiochemical changes in medicinal plants. Detailed studies should be carried out to understand the effects of these environmental factors on the chemical constituents and pharmacological activities in the future.

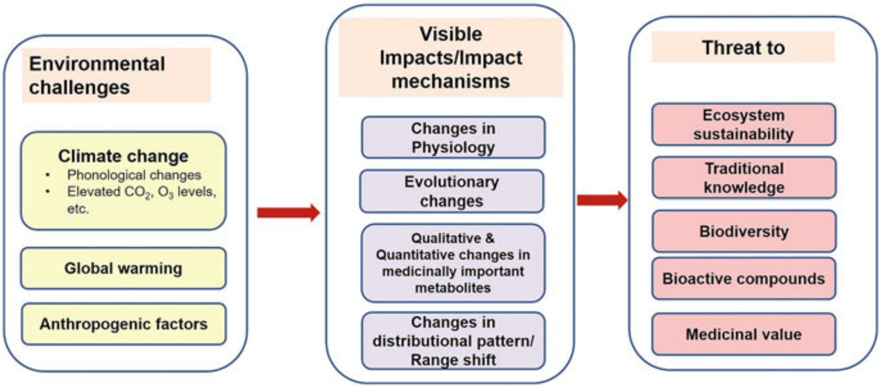

Fig. 2.1 Various environmental challenges and their impacts and threats to Himalayan medicinal plants

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Chapter 3
Wild-Growing Species in the Service of Medicine: Environmental Challenges and Sustainable Production

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Abstract Wild fruits are underutilized plants that are well adapted to the local climatic conditions. Extreme environmental conditions due to climate change or variability are a threat to wild-growing species, crop production, productivity, and livelihood. Wild fruit fields could be affected by not meeting winter chilling requirements, which is specific for every fruit species. On the other hand, the plants' secondary metabolites and other bioactive compounds can be attributed to the changing conditions as a response to various types of environmental stresses which affect their production. Secondary metabolites refer to small molecules that are non-essential for the growth and reproduction of plants, but have a wide range of effects on the plant itself and other living organisms. Blackthorn (*Prunus spinosa* L.), Cornelian cherry (*Cornus mas* L.), dog rose (*Rosa canina* L.), and hawthorn (*Crataegus monogyna* Jacq.) are important wild plants with powerful health-promoting properties. Due to their chemical composition and nutritive value, they have a strong effect on regional food security and poverty alleviation. Positive health effects, forceful impact on the quality of life, and market potential are additional attributes of these plants, which may have significant economic impact.

Keywords Blackthorn · Cornelian cherry · Dog rose · Hawthorn · Environmental conditions · Secondary metabolites · Health-promoting properties

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Abbreviations

GC	Gas chromatography
GC-FID	Gas chromatography with flame ionization detection
GC-MS	Gas chromatography-mass spectrometry
HPLC	High performance liquid chromatography
HPLC-DAD	High performance liquid chromatography coupled with diode-array detection
HPLC-DAD-ESI/MS	High performance liquid chromatography coupled with diode-array detection—electrospray ionization mass spectrometry
HPLC-ESIQ-TOF-MS	High performance liquid chromatography coupled to electro spray ionisation and quadrupole time of flight mass spectrometry
HPLC-MS	High performance liquid chromatography with mass spectrometry
HPLC-RI	High performance liquid chromatography with refractive index detectors
ICP-OES	Inductively coupled plasma—optical emission spectrometry
LC-DAD/ESI/MS	Gas chromatography with diode-array detection—electrospray ionization mass spectrometry
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
UHPLC-DAD-ESI-MS/MS	Ultrahigh performance liquid chromatography coupled with diode-array detection—electrospray ionization tandem mass spectrometry
UPLC-MS/MS	Ultra-performance liquid chromatography-tandem mass spectrometry
UPLC-MS^2	Ultra-performance liquid chromatography-tandem mass spectrometry
UV/Vis	Ultraviolet-visible spectroscopy.

3.1 Wild Fruits and Environmental Challenges

Wild fruits are underutilized, less known, polycarpic plants that bear fruits and are well adapted to the local climatic conditions. All these species have strong effect on regional food security and poverty alleviation. Due to their positive health effects and market potential, the gathering of these plants has a forceful impact on the quality of life and economics. These species that today have fallen somewhat into disuse were widely consumed in the past and especially in times of scarcity (Tardío et al. 2006).

The introduction of wild fruit species into the commercial fruit growing can be useful for several reasons: diversification of agriculture throughout the region, introduction of new raw materials into the food industry, as well as the preservation of agro-biodiversity. A number of wild relatives of cultivated fruit trees could be used in the future as rootstock in fruit production or for organized production, either organic or conventional. In the intensive fruit production, there are more and more problems with the susceptibility of cultivars to diseases and pests, which imposes numerous and obligatory applications of protective chemicals, which in addition to mineral fertilizers are reducing biological value of the cultivated fruits. Since wild fruit genotypes carry resistance genes for the most economically important pests, they can be used in breeding fruits and vines. Wild fruit species are also used as planting material for afforestation and prevention of erosion, some in cosmetic industry, and some as decorative forms in landscape architecture, while some species are important bee pastures (Mratinić and Fotirić-Akšić 2014).

Wild fruits are crucial for poor rural inhabitants and unemployed youths by giving them the possibility to make new varieties of edible products such as baking products, jelly, jam, preserves, marmalade, chocolate candy, dry berries, pickled (especially Cornelian cherry), pudding, butter, sauce, wine, juice, salad dressing, pie, tart, syrup, sauce, sherbet, candy, wine, cider, blossom fritters (especially from elderberry), and even beer. Wild fruits can be sold as potted trees; wood can be used for making furniture, jewelry, and traditional musical instruments. A community can be developed by starting small businesses. Wild fruit gathering can be organized as a recreation and tourism activity, promoting native recipes, cooking traditions, making festivals, and spreading local history.

In the last decade, wild fruits have received increasing interest due to their medicinal properties and nutritional value (Mikulic-Petkovsek et al. 2015; Li et al. 2016). They are a rich source of various bioactive compounds such as carbohydrates, organic acids, proteins, phenolic compounds, carotenoids, tocopherol, volatile oils, pectins, tannins, fatty acids, oils, aromatic substances, enzymes, vitamins, minerals (K, Ca, P, Fe, Mg, and Mn), dietary fibers, and others (Demir et al. 2014; Krstić et al. 2019, Popović-Djordjević et al. 2021).

All those phytochemicals together combat the oxidative stress which performs an essential role in multiple chronic diseases. Bioactive compounds from wild fruits have antioxidant, anti-inflammatory, antimicrobial, anti-ulcerogenic, antidiabetic, anti-mutagenic, and anti-cancer activities, and they act against rheumatoid arthritis, osteoporosis, hyperlipidemia, obesity, renal disturbances, skin disorders, and aging (Mármol et al. 2017; Tumbas et al. 2012). Therefore, wild fruits have the potential to become a functional food that will be used in preventing and treating chronic diseases. New application of wild fruits includes using natural antioxidants to extend the shelf life of food products and replace synthetic antioxidants, avoiding potential health risks and toxicity (Lourenço et al. 2019).

The wild fruits are very healthy food of high nutritional and, above all, vitamin value (Fernandez-Ruiz et al. 2017). The source of vitamin C among wild fruits is found in rose hips (*Rosa* sp. – 130–6694 mg/100 g), young walnut husk (500–2459 mg/100 g), and then currants, bilberry, and cranberry (Gergelezhiu 1937; Yoruk et al.

2008; Milivojević et al. 2013). Rowan (*Sorbus aucuparia*), seaberry (*Hippophae rhamnoides*), dog rose (*Rosa* sp.), and hawthorns (*Crataegus* sp.) are rich in carotenes. Rose hips, wild strawberries, and bilberries contain a relatively large amount of niacin. Vitamins B, K, and E are abundant in currants and gooseberries, dog rose, rowan, and guelder-rose. Fresh wild fruit extracts are an excellent source of polyphenolic compounds, acting as free radical scavengers, which can prevent neurodegenerative and cardiovascular diseases and even cancer (Zafra-Stone et al. 2007; Jing et al. 2008).

Wild fruits usually have more moisture, energy value, and more complex phytochemical composition than the cultivated varieties (Thole et al. 2006). Moreover, their main advantage is that they grow in nature without human influence, which means without watering, spraying with chemicals, or fertilization with synthetic fertilizers, and they are richer in the quantity of bioactive compounds. On the other hand, these fruits grow in optimal natural conditions, so they are more biologically resistant and less endangered by pathogens and pests, and their fruits are more abundant in bioactive substances. However, when collecting wild fruits, it should always be kept in mind that the picking should be done rationally, and some plants should leave in order to allow them to grow and reproduce.

Extreme environmental conditions due to climate change or variability are a threat to wildlife, crop production, productivity, and livelihood. In most cases, climate change is associated with elevated temperatures, solar radiation, drought, strong winds, and hails. The prediction is that the temperature will be higher by 1–3 °C, so the rainfall, snowfall, UV radiation, and amount of ozone, aerosols, and clouds in the atmosphere will be altered (Bais et al. 2014). According to Wessels et al. (2021), under a low-warming scenario, 60% of wild food plant species will experience an increase in the range extent, while 40% will experience a decrease, whereas in a high-warming scenario, reduction of 66% in wild-harvested food plant species will be observed. Therefore, according to Arslan et al. (2020), 170,596 km^2 is currently a "highly suitable" area for *Rosa canina* L., but in a "milder" scenario, the area will contract to 114,474 km^2 by 2070, and in the "warmer" scenario, it will be just 41,146 km^2 by 2070.

Climate change will diminish wild fruit fields, first of all, by not meeting winter chilling requirements, which is specific for every fruit species, delay of flowering and/or fruiting, and higher fruit abscission. Also, it can happen that due to the warming up, invasive species might divert pollinating insects away from the native wild fruit-producing plants. If winter precipitation decreases and if summer temperatures substantially increase, changing locations for wild fruits will be observed, which can be a problem for people who have developed traditional berry-gathering sites (Kellogg et al. 2010). On the other hand, climate change can positively affect the production of bioactive compounds, but in some cases, it would lead to deterioration in quality parameters such as fruit weight (Romero-Román et al. 2021).

3.2 Description of Selected Wild Fruit Plants

3.2.1 Blackthorn

Photo was taken by Nenad Mićanović, Serbia

Blackthorn or sloe (*Prunus spinosa* L.) belongs to the Rosaceae family. It occurs in most of South-Central Europe and northward to the southern part of the Scandinavian Peninsula and eastward to the Asia Minor, the Caucasus, and the Caspian Sea. Southward, it can be found in North Africa (in Tunisia and Algeria). It is naturalized in many other parts of the world (North America and New Zealand). The specific name "*spinosa*" comes from a Latin term indicating thorn-like spur shoots. The evidence of the early use of sloes by man is found in the famous case of a 5300-year-old human mummy discovered in 1991 in the Ötztal Alps along the Austrian-Italian border.

It is a deciduous shrub or, very rarely, small tree growing to 5 m. Since it readily produces suckers and 1-year branches have savage thorns, traditionally, it was used in making a cattle-proof hedge. Flowers are small, pentapetalous, single or in pairs, bisexual, and creamy-white. It blossoms very early (in March/April), much before leafing. The fruits ripen in late August and are called "sloe." It is a black drupe with a purple-blue waxy bloom which gives the young fruit a matt appearance. It contains one large and rough stone, either cling or noncling. It has a very strong astringent flavor, but best for consumption is after the first frosts. Fruits can remain attached to the plant for a prolonged period, even until the spring.

It is a heliophyte and xerothermophilic species which grows on glades, forest borders, gullies, and river valleys, in meadows and pastures, and on mountain slopes. It makes a dense belt of shrubs adjacent to the forest between woodland and grassland communities where it is left untrimmed and ungrazed. Blackthorn prefers deep and moist soils (except acid peats) regardless of geological basis. It is characterized by broad adaptability and good viability so it can survive on dry and eroded soils along the banks of gorges and on stony slopes (Dzhangaliev et al. 2003). It can be found at altitudes of up to 1600 m, and it can sustain frosts up to $-30\,^{\circ}\mathrm{C}$ (Mratinic and Fotiric-Akšic 2019). Crossing combination blackthorn × European plum

(*Prunus domestica*) forms *Prunus* × fruticans hybrids. Since the plant is hard and grows in a wide range of conditions, it is used as a rootstock for plum and apricot. It is also an important plant for wildlife, its early spring flowers provide nectar for early pollinators, and its branches create a spiny thicket, providing secure nesting for birds and protection and food for small mammals (Popescu and Caudullo 2016). It is suitable for stabilizing stony slopes of gorges and preventing landslides in the mountains (Dzhangaliev et al. 2003).

The fruits are mostly used in jellies, syrups, vinegar, and conserves and for liquor making (like gin, kvass, patxaran, flavored beers, vodka, "Porto" wine, "pacharán," "troussepinette," "bargnolino," "eau de vie de prunelle," "vin d'épine") or as ingredients of various pastries (Yuksel 2015; Popescu and Caudullo 2016). Sloes can also be made into jam and used in fruit pies, and if preserved in vinegar, they are similar in taste to Japanese "umeboshi." The flowers, petals, leaves, and dried fruits are used as herbal tea (Alarcón et al. 2015). The juice of the fruit dyes linen a reddish color that washes out to a durable pale blue. Blackthorn makes excellent firewood that burns slowly with good heat and little smoke. The wood has light yellow sapwood and brown heartwood. It is hard and tough and polishes up well. Traditionally, blackthorn wood is used for walking sticks and for the Irish shillelagh (Knaggs and Xenopoulou 2004).

3.2.2 Cornelian Cherry

Photo was taken by Milica Fotirić-Akšić, Serbia

Cornelian cherry or dogwood (*Cornus mas*) belongs to the genus *Cornus* (contains 45 species) and family Cornaceae. This species primarily grows in the temperate zone of Eurasia, and it is highly tolerant to diverse abiotic and biotic conditions. It grows spontaneously along oak forest edges and in woodland clearings; it is a light-loving plant and can live up to 300 years. In its distribution area, most individuals occur spontaneously as a result of open pollination, which varies widely in terms of productivity and fruit characteristics (Murrell 1993).

It is a deciduous shrub or a small tree 3–4 m high. The bark of older plants is gray, thin, and with shallow cracks, and juvenile branches are gray, smooth, and shiny (Mratinic and Fotiric-Akšic 2019). The flowers are small with four yellow petals, arranged in umbel inflorescence. It blooms at the end of winter/beginning of spring (February/March). The fruit is an oblong, olive-shaped, red drupe containing a single seed. When ripe (mid- to late summer), fruits resemble coffee berries. Fruits are edible but astringent. When ripe, the fruit is dark ruby red or a bright yellow.

This is a thermophyte and xerophilic species. It tolerates shade well, and it is very adaptive. It can be found on altitudes of up to 1300 m, on hills and slopes, and in forest clearings, as a companion tree in hornbeam, oak forests, and manna ash. *Cornus mas* can grow in all kinds of soils, from light sandy to heavy clay, with a pH ranging from slightly acid to very alkaline (Jaćimović et al. 2002; Bijelič et al. 2011). It can survive up to −30 °C, while it is sensitive to salt and marine exposures. The Cornelian cherry is free of disease and pest problems (Da Ronch et al. 2016).

Cornelian cherries can be consumed fresh or dried, but due to their acidic flavor, they are mainly used for making jams, juices, sauces, and alcoholic drinks such as vodka, brandy, and rakia (Bijelič et al. 2011). The oil-rich seeds of Cornelian cherry can be roasted, ground, and used as a coffee substitute (Facciola 1990), or the oil can be extracted and used in traditional medicine (Mamedov and Craker 2002). The stones and seeds can be converted to oil that can serve as biofuel (Akalın et al. 2012). The stones are sometimes made into beads (Smith and Branting 2014). The plant also has ornamental usage and is considered as a nectariferous, hedge, and shade plant (Mamedov and Craker 2002). Many European countries, such as Turkey, Ukraine, the Czech Republic, and Serbia, have breeding programs and select superior genotypes from natural populations (Mratinić et al. 2015).

Wood, leaves, fruits, and seeds have application in medical therapy (Hosseinpour-Jaghdani et al. 2017) and traditional Chinese medicine. The wood of *C. mas* is extremely dense and, unlike the wood of most other woody plant species, sinks in water (Demir and Kalyoncu 2003). Some ethnographic sources from southeast Europe describe *Cornus* tree as highly valued for its tough and durable wood that was used in the manufacturing of weapons, tools, instruments, and wickerwork (Filipović et al. 2020).

In the recent history of East European countries, Cornelian cherry tree had a special place in the life of rural communities and played an important role in celebrations and rituals. Some of the illustrative examples include the use of the inflorescence to make wedding wreaths for groom and bride, while the young shoots were eaten; the branches were placed on house roofs for protection against thunder, and they were soaked in bathing water; small pieces of wood were kneaded into Christmas bread; rods made from the branches were beating sticks for fighting off werewolves and witches; child swings were hung from Cornelian cherry tree (Čajkanović 1994).

3.2.3 Dog Rose

Photo was taken by Milica Fotirić-Akšić, Serbia

Dog rose (*Rosa canina*), which belongs to the Rosaceae family, is a climbing, wild rose species native to the northern hemisphere (Europe, Northwest Africa, and West Asia). It is a deciduous medium-developed shrub with a height of up to 3 m. Its stems are thin and covered with very strong, uneven, hooked prickles. The leaves are long, alternate- and odd-pinnate, and composed of 7–9 leaflets. The leaflets are usually elliptical, bare, and smooth on both sides. The flowers are large, pale pink, bisexual, and usually in large corymb-like inflorescence of 3–7 blossoms. The corolla has five white petals. The gynaeceum has many free, fairly protruding styles. A pseudo fruit is a red-orange "hip," which is an aggregate fruit consisting of several achenes (30–35% of fruit weight) enclosed by an enlarged, red, fleshy floral cup (hypanthium) (65–70% of fruit weight) (Pećinar et al. 2021).

Rose hip achenes contain neurotoxic substances, and the hairs are extremely irritable for the skin and mucous membranes (Ghrabi 2005). It blossoms in May and June and ripens in September and October. Fruits persist on the plant for several months and become black. Plants reproduce sexually by seed and vegetatively by suckering and layering.

Rosa canina can be found in lowlands, hills, or mountain regions, in deciduous and coniferous forests, along roadsides, in pastures, on forest clearings and edges, and among bushes. It is very adaptive to different geological rocks (silicate and limestone) and different soil types. It can grow in semi-shade or on direct sun. It can tolerate strong winds but not maritime exposure. Birds and other wildlife consume the hips of dog rose and spread the seed. In some areas, it is an invasive species (Pavek 2012).

The plant was described for the first time by Pliny the Elder (23–79 BC), who attributed the plant's name to a belief that the root could cure the bite of a mad dog (Hass 1995). The plant had also been known by sailors as a means of protection against scurvy, due to its high concentration of vitamin C (up to 1500 mg/100 g), and thus it spread to several continents (Winther et al. 2016). Today, these deciduous flowering shrubs are widely grown in gardens for their flowers and fruits (Ercisli 2005).

The fruit is used in the making of traditional probiotic drinks, beverages, soups, and yogurts, herbal teas, pies, stews, and wine, whereas the flowers can be made into syrup, eaten in salads, candied, or preserved in vinegar, honey, and brandy (Ahmad et al. 2016; Chrubasik et al. 2008). Distilling 1 kilo of flowers gives 1 liter of pure rose water (Ghrabi 2005). In traditional folk medicine, aqueous extracts of petals, fruit, and leaves of *Rosa canina* plants are applied in the treatment of various diseases such as nephritis, common cold, the flu, coughing, bronchitis, eczema, itching, and biliary diseases (Kultur 2007). The substances within the dog rose hips are endowed with vitaminisant, astringent, colagogue, choleretic, diuretic, antidiarrhoea, antioxidant properties (Roman et al. 2013).

3.2.4 Hawthorn

Photo was taken by Milica Fotirić-Akšić, Serbia

Common hawthorn or single-seed white hawthorn (*Crataegus monogyna*) is an endemic member of the Rosaceae family (Chang et al. 2002). The generic name *Crataegus* stems from the Greek "Kratos" meaning strength, and the species' name *monogyna* reveals that this species contains one ("mono") seed ("gyna"). It is native to Europe, Northwest Africa, and West Asia, but has been introduced in many other parts of the world. The plant forms a bush or a small tree, 3–10 m high. The crown is round and dense. One-year-old shoots, the leaves, stems, and flowers are completely bare or slightly ciliate on the receptacle and the flower's stem. The younger stems bear sharp thorns. The leaves are obovate and lobed at a wide angle. The flowers are bisexual, white, or pink and organized in corymbs with 4–33 blossoms together. The fruits are tiny (10 mm). Oval dark red haws, delicate in taste, contain only one seed (Mratinic and Fotiric-Akšic 2019).

It blossoms from late April until mid-June, and it is a sign that spring is turning to summer. The fruits ripen in the second half of September. The most widely known hybrid is *C.* × *media* (*C. monogyna* × *C. laevigata*), from which "Paul's Scarlet" genotype (with dark pink double flowers) was derived. Seedling trees take from 5 to 8 years before they start bearing fruit. The plant may be invasive.

This plant is an extreme heliophyte and can be found at altitudes of up to 1600 m. The tree is quite adaptive and can thrive in both carbonate and silicate soil. It appears most frequently on the fringes of forests and on waysides and roadsides. The hawthorn has developed resistance to drought, overly moist environments, wind, and atmospheric pollution. Common hawthorn can live long. In Mayenne (France), there is one hawthorn tree that is traced back to the third century.

Traditionally, *C. monogyna* has been used in folk medicine as a primary heart tonic, to correctly balance high and low blood pressure. Hawthorn is mainly used for hedging, especially in agriculture because it is stock- and human-proof. Being small in size, the use of its timber covers wood engravers' blocks, mallet heads, and tool handles. Since it grows in twisted shape, it is an excellent wood for carving ornaments. It is good firewood which burns with good heat and little smoke (Knaggs and Xenopoulou 2004).

The fruits of *C. monogyna* are used for different culinary purposes, such as the preparation of jellies, jams, syrups, candies, and pickles, and they are used to make wine or to add flavor to brandy (Sallabanks 1992). The fruit can be dried, ground, mixed with flour, and used for making bread and roasted seed for "coffee." The petals are also edible, as are the leaves, which if picked in spring when still young are tender enough to be used in salads (Kunkel 1984).

In folk medicine, the hawthorn is the center of many folklore tales, legends, and beliefs. It was primarily used to protect against all forms of evil spirits and demons. To ward them off, hawthorn amulets were carved and hung above doors or worn for protection. For example, in Serbian and Croatian folklore, hawthorn stakes were used to slay vampires, while in Gaelic folklore, the hawthorn symbolized the entrance into the other world and was strongly connected to fairies. Hawthorn bears both Pagan and Christian symbolism since it is believed that the stems that were used to make the crown of thorns given to Jesus before his crucifixion were made of hawthorn (Eberly 1989).

3.3 Chemical Composition of Selected Wild Plants

Compounds from different classes were found in fruits and other plants' parts of blackthorn, Cornelian cherry, dog rose, and hawthorn. These wild plants are good source of sugars, organic acids, fatty acids, amino acids, essential elements, and vitamins. The common sugars are fructose, glucose, sucrose, pectines, and cellulose. Among organic acids, malic, citric, oxalic, tartaric, quinic, and succinic are most represented in various parts of described plants (Barros et al. 2010; Babalau-Fuss et al. 2018; Cunja et al. 2016; De Biaggi et al. 2018; Ilyasoğlu 2014; Kubczak et al. 2020; Milić et al. 2020; Nađpal et al. 2016; Özderin et al. 2016; Paunović et al. 2018; Popović-Djordjević et al. 2021; Sikora et al. 2013; Vasić et al. 2020). Moreover, they are rich in secondary metabolites especially polyphenolic compounds including phenolic acids, flavonoids, anthocyanins, and tannins (Alirezalu et al. 2020; Bajić-Ljubičić et al. 2018; Bekbolatova et al. 2018; Cunja et al. 2016; Garofulić et al. 2018; Gironés-Vilaplana et al. 2012; Guimaraes et al. 2013, 2014; Jiménez et al. 2017; Kerasioti et al. 2019; Kubczak et al. 2020; Liu et al. 2011; Medveckiene et al. 2020; Milenković-Andjelković et al. 2015; Moldovan et al. 2016; Nađpal et al. 2016; Okan et al. 2019; Ouerghemmi et al. 2016; Natić et al. 2019; Popović et al. 2020; Pozzo et al. 2020; Polumackanycz et al. 2020; Szumny et al. 2015; Veličković et al. 2014; Zhang et al. 2020; Živković et al. 2015). These compounds contribute to antioxidant activity and other health-beneficial properties of fruit extracts as well as extracts obtained from flowers, leaves, twigs, and seeds. Other important secondary metabolites found in selected wild plants are carotenoids, terpenes, terpenic acids, and sterols (Cunja et al. 2016; De Biaggi et al. 2018; Kerasioti et al. 2019; Medveckiene et al. 2020; Ouerghemmi et al. 2016).

Secondary metabolites are substances produced by plants in response to various types of environmental stress and mediate interactions between organisms. Unlike primary metabolites, secondary metabolites are associated with small molecules that are non-essential for the growth and reproduction of the plants, but have a wide range of effects on the plant itself and other living organisms. They cause flowering, fruit set, and shedding; maintain perennial growth or signal deciduous behavior; have a defensive function in protecting plants from pathogens, pests, and herbivores; act as antimicrobial drugs; and act as attractants or as repellents. Over 50,000 secondary metabolites have been discovered in the plant world. The basis for the positive health effects of medicinal plants and many modern medicines lay in secondary herbal metabolites (Pang et al. 2021; Teoh 2016).

Chemical compositions of extracts of different parts of blackthorn, Cornelian cherry, dog rose, and hawthorn plants obtained by various solvents (or solvent mixtures) are presented in Tables 3.1, 3.2, 3.3, and 3.4, respectively.

Chemical structures of most prominent compounds (major compounds and secondary metabolites) isolated from described wild plants are given in Figs. 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, and 3.8.

Table 3.1 Chemical composition of blackthorn (*Prunus spinosa* L.)

Country of origin	Plant part	Analytical technique	Type of extract	Compound(s)	References
Serbia (Belgrade)	Fruit	UHPLC-DAD-ESI-MS/MS	70% methanol	*Phenolic acids* (mg/kg): protocatechuic acid (1.14); vanillic acid (3.17); ellagic acid (1.87) *Flavonoids* (mg/kg): rutin (33.92); quercetin 3-*O*-galactoside (3.30); naringin (1.83); kaempferol 3-*O*-glucoside (6.16)	Natić et al. (2019)
Serbia (Fruška Gora)	Fruit	HPLC	Water	*Phenolic acids* (mg/100 g): 3-caffeoylquinic acid (422.04); 3-*p*-coumaroylquinic acid (9.99); 5-caffeoylquinic acid (4.09) *Flavonols* (mg/100 g): quercetin 3-galactoside (1.82); quercetin 3-glucoside (11.22); quercetin 3-rutinoside (32.85); quercetin (0.20) *Anthocyanins* (mg/100 g): cyanidin 3-glucoside (157.85); cyanidin 3-rutinoside (185.62); peonidin 3-glucoside (17.58); peonidin 3-rutinoside (48.61)	Popović et al. (2020)
Southeast Serbia	Fruit	HPLC-DAD	Ethanol	*Phenolic acids* (mg/L): neochlorogenic acid (12.26); caffeic acid (2.12) *Flavonol* (mg/L): quercetin (4.02) *Anthocyanins* (mg/L): cyanidin-3-*O*-glucoside (1.10); cyanidin-3-*O*-rutinoside (1.10)	Veličković et al. (2014)
			Ethanol/water (1:1)	*Phenolic acids* (mg/L): neochlorogenic acid (16.95); caffeic acid (9.73) *Flavonols* (mg/L): quercetin (3.83) *Flavone* (mg/L): myricetin (8.86) *Anthocyanins* (mg/L): cyanidin-3-*O*-glucoside (0.90); cyanidin-3-*O*-rutinoside (3.10); peonidin-3-*O*-glucoside (1.20)	
			Methanol	*Anthocyanins* (mg/L): cyanidin-3-*O*-glucoside (1.10); cyanidin-3-*O*-rutinoside (1.50); peonidin-3-*O*-glucoside (2.20)	
Portugal	Fruit	HPLC-DAD-ESI/MS	Methanol	*Phenolic acids* (mg/100 g): 3-*O*-caffeoylquinic acid (22.09), 3-*p*-coumaroylquinic acid (0.80), 4-*O*-caffeoylquinic acid (3.41), 3-*O*-feruloylquinic acid (1.76), 4-*p*-coumaroylquinic acid (0.64)	Guimaraes et al. (2013, 2014)

				Flavonols (mg/100 g): quercetin pentosylhexoside (1.36); quercetin rhamnosylhexoside (2.22); quercetin 3-*O*-rutinoside (15.63); quercetin pentosylhexoside (6.83); quercetin 3-*O*-glucoside (1.36); quercetin hexoside (4.70); kaempferol 3-*O*-rutinoside (1.90); isorhamnetin 3-*O*-rutinoside (0.87) *Anthocyanins* (µg/100 g): cyanidin 3-*O*-glucoside (19.83); cyanidin 3-*O*-rutinoside (31.12); peonidin 3-*O*-glucoside (10.73); peonidin 3-*O*-rutinoside (34.47); cyanidin 3-*O*-pentoside (1.49); peonidin 3-*O*-pentoside (0.26); cyanidin 3-*O*-acetylglucoside (1.77); peonidin 3-*O*-acetylglucoside (0.73)	
Spain	Fruit	HPLC-DAD-ESI/MS	Citric acid buffer (pH 2.46)	*Phenolic acids* (mg/100 mL): caffeoyldihydrocaffeoylquinic acid (1.96); 3-*O*-caffeoylquinic acid (31.26); 3-*O*-*p*-coumaroylquinic acid (3.42); 4-*O*-caffeoylquinic acid (4.42); 3-*O*-feruloylquinic acid (1.44) *Flavonols* (mg/100 mL): quercetin 3-*O*-rutinoside (1.11); quercetin 3-*O*-hexoside-5-*O*-pentoside (1.21); quercetin 3-*O*-xyloside (1.52) *Anthocyanins* (mg/100 mL): cyanidin 3-*O*-glucoside (0.70); cyanidin-3-*O*-rutinoside (1.59); peonidin 3-*O*-glucoside (0.24); peonidin 3-*O*-rutinoside (1.18)	Gironés-Vilaplana et al. (2012)
		GC-FID	Methanol/sulfuric acid/toluene (2:1:1)	*Fatty acids* (*predominant*) (%): oleic acid (57.58); linoleic acid (23.57); α-linolenic acid (2.79); palmitic acid (6.50)	Barros et al. (2010)
		HPLC-RI	80% ethanol	*Sugars* (g/100 g): fructose (6.95); glucose (29.84); sucrose (0.27)	
		HPLC	Hexane	*Tocopherols* (mg/100 g): α-tocopherol (7.18); β-tocopherol (0.06); γ-tocopherol (1.91); δ-tocopherol (0.10)	
		Spectrophotometer	1% metaphosphoric acid	*Ascorbic acid* (15.69 mg/100 g)	
		UV/Vis	Acetone/hexane (4:6)	*β-Carotene* (0.78 mg/100 g)	

(continued)

Table 3.1 (continued)

Country of origin	Plant part	Analytical technique	Type of extract	Compound(s)	References
Italy	Fruit	HPLC-DAD	Water	*Phenolic acids* (mg/kg): gallic acid (41.10); 4-hydroxybenzoic acid (73.93); caffeic acid (3.36); *trans p*-coumaric acid (2.99); *trans*-ferulic acid (4.93); *trans*-sinapic acid (37.69); rosmarinic acid (3.23) *Flavonoids* (mg/kg): rutin (183.94); myricetin (1.47); quercetin (9.94); genistin (1.74)	Pozzo et al. (2020)
Croatia	Flower	UPLC-MS^2	Ethanol (ultrasound-assisted)	*Phenolic acids* (mg/100 g): gallic acid (1.75); *3*-O-caffeoylquinic acid (192.00); 3-*p*-coumaroylquinic acid (216.28); chlorogenic acid (55.47); feruloylquinic acid (132.20); caffeic acid (34.32); 4-*p*-coumaroylquinic acid (61.53); ferulic acid (8.69) *Flavonoids* (mg/100 g): catechin (85.67); epicatechin (70.16); kaempferol rhamnosylhexoside (49.79); quercetin-pentosyl-hexoside (56.81); quercetin-3-rutinoside (82.35); quercetin-3-glucoside (31.29); quercetin-pentoside (226.75); kaempferol-3-rutinoside (51.84); kaempferol-pentosylhexoside (50.27); kaempferol-pentoside (494.94); quercetin-rhamnoside (81.15); kaempferol-rhamnoside (436.62); quercetin-acetylhexoside (2.34); kaempferol-acetylhexoside (0.92); luteolin (6.68); apigenin-pentoside (3.23) *Procyanidins* (mg/100 g): procyanidin B1 (70.21)	Garofulić et al. (2018)
Romania	Fruit	GC-FID	Chloroform/methanol (2:1)	*Fatty acids* (%): oleic acid (57.4); linoleic acid (23.4); α-linolenic acid (2.62)	Babalau-Fuss et al. (2018)
Poland	Fruit	UV/Vis	Methanol	*β-Carotene* (0.04 mg/100 g) *Vitamin C* (23.84 mg/100 g)	Sikora et al. (2013)

Table 3.2 Chemical composition of Cornelian cherry (*Cornus mas*)

Country of origin	Plant part	Analytical technique	Type of extract	Compounds	References
Serbia (Fruška Gora)	Fruit	UHPLC-DAD-ESI-MS/MS	70% methanol	*Phenolic acids* (mg/kg): gallic acid (6.56); protocatechuic acid (0.83); *O*-caffeoylquinic acid (16.17); caffeic acid (0.85); syringic acid (1.11); *p*-coumaric acid (0.88); ellagic acid (15.17); ferulic acid (0.18) *Flavonoids* (mg/kg): rutin (10.62); quercetin 3-*O*-galactoside (1.81); naringin (0.27); phlorizin (1.16)	Natić et al. (2019)
Southeast Serbia	Fruit	HPLC-DAD	Methanol/acetone/water/formic acid	*Phenolic acids* (mg/g): gallic acid (0.62); ellagic acid (2.11); chlorogenic acid (0.85) *Flavonoids* (mg/g): quercetin-3-glucoside (0.20); quercetin-3-galactoside (0.57); rutin (0.81); kaempferol-3-glucoside (1.11); catechin (3.91); epicatechin (2.11) *Procyanidins* (mg/g): procyanidin B2 (1.55) *Anthocyanins* (mg/g): cyanidin 3-galactoside (3.27); pelargonidin 3-glucoside (10.23); delphinidin-3-galactoside (0.53)	Milenković-Andjelković et al. (2015)
	Leaf			*Phenolic acids* (mg/g): gallic acid (0.37); ellagic acid (2.62); chlorogenic acid (0.33) *Flavonoids* (mg/g): quercetin-3-glucoside (9.37); rutin (6.09); luteolin-3-glucoside (0.15); kaempferol-3-glucoside (4.37); catechin (2.28); epicatechin (4.15)	
Western Serbia	Fruit	LC-MS/MS	70% methanol	*Phenolic acids* (µg/g): neochlorogenic acid (37.64) *Flavonoids* (µg/g): quercetin 3-*O*-glucuronide (151.82); quercetin 3-*O*-galactoside (13.55); quercetin 3-*O*-glucoside (7.42); quercetin 3-*O*-rutinoside (9.12); quercetin 3-*O*-rhamnoside (0.23)	Bajić-Ljubičić et al. (2018)
Poland	Fruit	UPLC-MS/MS	80% ethanol	*Phenolic acids* (mg/g): 3-*O*-caffeoylquinic acid (3.47); 5-*O*-caffeoylquinic acid (10.89) *Flavonoids* (mg/g): quercetin 3-*O*-glucuronide (4.86); kaempferol 3-*O*-galactoside (4.03) *Anthocyanins* (mg/g): delphinidin 3-*O*-galactoside (0.63); cyanidin 3-*O*-galactoside (15.79); cyanidin 3-*O*-robinobioside	Szumny et al. (2015)

(continued)

Table 3.2 (continued)

Country of origin	Plant part	Analytical technique	Type of extract	Compounds	References
				(6.38); pelargonidin 3-*O*-galactoside (29.94); pelargonidin 3-*O*-robinobioside (5.95)	
Italy	Fruit	HPLC		*Phenolic acids* (mg/100 g): gallic acid (0.05); ellagic acid (23.56); caffeic acid (0.66); chlorogenic acid (11.27); coumaric acid (3.86); ferulic acid (2.14) *Flavonoids* (mg/100 g): hyperoside (1.00); rutin (0.29); catechin (14.38); epicatechin (21.74) *Tannins* (mg/100 g): vescalagin (4.66) *Organic acids* (mg/100 g): citric acid (58.24); malic acid (48.59); oxalic acid (2.11); succinic acid (2.67); tartaric acid (40.35) *Monoterpenes* (mg/100 g): limonene (115.63); phellandrene (18.49); γ-terpinene (18.44); terpinolene (1.42) *Vitamin C* (mg/100 g): ascorbic acid (41.98); dehydroascorbic acid (19.44)	De Biaggi et al. (2018)
Romania	Fruit	HPLC	Acetone	*Phenolic acids* (mg/100 g): ellagic acid (187.91); caffeic acid (27.12); chlorogenic acid (32.76) *Flavonoids* (mg/100 g): quercetin-3-*O*-glucuronide (471.01); kaempferol-3-*O*-galactoside (366.88); catechin (37.06); epicatechin (66.89) *Anthocyanins* (mg/100 g): cyanidin-3-*O*-galactoside (3.82); pelargonidin-3-*O*-glucoside (58.62); pelargonidin-3-*O*-rutinoside (33.8)	Moldovan et al. (2016)
Turkey	Fruit	HPLC	Methanol	*Phenolic acids* (μg/g): gallic acid (12.60); protocatechuic acid (9.88); chlorogenic acid (0.81); ferulic acid (0.92) *Flavonols* (μg/g): quercetin (3.34)	Okan et al. (2019)
			80% ethanol	*Sugars* (%): fructose (2.00); glucose (2.50); sucrose (0.57)	

Table 3.3 Chemical composition of dog rose (*Rosa canina* L.)

Country of origin	Plant part	Analytical technique	Type of extract	Compound(s)	References
Northern Serbia	Fruit	LC-MS/MS	Water	*Phenolic acids* (µg/g): gallic acid (11.3); protocatechuic acid (9.79) *Flavonoids* (µg/g): quercitrin (40.4); quercetin-3-*O*-glucoside (2.54); hyperoside (2.53); epicatechin (2.35); catechin (7.83)	Nađpal et al. (2016)
		UV/Vis		*Vitamin C*: 1.96 mg/g	
		LC-MS/MS	Methanol	*Phenolic acids* (µg/g): gallic acid (1.86); protocatechuic acid (8.04); *p*-coumaric acid (1.53) *Flavonoids* (µg/g): kaempferol-3-*O*-glucoside (1.77); quercitrin (95.2); quercetin-3-*O*-glucoside (9.40); hyperoside (7.73); epicatechin (2.92); catechin (4.23)	
		UV/Vis		*Vitamin C*: 1.87 mg/g	
Serbia	Seed	ICP-OES		*Major and trace elements* (mg/kg): Mg (848–1072); Ca (3236–3567); K (2494–3481); Ba (4.86–5.26); Cu (4.16–5.37); Fe (15.87–19.30); Mn (17.76–31.97); P (1178–2184); S (567–959); Zn (0.36–3.53)	Popović-Djordjević et al. (2021)
	Mesocarp			*Major and trace elements* (mg/kg): Mg (675–1670); Ca (2310–5042); K (8750–8953); Ba (4.35–9.60); Cu (0.75–1.60); Fe (3.03–3.90); Mn (25.50–34.67); P (731–872); S (279–427); Zn (0.85–1.10)	
Southern Serbia	Leaf	HPLC-DAD	Methanol	*Flavonoids* (µg/g): rutin (343.81); hyperside (585.24); isoquercetin (1022.95); isorhamnetin-3-*O*-rutinoside (1568.34)	Živković et al. (2015)
Serbia	Seed	GC	*n*-Hexane	*Fatty acids* (%): linoleic acid (51.1–53.4); oleic acid (18.4–19.6); α-linolenic acid (19.4–20.8); palmitic acid (2.6–4.9); arachidic acid (1.1–1.5)	Milić et al. (2020)
Western Serbia	Seed	GC-FID	*n*-Heptane	*Fatty acids* (%): linoleic acid (35.4); arachidic acid (28.2); palmitoleic acid (18.2); *cis*-8,11,14-eicosatrienoic acid (23.1); *cis*-11,14,17-eicosatrienoic acid (14.7); arachidonic acid (13.2); heneicosylic acid (21.4)	Vasić et al. (2020)

(continued)

Table 3.3 (continued)

Country of origin	Plant part	Analytical technique	Type of extract	Compound(s)	References
Slovenia	Fruit	HPLC-MS	Methanol (with 3% formic acid)	*Phenolic acids* (μg/g): ellagic acid derivatives (50.6); total hydroxybenzoic acids (499); hydroxycinnamic acids (195) *Flavanols* (μg/g): catechin and derivatives (1111) *Flavonols* (μg/g): isorhamnetin glycosides (11.1); kaempferol derivatives (3.07); quercetin glycosides (68.0) *Flavanones* (μg/g): eriodictyol derivatives (77.7); naringenin derivatives (98.9); taxifolin derivatives (177) *Anthocyanins* (μg/g): cyanidin glucoside (83.0)	Cunja et al. (2016)
		HPLC	Water	*Sugars* (mg/100 g): glucose (20.5); fructose (20.8); sucrose (5.14) *Organic acids* (mg/100 g): citric acid (11.6); quinic acid (1.49); malic acid (10.3); tartaric acid (341); shikimic acid (8.50); fumaric acid (9.41)	
			2% metaphosphoric acid	*Ascorbic acid:* 1835 mg/100 g	
		HPLC-MS	Acetone	*Carotenoids* (μg/g): β-carotene (2126); lycopene (1550)	
Tunisia	Leaf	HPLC-DAD	Ethyl acetate	*Flavonoids* (mg/100 mg): catechin (0.07); epicatechin gallate (0.17); rutin (0.02); quercetin 3-*O*-glucoside (0.67); kaempferol *O*-hexoside-deoxyhexoside (0.60); kaempferol 3-*O*-rutinoside (0.03); kaempferol 7-*O*-glucoside (0.02); kaempferol 3-*O*-glucoside (0.14)	Ouerghemmi et al. (2016)
			Methanol	*Flavonoids* (mg/100 mg): catechin (0.33); quercetin di-*O*-hexoside (0.17); epicatechin gallate (0.46); epicatechin (0.46); rutin (0.15); quercetin 3-*O*-glucoside (2.27); kaempferol *O*-hexoside-deoxyhexoside (0.83); kaempferol 3-*O*-rutinoside (0.11); kaempferol 7-*O*-glucoside (0.12); kaempferol 3-*O*-glucoside (0.28); quercitrin (0.10)	
Iran	Fruit	HPLC	80% methanol	*Phenolic acids* (μg/g): *p*-coumaric acid (11.7); cinnamic acid (8.3); chlorogenic acid (5.7 ± 0.02); caffeic acid (13.1); gallic acid (81.3) *Flavonoids* (μg/g): apigenin (2.7); quercetin (1.1); rutin (19.6)	Shameh et al. (2019)

		UV/Vis	Acetone	*β-Carotene*: 9.1 μg/g	
		UV/Vis	1% metaphosphoric acid	*Vitamin C*: 62.90 mg/g	
Kazakhstan	Leaf	HPLC	Ethanol/water (1:1)	*Phenolic acids* (mg/g): gallic acid (0.805); α-resorcylic acid (0.138); protocatechuic acid 0.153); neochlorogenic acid (57.148); 4-hydroxybenzoic acid (1.182); gentisic acid (1.577); chlorogenic acid (4.609); vanillic acid (0.102); caffeic acid (0.035); syringic acid (0.613); *p*-coumaric acid (0.520); ferulic acid (0.439); sinapic acid (0.214); ellagic acid (35.881); *o*-coumaric acid (0.711); rosmarinic acid (4.406); salicylic acid (0.457) *Flavonols* (mg/g): epigallocatechin (0.207); catechin (2.804); epicatechin (1.822); rutin (26.66); myricetin (3.928); quercetin (0.156) *Flavone* (mg/g): coumarin (1.285); luteolin 7-*O*-β-D-glucoside (1.614); hesperidin (4.013); luteolin (0.911); kaempferol (0.148); 3-hydroxyflavone (0.103) *Anthocyanins* (mg/g): cyanidin (47.448) *Vitamins* (mg/g): B1 (1.12); B2 (0.48); B3 (2.10); B5 (3.30); B6 (5.70); Bc (0.97); α-tocopherol (0.54); β-tocopherol (0.13); γ-tocopherol (0.18) *Amino acids* (mg/g): arginine (4.979); lysine (3.378); tyrosine (3.023); phenylalanine (6.045); histidine (0.960); leucine + isoleucine (6.900); methionine (1.494); valine (0.460); proline (10.491); threonine (4.445); serine (4.801); alanine (5.334); glycine (4.801)	Kubczak et al. (2020)
	Twig	HPLC	Ethanol/water (1:1)	*Phenolic acids* (mg/g): gallic acid (0.357); α-resorcylic acid (0.117); protocatechuic acid (13.911); neochlorogenic acid (0.258); 4-hydroxybenzoic acid (0.323); gentisic acid (0.340); chlorogenic acid (0.934); vanillic acid (0.379); caffeic acid (0.203); syringic acid (0.134); *p*-coumaric acid (0.247); ferulic acid (0.081); sinapic acid (0.140); ellagic acid (14.448); *o*-coumaric acid (0.149); rosmarinic acid (1.851); salicylic acid (0.474) *Flavonols* (mg/g): epigallocatechin (1.680); catechin (17.798);	

(continued)

Table 3.3 (continued)

Country of origin	Plant part	Analytical technique	Type of extract	Compound(s)	References
				epicatechin (1.379); rutin (4.431); myricetin (7.175); quercetin (0.241) *Flavones* (mg/g): coumarin (0.170); luteolin 7-*O*-β-D-glucoside (1.417); naringenin (0.633); luteolin (0.364); kaempferol (0.167); 3-hydroxyflavone (0.128) *Anthocyanins* (mg/g): cyanidin (4.453) *Vitamins* (mg/g): B1 (0.77); B2 (0.51); B3 (2.70); B5 (3.10); B6 (6.20 ± 0.10); Bc (0.86); α-tocopherol (0.31); β-tocopherol (0.15); γ-tocopherol (0.09) Amino acids (mg/g): lysine (1.426); tyrosine (0.713); phenylalanine (2.262); histidine (0.565); leucine + isoleucine (1.819); methionine (0.787); valine (0.713); proline (3.196); threonine (1.770); serine (2.040); alanine (1.573)	
Lithuania	Fruit	HPLC	Methanol	*Phenolic acids* (mg/100 g): gallic acid (22.67); chlorogenic acid (9.80); caffeic acid (22.08); *p*-coumaric acid (48.22); ferulic acid (19.03) *Flavonoids* (mg/100 g): rutin (11.62); kaempferol-3-*O*-glucoside (4.40); luteolin (7.46); quercetin (9.77); quercetin-3-*O*-glucoside (2.35)	Medveckiene et al. (2020)
			n-Hexane	*Carotenoids* (mg/100 g): β-carotene (3.95); α-carotene (0.80); lutein (1.55); zeaxanthin (0.23); *cis*-lycopene (0.55); *trans*-lycopene (1.59)	
		Titration		*Ascorbic acid*: 385.82 mg/100 g	
	Seed	HPLC	Methanol	*Phenolic acids* (mg/100 g): gallic acid (88.69); chlorogenic acid (45.83); caffeic acid (12.73); *p*-coumaric acid (22.04); ferulic acid (4.83) *Flavonoids* (mg/100 g): rutin (19.11); kaempferol-3-*O*-glucoside (5.29); luteolin (1.89); quercetin (2.87); quercetin-3-*O*-glucoside (3.02)	

			n-Hexane	*Carotenoids* (mg/100 g): β-carotene (0.58); zeaxanthin (0.21); *cis*-lycopene (0.13); *trans*-lycopene (0.24)	
		Titration		*Ascorbic acid*: 22.99 mg/100 g	
Spain	Fruit	HPLC	Acetone/water (4:1)	*Phenolic acids* (mg/g): vanillic acid (0.26); caffeic acid (0.002); syringic (0.11); gallic acid (0.298); ellagic acid (0.080); protocatechuic acid (0.21) *Flavonoids* (mg/g): myricetin (5.4); rutin (22); catechin (11.9); quercetin (1.5) *Vitamin C*: 101 μg/g	Jiménez et al. (2017)
Portugal	Fruit	HPLC-DAD-ESI/MS	Methanol/water (4:1)	*Flavone/flavonols* (mg/100 g): quercetin glucuronide 0.24); quercetin rhamnoside (0.46); quercetin 3-*O*-rutinoside (0.47); taxifolin pentoside (1.18); eriodictyol hexoside (0.50) *Flavans* (mg/100 g): catechin (3.59) *Procyanidins* (mg/100 g): procyanidin dimer B1 (1.68); procyanidin dimer B3 (1.50) *Anthocyanins* (mg/100 g): cyanidin 3-*O*-glucoside (0.68)	Guimaraes et al. (2013)
Greece	Fruit	UPLC-MS-MS	Methanol	*Phenolic acids* (μg/g): gallic acid (2.12); protocatechuic acid (2.09); *p*-coumaric acid (2.44) *Flavonoids* (μg/g): catechin (134.75); epicatechin (120.99); hyperoside (308.11); rutin (25.64); astragalin (172.48); phloridzin (3.41); quercetin (0.67); kaempferol (0.46) *Organic acids* (μg/g): quinic acid (1102.59 ± 38.91) *Triterpenoids* (μg/g): betulinic acid (0.47); ursolic acid (138.23)	Kerasioti et al. (2019)
Poland	Fruit	LC-DAD/ESI/MS	Water	*Phenolic acids* (μg/g): gallic acid (2.08); protocatechuic acid (1.07); vanillic acid (2.35); chlorogenic acid (1.45); syringic acid (1.11); *p*-coumaric acid (9.05); ferulic acid (0.93); sinapic acid (1.54); rosmarinic acid (0.62); cinnamic acid (9.53) *Flavonoids* (μg/g): rutin (3.04); quercetin (7.64)	Polumackanycz et al. (2020)
			Methanol/water (4:1)	*Phenolic acids* (μg/g): gallic acid (8.77); protocatechuic acid (2.60); vanillic acid (0.08); chlorogenic acid (0.16); cinnamic acid (0.11) *Flavonoids* (μg/g): rutin (0.33); quercetin (0.18)	

(continued)

Table 3.3 (continued)

Country of origin	Plant part	Analytical technique	Type of extract	Compound(s)	References
	Leaf		Water	*Phenolic acids* (μg/g): gallic acid (7.65); protocatechuic acid (5.52); vanillic acid (2.58); chlorogenic acid (9.52); syringic acid (6.75); *p*-coumaric acid (3.56); ferulic acid (1.46); sinapic acid (2.51); rosmarinic acid (0.65); cinnamic acid (10.53 ± 1.65) *Flavonoids* (mg/g): rutin (5.68); quercetin (6.82)	
			Methanol/water (4:1)	*Phenolic acids* (μg/g): gallic acid (8.92); vanillic acid (9.61); chlorogenic acid (4.74); cinnamic acid (9.03) *Flavonoids* (mg/g): rutin (3.13); quercetin (0.63)	
Turkey	Seed	UV/Vis	2% metaphosphoric acid	*Vitamin C*: 1793 μg/g	Ilyasoğlu (2014)
		GC	Chloroform	*Fatty acids* (%): palmitic (3.34); stearic (1.69); oleic (19.50); linoleic (54.05); α-linolenic (19.37); arachidic (1.00) *Sterols* (mg/100 g): campesterol (23.3); stigmasterol (18.9); clerosterol (1.4); β-sitosterol (544); 5-avenasterol (31.6); 7-stigmastenol (41.1); 7-avenasterol (1.9)	

Table 3.4 Chemical composition of hawthorn (*Crataegus monogyna*)

Country of origin	Plant part	Analytical technique	Type of extract	Compounds	References
Serbia (Belgrade)	Fruit	UHPLC-DAD-ESI-MS/MS	70% methanol	*Phenolic acids* mg/kg): protocatechuic acid (0.78); *p*-hydroxybenzoic acid (1.56); 5-*O*-caffeoylquinic acid (4.36); caffeic acid (1.35); vanillic acid (6.66); *p*-coumaric acid (0.56); ellagic acid (2.49); ferulic acid (0.52) *Flavonoids* (mg/kg): rutin (30.92); quercetin 3-*O*-galactoside (77.31); phlorizin (0.32); kaempferol (0.59); aesculin (3.79)	Natić et al. (2019)
Western Serbia				*Phenolic acids* (mg/kg): 5-*O*-caffeoylquinic acid (20.71); caffeic acid (0.61); *p*-coumaric acid (0.14); ellagic acid (0.63); ferulic acid (0.11) *Flavonoids* (mg/kg): arbutin (12.28); catechin (3.29); rutin (66.54); quercetin 3-*O*-galactoside (117.35); naringin (0.41); kaempferol 3-*O*-glucoside (29.49); phlorizin (3.91); kaempferol (0.25); aesculin (14.69)	Natić et al. (2019)
Poland	Fruit	HPLC-ESI-Q-TOF-MS and HRMS/MS	Ethanol/water (1:1)	*Phenolic acids* (mg/g): protocatechuic acid (0.035); chlorogenic acid (0.326); sinapinic acid (0.363) *Flavonoids* (mg/g): quercetin (0.4907); quercitrin (0.0385); rutin (0.368); quercetin 3-galactoside (0.6428) *Anthocyanin* (mg/g): cyanidin-3-glucoside (0.5357)	Bekbolatova et al. (2018)
			96% ethanol	*Phenolic acids* (mg/g): sinapinic acid (0.1208); chlorogenic acid (0.279); *p*-coumaric acid (0.022); *m*-coumaric acid (0.0229); *o*-coumaric acid (0.018) *Flavonoids* (mg/g): quercetin (0.392); quercitrin (0.0451); rutin (0.103); quercetin 3-galactoside (0.1709) *Anthocyanins* (mg/g): cyanidin-3-glucoside (0.721)	
	Flower		Ethanol/water (1:1)	*Phenolic acids* (mg/g): gentisic acid (0.086); sinapinic acid (0.057); chlorogenic acid (1.496); *p*-coumaric acid (0.0359); *o*-coumaric acid (0.027); caffeic acid 3-glucoside (0.0046) *Flavonoids* (mg/g): quercetin (0.572); quercitrin (0.5117); rutin (0.73); quercetin 3-galactoside (3.712)	

(continued)

Table 3.4 (continued)

Country of origin	Plant part	Analytical technique	Type of extract	Compounds	References
			96% ethanol	*Phenolic acids* (mg/g): gentisic acid (0.095); chlorogenic acid (0.749); *p*-coumaric acid (0.044); *m*-coumaric acid (0.0399); *o*-coumaric acid (0.031); caffeic acid 3-glucoside (0.0011), caffeic acid (0.0076) *Flavonoids* (mg/g): quercetin (0.1234); quercitrin (0.26); rutin (0.563); quercetin 3-galactoside (0.105) *Anthocyanins* (mg/g): cyanidin-3-glucoside (0.0052)	
	Leaf		Ethanol/water (1:1)	*Phenolic acids* (mg/g): gentisic acid (0.0422); chlorogenic acid (0.479); protocatechuic acid (0.0126); *m*-coumaric acid (0.0139); *o*-coumaric acid (0.01) *Flavonoids* (mg/g): quercetin (1.094); quercitrin (0.83); epigallocatechin (0.0006); rutin (0.427); quercetin 3-galactoside (2.671) *Anthocyanins* (mg/g): cyanidin-3-glucoside (0.0026)	
			96% ethanol	*Phenolic acids* (mg/g): sinapinic acid (0.0389); chlorogenic acid (0.6087) *Flavonoids* (mg/g): quercetin (0.2024); quercitrin (0.1603); catechin (0.0036); epigallocatechin (0.0009); rutin (0.372); quercetin 3-galactoside (1.6328)	
China	Fruit	HPLC	Methanol	*Phenolic acids* (mg/100 g): chlorogenic acid (84.2); *p*-coumaric acid (24.9) *Flavonoids* (mg/100 g): catechin (27.1); epicatechin (281.6); quercetin (78.4); isoquercitrin (9.6) *Procyanidins* (mg/100 g): procyanidin B2 (243.5)	Zhang et al. (2020)
Iran	Fruit	HPLC	Methanol/ water (4:1)	*Phenolic acids* (mg/g): chlorogenic acid (0.40) *Flavonoids* (mg/g): vitexin (0.18); hyperoside (1.15); isoquercetin (0.68); quercetin (0.05)	Alirezalu et al. (2020)

Finland	Fruit	HPLC-DAD-ESI-MS	Methanol	*Phenolic acids*: chlorogenic acid; neochlorogenic acid *Procyanidins*: procyanidin B2; procyanidin B5 *Flavonols*: hyperoside; quercetin-pentosides; quercetin-hexoside acetate; quercetin-rhamnosylhexoside; epicatechin *Flavones*: luteolin-*C*-hexoside; methyl luteolin-*C*-hexoside	Liu et al. (2011)
	Leaf			*Phenolic acid*: chlorogenic acid *Procyanidins*: procyanidin B2; procyanidin B5 *Flavonols*: hyperoside; quercetin-pentosides; quercetin-hexoside acetate; quercetin-rhamnosylhexoside; epicatechin *Flavones*: luteolin-*C*-hexoside; methyl luteolin-*C*-hexoside	
Turkey	Seed	GC-MS	Hexane	*Fatty acids* (%): palmitic (5.61–6.76); palmitoleic (0.07–0.13); stearic (1.43–1.91); oleic (33.48–39.36); linoleic (50.53–52.51); γ-linolenic (1.26–1.47); eicosenoic (0.40–0.45); tricosanoic (0.64–0.89); docosadienoic (0.25–0.29)	Özderin et al. (2016)

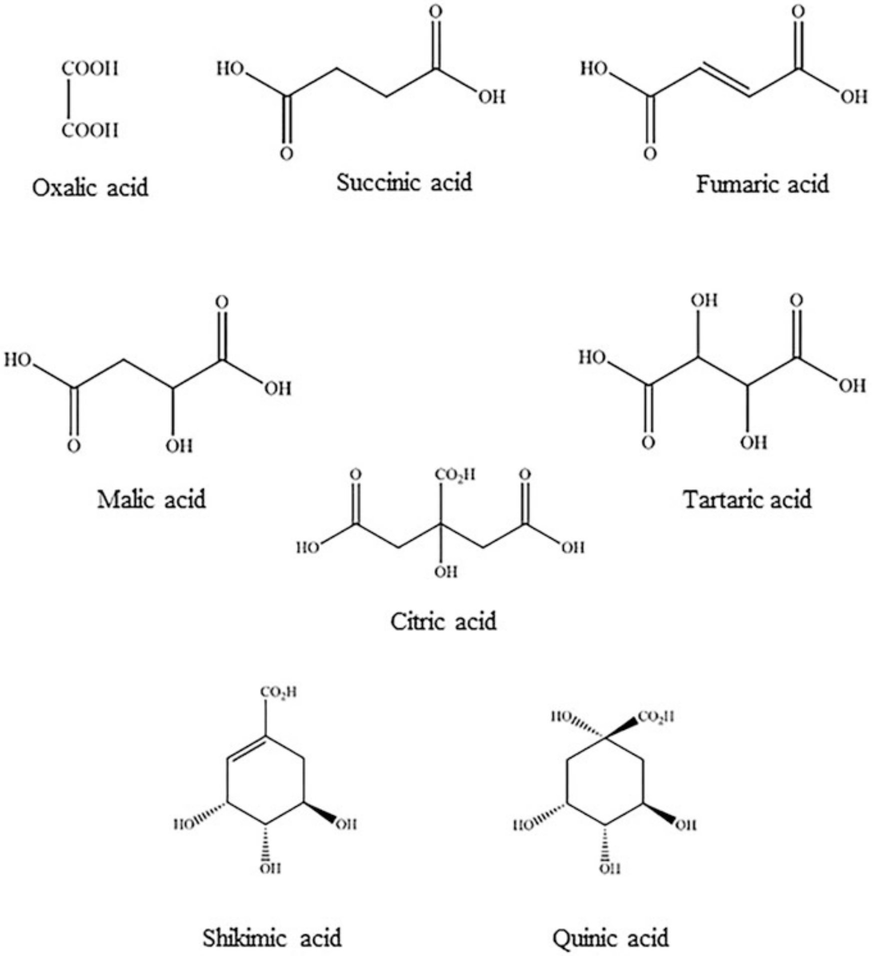


Fig. 3.1 Chemical structures of organic acids isolated from selected wild plants

3.4 Biological Activity and Medicinal Application of Selected Plants

Blackthorn (*Prunus spinosa* L.) is used in the traditional medicine of many European countries, and it is native in West Asia and Northwest Africa as well (Marchelak et al. 2017). In Europe and Near East countries, the blackthorn fruits have been used since prehistoric times (Balta et al. 2020). Blackthorn fruits, despite their pungent acid taste, were dominantly used as phytotherapeutics in the treatment of diseases of the circulatory system, based on their anti-inflammatory, diuretic, laxative, and astringent characteristics, but also for curing coughs (Marchelak et al.

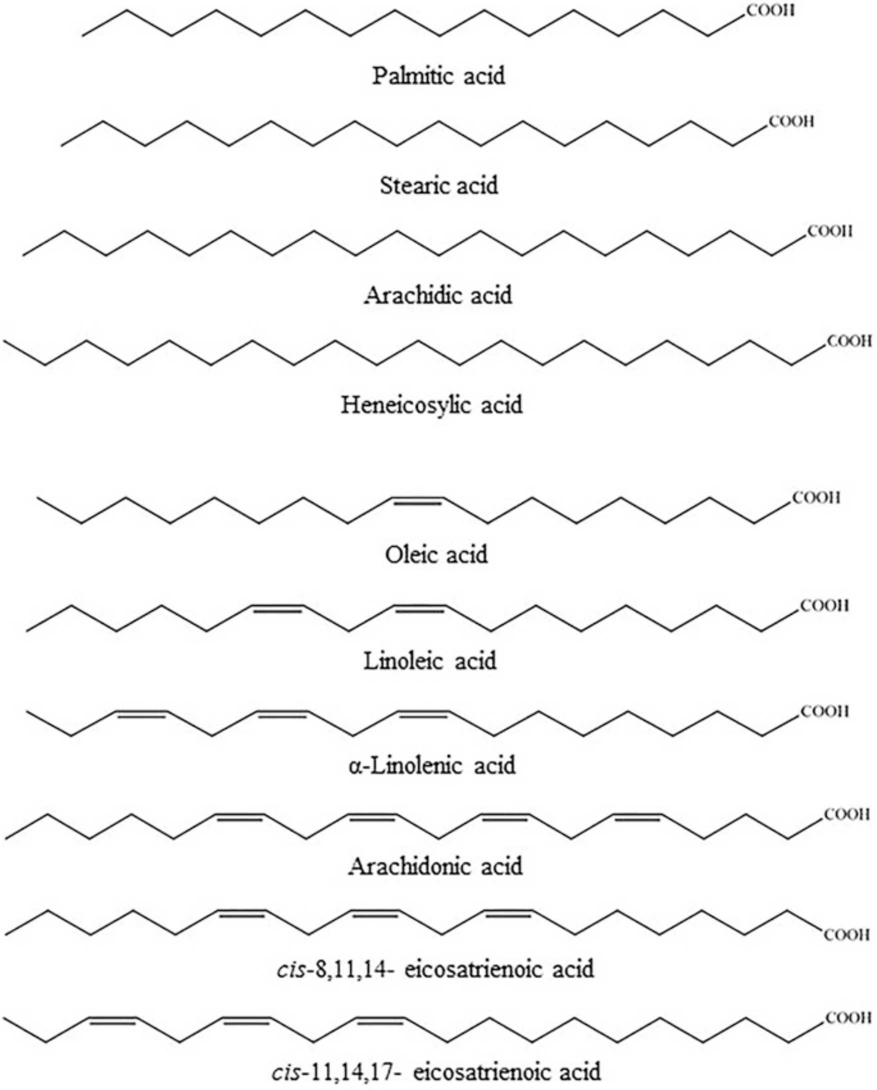


Fig. 3.2 Chemical structures of the most represented saturated and unsaturated fatty acids (FAs) of selected wild plants

2017; Sabatini et al. 2020). Fruits also possess heart-strengthening properties and are used in the treatment of myocarditis, cardiac neurosis, and atherosclerosis (Jarić et al. 2015).

The other parts of the plant (e.g., roots, flowers, and laxative and bark) have similar effects (laxative and diuretic) (Balta et al. 2020). Flowers showed many beneficial properties, such as detoxifying, anti-inflammatory, vasoprotective, and

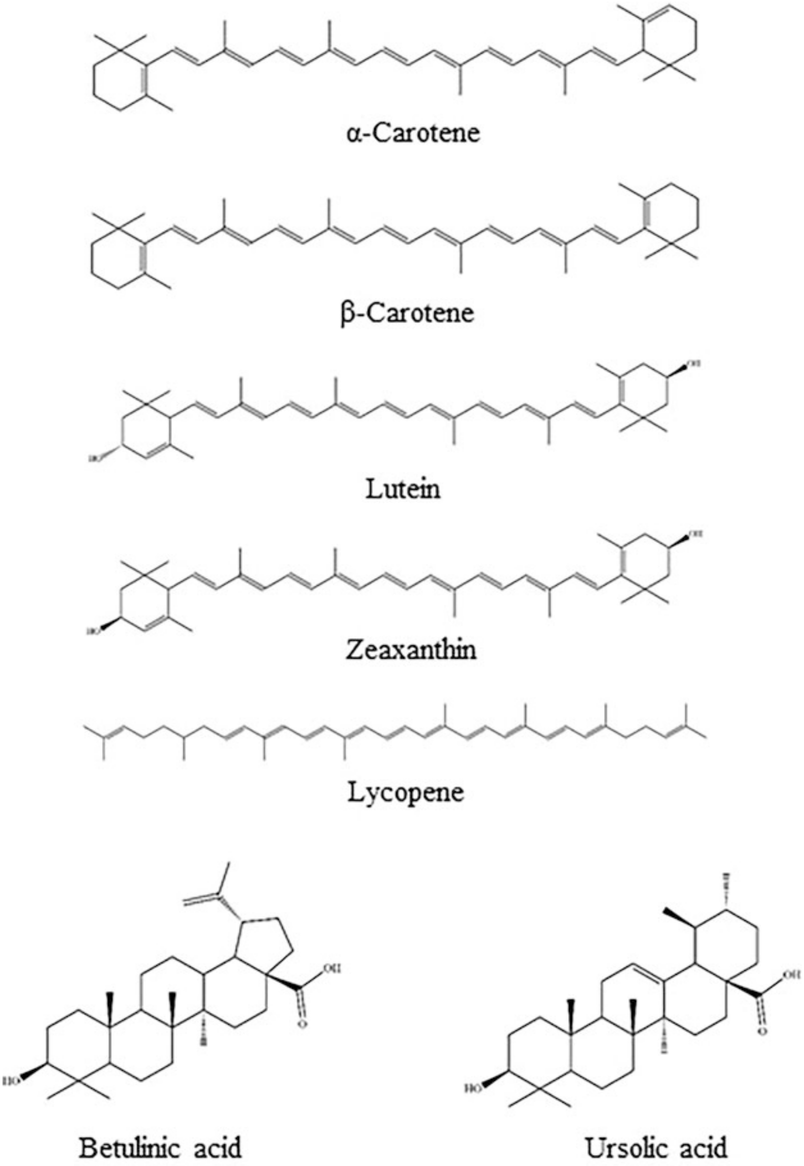
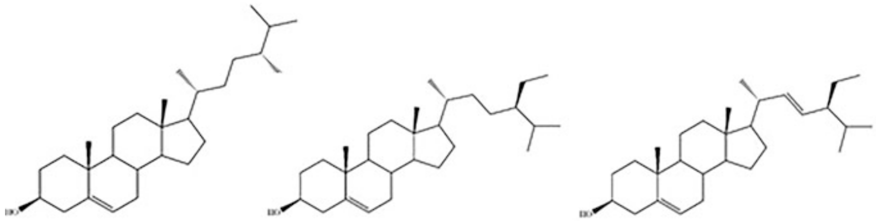
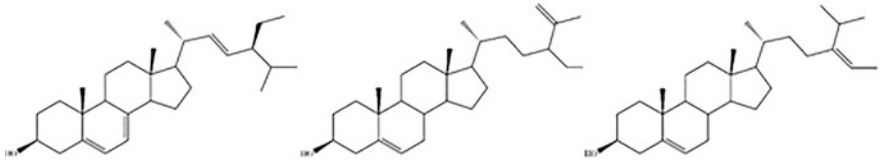


Fig. 3.3 Chemical structures of carotenoids and triterpenoid acids (betulinic and ursolic) isolated from selected wild plants

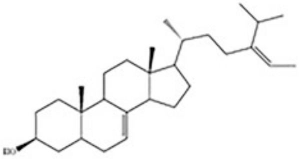
spasmolytic actions, and may be used for various disorders of the respiratory tract and intestinal problems, but also in the treatment of cardiovascular diseases (Marchelak et al. 2021).



Campesterol β-sitosterol Stigmasterol



Δ-7-Stigmasterol Clerosterol Δ-5-Avenasterol

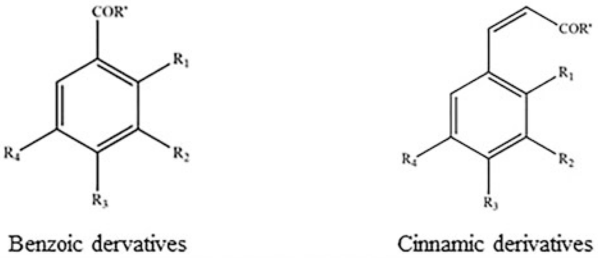


Δ-7-Avenasterol

Fig. 3.4 Chemical structures of phytosterols isolated from selected wild plants

Although the astringent *P. spinosa* fruits have many benefits for human health, their consumption is limited to the prepared product such as teas, juices, wines, liqueurs, jams, and compote, because heat treatment contributes to the richness of taste (Balta et al. 2020; Sabatini et al. 2020).

Main compounds that are associated with blackthorn pharmacological potential are several classes of polyphenols – anthocyanins, A-type proanthocyanidins, tannins, flavonoids, and phenolic acids (Marchelak et al. 2017). All these phytochemicals are well-known for their significant activities in terms of antioxidant defense, anti-inflammatory, and antimicrobial effects (Katanić et al. 2015a, 2016), and many compounds, including anthocyanins, showed excellent cardioprotective properties (Di Lorenzo et al. 2021; Najjar and Feresin 2021; Verediano et al. 2021). Various biological activities of many plants from Rosaceae family containing anthocyanins, hydroxycinnamic acids, flavonoids, and tannins are well-known, not just the species



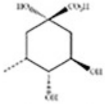
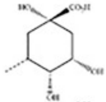
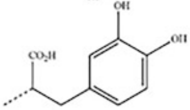
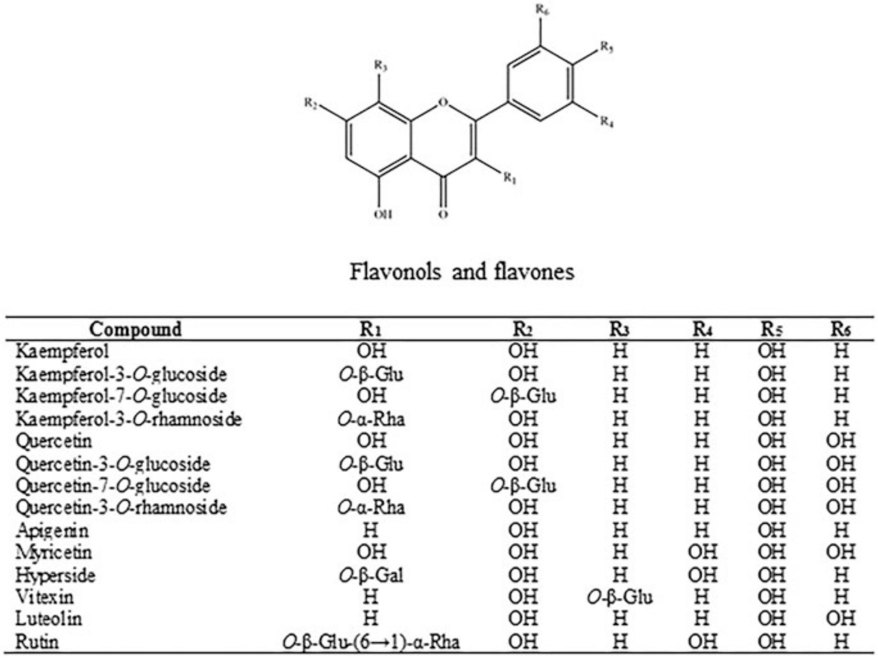
Benzoic dervatives	R'	R_1	R_2	R_3	R_4	Cinnamic derivatives
Benzoic acid	OH	H	H	H	H	Cinnamic acid
p-hydroxybenzoic	OH	H	H	OH	H	*p*-Coumaric acid
Salicylic acid	OH	OH	H	H	H	*o*-Coumaric acid
α-Resorcylic acid	OH	H	OH	H	H	*m*-Coumaric acid
Protocatechuic acid	OH	H	OH	OH	H	Caffeic acid
Gentisic acid	OH	OH	H	H	H	
Gallic acid	OH	H	OH	OH	OH	
Vanillic acid	OH	H	OCH_3	OH	H	Ferulic acid
Syringic acid	OH	H	OCH_3	OH	OCH_3	Sinapic acid
Gentisic acid	OH	OH	H	H	OH	
		H	OH	OH	H	Chlorogenic acid
		H	OH	OH	H	Neochlorogenic acid
		H	OH	OH	H	Rosmarinic acid

Fig. 3.5 Chemical structures of most represented phenolic acids in selected wild plants

with edible fruits (Mihailović et al. 2018, 2019) but also the aerial parts, flowers, and roots of herbaceous Rosaceae plants (Katanić et al. 2015a, b; Boroja et al. 2018).

The antioxidant activity of blackthorn fruits was assessed in vitro by many research groups in the last decade, starting with Barros et al. (2010), who showed significant amount of tocopherols and vitamin C in blackthorn fruit extract, but also interesting free radical scavenging properties, especially in terms of lipid peroxidation inhibition. This was also confirmed by Morales et al. (2013). The antioxidant action of aqueous *P. spinosa* fruit extract toward DPPH radical was shown by Gegiu et al. (2020) as well as by Sabatini et al. (2020) who demonstrated concentration-dependent antioxidant potential as well as antimicrobial and anti-inflammatory



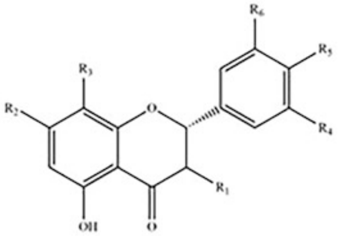
Flavonols and flavones

Compound	R1	R2	R3	R4	R5	R6
Kaempferol	OH	OH	H	H	OH	H
Kaempferol-3-*O*-glucoside	*O*-β-Glu	OH	H	H	OH	H
Kaempferol-7-*O*-glucoside	OH	*O*-β-Glu	H	H	OH	H
Kaempferol-3-*O*-rhamnoside	*O*-α-Rha	OH	H	H	OH	H
Quercetin	OH	OH	H	H	OH	OH
Quercetin-3-*O*-glucoside	*O*-β-Glu	OH	H	H	OH	OH
Quercetin-7-*O*-glucoside	OH	*O*-β-Glu	H	H	OH	OH
Quercetin-3-*O*-rhamnoside	*O*-α-Rha	OH	H	H	OH	OH
Apigenin	H	OH	H	H	OH	H
Myricetin	OH	OH	H	OH	OH	OH
Hyperside	*O*-β-Gal	OH	H	OH	OH	H
Vitexin	H	OH	*O*-β-Glu	H	OH	H
Luteolin	H	OH	H	H	OH	OH
Rutin	*O*-β-Glu-(6→1)-α-Rha	OH	H	OH	OH	H

Fig. 3.6 Chemical structures of flavonoids (flavonols and flavones) isolated from selected wild plants

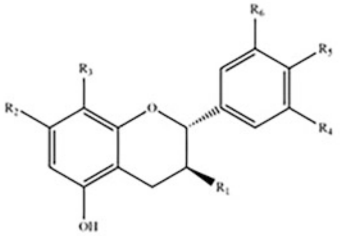
properties. The antimicrobial effects of *P. spinosa* fruit from Italy showed antibacterial potential against both Gram+ and Gram− bacteria, e.g., *E. coli*, *S. typhimurium*, *E. aerogenes*, *E. faecalis*, and *S. aureus* (Pozzo et al. 2020), along with in vitro antioxidant properties and against in vivo streptozotocin-induced oxidative stress in liver and brain tissues.

Many Serbian authors confirmed antioxidant properties of blackthorn fruit, predominantly used in Serbia as an infusion or alcoholic extract in the treatment of heart problems and for reducing high blood cholesterol and triglyceride levels (Šavikin et al. 2013). Mitic et al. (2014) reported high antioxidant potential of blackthorn extract in FRAP, DPPH, and ABTS assays compared with blackberries, raspberries, and cherries from southern Serbia, which correlated with the content of anthocyanins. Blackthorn fruits from the region of Southeast Serbia were also tested for antioxidant activity as well as antimicrobial potential toward *E. coli*, *P. aeruginosa*, *S. aureus*, and *C. albicans* (Veličković et al. 2014). Blackthorn fruits from Central Serbia showed a high antioxidant potential in DPPH and nitric oxide scavenging activity, ferro-chelating capacity, and ferric-reducing capacity assays (Natić et al. 2019). The study of ultrasonic blackthorn fruit extracts showed that 45% propylene glycol extract had the highest antioxidant activity against DPPH radicals, nitric oxide radical scavenging, and non-site-specific hydroxyl radical scavenging activity, along with tyrosinase inhibitory potential (Stanković et al. 2019). *P. spinosa* fruit extracts



Flavanones

Compound	R1	R2	R3	R4	R5	R6
Epicatechin	OH	OH	H	H	OH	OH
Naringenin	H	OH	H	H	OH	H
Taxifolin	OH	OH	H	H	OH	OH
Hesperidin	H	*O*-α-Rha-(1→6)-β-Glu	H	H	OCH_3	OH



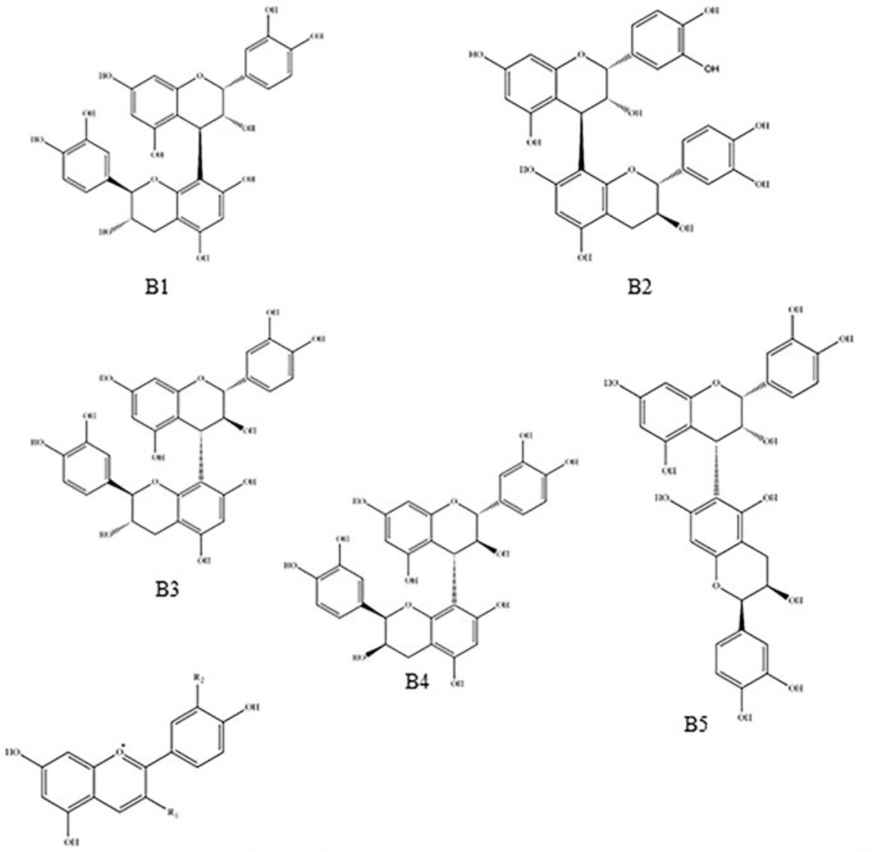
Flavans

Compound	R1	R2	R3	R4	R5	R6
Catechin	OH	OH	H	H	OH	OH
Catechin-3-*O*-gallate	OH	OH	H	H	OH	*O*-galloyl

Fig. 3.7 Chemical structures of flavonoids (flavanones and flavans) isolated from selected wild plants

collected in north Serbia demonstrated FRAP and DPPH antioxidant potential ranging from 7.06 to 25.27 mg ascorbic acid equivalents/g and IC_{50} values from 0.62 to 3.46 mg/mL, respectively, with significant positive correlation with total phenolic content (Popović et al. 2020). The same extracts showed antidiabetic potential toward inhibition of α-amylase and α-glucosidase enzymatic activity with strong positive correlation with total content of phenolics and individual polyphenols. The antiproliferative effects of tested fruit samples were demonstrated on HT29 cell line, with the most pronounced potential of samples from Beška (Popović et al. 2020).

Other parts of *P. spinosa* plant are also interesting given the biological activities they exerted. It was reported that the extracts of *P. spinosa* branches showed high DPPH scavenging capacity and that simulated in vitro digestion lead to the alteration



Anthocyanidins

Compound	R1	R2
Cyanidin	OH	OH
Cyanidin-3-*O*-glucoside	*O*-β-Glu	OH
Cyanidin-3-*O*-rutinoside	*O*-β-Glu-(6→1)-α-Rha	OH
Peonidin	OH	OCH_3
Peonidin-3-*O*-glucoside	*O*-β-Glu	OCH_3
Peonidin-3-*O*-rutinoside	*O*-β-Glu-(6→1)-α-Rha	OCH_3

Fig. 3.8 Chemical structures of procyanidins (B1-B5) and anthocyanidins from wild plant extracts

of phenolic compounds but with no significant changes in the total content of polyphenolics (Pinacho et al. 2015). The flowers of *P. spinosa* demonstrated significant antioxidant potential, pro-inflammatory enzymes' (lipoxygenase and hyaluronidase) inhibitory activity, with the protection of human plasma components against peroxynitrite-induced damage (Marchelak et al. 2017). The flower extracts showed scavenging effects toward harmful reactive oxygen species such as $OH^{\cdot}$, O_2^-, H_2O_2, $NO^{\bullet}$, $ONOO^-$, and HOCl, along with their phenolic metabolites (Marchelak et al. 2019). Regarding the antioxidant mechanism of action of *P. spinosa* flower extracts and phenolic metabolites, the same research group recently reported the amelioration of fibrinogen changes under peroxynitrite-induced oxidative stress (Marchelak et al. 2021). They showed that low molecular weight blackthorn polyphenolic compounds were the most responsible for the protection of fibrinogen and other plasma components.

It was recently reported that *P. spinosa* leaf extracts (aqueous and ethanol) showed prominent antioxidant, antimicrobial, and cytotoxic properties on malignant cell lines: HeLa, K562, and MDA-MB-453 (Veličković et al. 2021). In addition to this, they exerted significant inhibition of α-amylase and α-glucosidase enzymes showing the potential antidiabetic activity. The cytotoxic effects of blackthorn flower extract were proven by Murati et al. (2019) in non-neoplastic hepatocytes and hepatoblastoma cells with cell death primarily through necrosis. The *P. spinosa* drupes also showed cytotoxic activity against in vitro 3D and in vivo colon cancer models. Flower extract also showed significant amelioration in oxidative status of C57/BL6 mice in an in vivo experiment (Balta et al. 2020) affecting the levels of internal antioxidants such as catalase, superoxide dismutase, reduced glutathione, and levels of tissue lipid oxidation.

The biological benefits of blackthorn branch extract were applied in the gel emulsion for the incorporation in beef patties (Alejandre et al. 2019). The extract enhanced the antioxidant benefits, and it was able to inhibit lipid peroxidation in beef patties. Moreover, Gironés-Vilaplana et al. (2012) tested adding lyophilized blackthorn fruits to lemon juice toward DPPH, superoxide radical, hydroxyl radical, and hypochlorous acid scavenging activity and additionally on AChE and BChE inhibition. It showed high activity considering a significant level of bioactive anthocyanins as well as other important polyphenolics quantified in blackthorn fruits. Modern aspects of application of *P. spinosa* fruits may be based on the use of biomimetic nanoparticles loaded with the extract (Tiboni et al. 2021). Multiple benefits of nanoparticle synthesis are reflected, not only in increased bioavailability and biocompatibility but also in the potential to be accumulated in specific tissue exerting their biological activity. Tiboni et al. (2021) recently showed excellent properties of *P. spinosa* fruit nanoparticles in wound-healing activity accompanied by increased anti-inflammatory effects. Although there are numerous data regarding the positive effects of blackthorn on human health, there is still enough space to evaluate its most efficient form, to expand research in many directions, and especially to consider new ways of application for better utilization and higher bioactivity. The overview of major chemical constituents, secondary metabolites, and bioactivity of blackthorn is presented in Fig. 3.9.

***Cornelian cherry* (*Cornus mas* L.)** ethnomedicinal use has been well-known for more than 1000 years in different regions of Europe and Asia. The most used preparations in traditional medicine from *C. mas* are made from fruits, but there are also some galenic formulations prepared from flowers, leaves, and fruit stones of *C. mas* (Dinda et al. 2016; Przybylska et al. 2020). In ethnobotanical studies, Cornelian cherry is reported as the most commonly used medicine for gastrointestinal disorders in many countries. Especially the preparations made from Cornelian cherry are reported to act against diarrhea and colitis (Dinda et al. 2016; Süntar et al. 2020). There are also some traditional medicines prepared from *C. mas* for

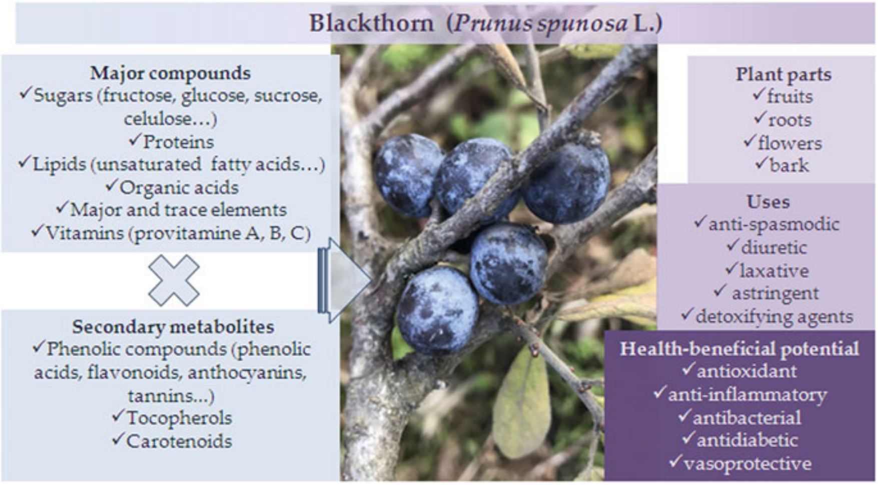


Fig. 3.9 Chemical composition and health-promoting properties of blackthorn

inflammatory bowel disease, sore throats, wound healing, stomach ulcers, fever, malaria, and kidney stones (Dinda et al. 2016). According to literature reports, Cornelian cherry fruit has exhibited anti-inflammatory, antimicrobial, antioxidant, antidiabetic, and nephron-, hepato-, cardio-, and neuroprotective activities in pharmacological studies (Bayram and Arda Ozturkcan 2020; Süntar et al. 2020; Nowak et al. 2021). Considering the wide range of traditional use and abundant evidence for pharmacological effects of *C. mas* fruits, there are also researches based on their application as ingredients of some functional foods (Szczepaniak et al. 2019). The literature search about biological properties of *C. mas* showed a thorough evaluation of different preparations (whole fruits, extracts, juice, or isolated compounds) from *C. mas* in in vitro, in vivo, toxicological, and clinical studies, as well as nanotechnological application.

The most studied biological activity of *C. mas* is its antioxidant potential using different in vitro methods including free radical scavenging activities, reducing antioxidant capacity, β-carotene bleaching properties, and antioxidant activity in the lipid system (Dinda et al. 2016; Szczepaniak et al. 2019; Tiptiri-Kourpeti et al. 2019; Bayram and Arda Ozturkcan 2020; Przybylska et al. 2020; Moussouni et al. 2020; Blagojević et al. 2021). The fruit extracts were the subject of the largest number of studies dealing with antioxidant potential of *C. mas* and showed remarkable antioxidant characteristics of this fruit from different areas of Europe and Asia. Some fruit extracts of *C. mas* showed antioxidant activity comparable to activities of synthetic standard antioxidants, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) (Szczepaniak et al. 2019; Bayram and Arda Ozturkcan 2020). This property of Cornelian cherry is related to high phenolic and vitamin C content in the studied extracts (Dinda et al. 2016; Szczepaniak et al. 2019; Blagojević et al. 2021; Mishra et al. 2017). In addition to the antioxidant activity of the fruit, it has also been found that *C. mas* leaves (Dinda et al. 2016; Szczepaniak et al. 2019; Grygorieva et al. 2020; Efenberger-Szmechtyk et al. 2021a, b) and stones (Przybylska et al. 2020) possess antioxidant properties.

Among in vitro studies conducted to determine the biological properties of *C. mas*, some researches focused on its antimicrobial properties. The extracts from fruit and leaves of *C. mas* showed the potential to inhibit the growth of different pathogenic bacteria and fungi (Dinda et al. 2016; Szczepaniak et al. 2019; Bayram and Arda Ozturkcan 2020; Efenberger-Szmechtyk et al. 2020a; Savaş et al. 2020; Efenberger-Szmechtyk et al. 2021a, b). Recent study showed that *C. mas* leaf extract possessed the most potent antimicrobial activities compared with *Aronia melanocarpa* (black chokeberry) and *Chaenomeles superba* leaf extracts (Efenberger-Szmechtyk et al. 2020a). The Cornelian cherry leaf extract also showed potential for use as a natural preservative in pork meat products extending their shelf life (Efenberger-Szmechtyk et al. 2021a, b).

The extracts of different parts of *C. mas* displayed cytotoxic activity toward some cancer cell lines (Dinda et al. 2016; Tiptiri-Kourpeti et al. 2019; Bayram and Arda Ozturkcan 2020). For example, *C. mas* leaf aqueous extract caused morphological changes and DNA damage in the Caco-2 cells with an IC_{50} value of 0.6% (Efenberger-Szmechtyk et al. 2020b). In another study, *C. mas* juice showed pronounced antiproliferative activity against HepG2 human cancer cells (IC_{50} 0.08%) (Tiptiri-Kourpeti et al. 2019). The study conducted by Popović et al. (2021) showed low cytotoxicity of 50% ethanol *C. mas* fruit extract. Cornelian cherry fruit extract was successfully applied for green synthesis of gold nanoparticles with low cytotoxic activity against human skin (Perde-Schrepler et al. 2016), while biosynthesized gold and silver nanoparticles using fruit extract showed non-cytotoxicity to normal oral cells, but induced cell death of dysplastic cells (Baldea et al. 2019). Gold nanoparticles synthesized using Cornelian cherry fruit extract and luteolin, in a study published by Domsa et al. (2020), showed possibility to modulate oxidative stress and inflammation process on Caco-2 cells treated with gliadin simulating celiac disease. This use of *C. mas* in the preparation of nanomaterials also shows the possibility of its application in an environmentally friendly synthesis and production of nanoparticles with capping molecules that make them less toxic to normal cells.

Cornelian cherry extracts were evaluated as potential inhibitors of some enzymes in vitro. The fruit extract showed the ability to inhibit α-glucosidase suggesting its potential application in the prevention and treatment of type 2 diabetes (Blagojević et al. 2021; Szczepaniak et al. 2021a). Also, *C. mas* flower infusion possesses an inhibitory effect of aldose reductase which reduces glucose to sorbitol under hyperglycemic conditions contributing to the development of chronic diabetic complications (Forman et al. 2020). Fruit extract was described as an arginase inhibitor which has an important role in normal vascular function (Bujor et al. 2019). It has also been proven that Cornelian cherry fruit extract may inhibit the TAS2R3 and TAS2R13 bitter taste receptors and can be effectively applied for masking the bitter taste of probiotic dark chocolate (Szczepaniak et al. 2021b).

Aside from in vitro studies, there are in vivo, clinical, and toxicological studies, as well as studies about justification of ethnomedicinal uses of *C. mas*. The latest results showed that various preparations from *C. mas* or its extracts may reduce oxidative stress in mice with tumor developed by Ehrlich ascites tumor cell injection (Yilmaz et al. 2020a, b) and in rats treated with a chemotherapeutic agent (Zarei and Shahrooz 2019; Mesgari Abbasi et al. 2020). There is also evidence for Cornelian cherry influence on tumor proliferation in mice (Yilmaz et al. 2020a, b). As in vitro studies showed the potential of Cornelian cherry type 2 diabetes treatment, in vivo studies demonstrated antidiabetic effects of Cornelian cherries' extracts and improvement of diabetes manifestations (Capcarova et al. 2019; Dzydzan et al. 2019, 2020; Omelka et al. 2020). A recent study justified the ethnomedical use of Cornelian cherry for the treatment of ulcerative colitis (Süntar et al. 2020). *C. mas* extracts were also reported to possess anti-inflammatory effects (Szczepaniak et al. 2019; Bayram and Arda Ozturkcan 2020), positive influence on hypercholesterolemia (Nowak et al. 2021), and atherosclerosis (Lietava et al. 2019) in some in vivo studies.

Only a few clinical studies have included *C. mas* supplements. Human clinical studies showed that consumption of Cornelian cherry fruits may prevent hyperlipidemia (Asgary et al. 2013) and hyperglycemia in humans (Soltani et al. 2015). A randomized clinical trial that included effects of *C. mas* extract supplementation (900 mg daily) in postmenopausal women showed that this extract may improve some aspects of postmenopausal complications (bone resorption, osteoporosis, lipid profile, and glycemic indices) (Gholamrezayi et al. 2019; Aryaeian et al. 2021).

According to all literature data about *C. mas* biological activities and potential use, there is a need for further research for its application as a food supplement or raw material for the pharmaceutical industry, especially clinical trials. Also, it seems that more research should be directed toward the use of waste products which remain after *C. mas* fruit exploitation. The overview of major chemical constituents, secondary metabolites, and bioactivity of Cornelian cherry is presented in Fig. 3.10.

***Dog rose* (*Rosa canina* L.)** is a wild plant, well-known as a component of traditional medicine in Europe, Asia, and North America. The pseudo fruits of *R. canina* (the rose hips) are often used as food or medicine in many countries. The health benefit of the rose hip is attributed to its high vitamin C and polyphenolic content (Fan et al. 2014; Patel et al. 2017). Usually, decoctions of *R. canina* hips are used as remedies for the treatment and prevention of cold and flu, as well as for infectious diseases, inflammation, stomach disorders, arthritis, and rheumatoid disorders in traditional folk medicine (Chrubasik et al. 2008; Patel 2013; Živković et al. 2020, 2021). *R. canina* hip seeds are a valuable source of oil popular in natural skin care products. Rose hip seed oil is used in cosmetic preparation as a skin vitalizing agent, usually for reducing scars, wrinkles, and pigmentation on the skin. There are several commercial products based on dog rose hips on the market such as supplements, rose hip tea bags, or cosmetic preparations (Patel 2013). The biological activities of *R. canina* reported so far included antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and osteoarthritis treatments, as well as the use for

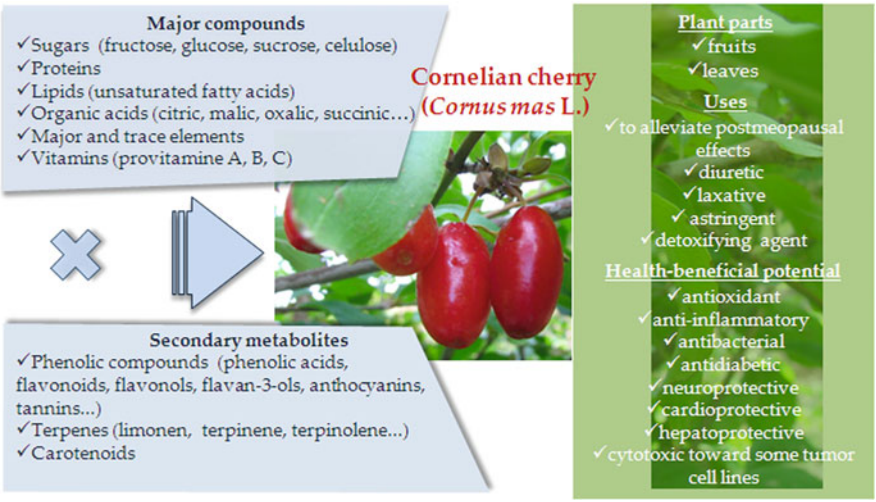


Fig. 3.10 Chemical composition and health-promoting properties of Cornelian cherry

immunomodulation, and in cosmetics (Chrubasik et al. 2008; Patel 2013, 2017; Gruenwald et al. 2019).

R. canina hips displayed strong antioxidant activity in vitro in radical scavenging methods similar to the antioxidant activity of well-known antioxidants, quercetin and Trolox (Fetni et al. 2020a, b; Rovná et al. 2020). The dog rose hips in comparative studies proved to be one of the most potent antioxidants among different wild fruits and berries (Ungurianu et al. 2019; Hendrich et al. 2020; Ouerghemmi et al. 2020; Smanalieva et al. 2020; Tabaszewska and Najgebauer-Lejko 2020; Moldovan et al. 2021). The animal study also showed the antioxidant potential of dog rose hips lowering oxidative manifestations on vancomycin-induced nephrotoxicity in rats (Sadeghi et al. 2021). The antioxidant potential in vitro also showed pasteurized dog rose nectar (Atalar et al. 2020) and flower extract (Demasi et al. 2021), while *R. canina* distilled water improved antioxidant and biochemical parameters in tamoxifen-treated male Wistar rats (Karimimojahed et al. 2020). Interestingly, dog rose hip powder showed the possibility to improve oxidative and microbiological stability of gingerbread (Ghendov-Mosanu et al. 2020). *R. canina* hip extracts showed moderate antimicrobial properties in different studies especially against *E. coli* growth (Hendrich et al. 2020; Rovná et al. 2020). The extracts of dog rose hips showed no cytotoxicity to some carcinoma cells in concentrations up to 400 µg/mL, as well as inhibition of NO production in mouse macrophage-like cell line RAW 264.7 (Moldovan et al. 2021). Fetni et al. (2020a) reported that methanolic extract of dog rose hips applied at a concentration of 250 µg/mL significantly reduced the growth of HepG2 and SH-SY5Y cancer cells. The determination of biological activities of *R. canina* hips in vitro demonstrated that hip extract possessed inhibitory activity of α-glucosidase (IC_{50} 0.54 mg/mL) (Moldovan et al. 2021) and lipoxygenase (Hendrich et al. 2020).

In recent studies, *R. canina* hip extracts were described as agents for the biosynthesis of metal nanoparticles. Green synthesized nanoparticles obtained using *R. canina* were utilized for different applications, e.g., palladium and copper oxide nanoparticles were successfully applied as recyclable nano-catalysts in organic synthesis reactions (Hekmati 2019; Hemmati et al. 2019), and silver and gold nanoparticles showed antimicrobial activity (Gulbagca et al. 2019; Cardoso-Avila et al. 2021), catalytic degradation potential (Cardoso-Avila et al. 2021), and antioxidant potential (Gulbagca et al. 2019).

One of the newest studies demonstrated that methanol extract of dog rose hips is nontoxic in subchronic intraperitoneal toxicity examination on female Wistar albino rats. The methanol extract possessed $LD_{50} > 5000$ mg/kg of body weight, and that result classified this extract as nontoxic. Also, there were no statistically significant differences in biochemical and hematological parameters between the groups treated with different doses of this extract and the untreated group (Fetni et al. 2020a).

Several recent studies demonstrated the positive influence of dog rose hips on diabetic management. Sajadimajd et al. (2020) proved that oligosaccharides from *R. canina* hips improved streptozotocin (STZ)-induced diabetic condition with an increase in the expression of autophagy markers in rat pancreatic Rin-5F cells in vitro. Also, in vivo studies showed significant effects of oligosaccharides from *R. canina* hips on lowering the glucose levels in SZT-induced diabetic rats (Bahrami et al. 2020a, b; Rahimi et al. 2020). One of the most studied biological activities of dog rose in in vivo and clinical studies is its use in osteoarthritis treatment. Several clinical studies showed that dog rose hips (5 g of powder/day) successfully reduced pain associated with osteoarthritis. This beneficial effect of dog rose on osteoarthritis can be attributed to its powerful antioxidant and anti-inflammatory effects (Patel 2013; Cheng et al. 2016; Gruenwald et al. 2019). *R. canina* hip extract has potential for application in cosmetics and dermatology. It has been demonstrated that dog rose hip extract inhibited melanogenesis and reduced skin pigmentation (Patel 2013, 2017).

R. canina has great potential for the development of food supplements and pharmaceutical products, but additional research is needed, especially in vivo studies and randomized controlled clinical trials for more evidence about its biological activities determined in in vitro and animal studies. The most important compounds and bioactivity of rose dog are summarized in Fig. 3.11.

Hawthorn (*Crataegus monogyna* Jacq.) has been continuously used in traditional medicine for centuries. The first written mention of hawthorn was in Dioscorides *De Materia Medica* in the first century where it was described to be used against cardiac disorders (Nabavi et al. 2015). The beneficial effects of hawthorn leaves, flowers, and fruits in the prevention and treatment of heart diseases are mainly due to their hypotensive, antispasmodic, cardiotonic, anti-hyperlipidemic, and anti-atherosclerotic activities (Pawlaczyk-Graja 2018). It was used for relieving symptoms of arrhythmia and hypertension; to treat chronic heart failure, angina pectoris, and myocardial injuries; and to improve blood circulation (Abuashwashi et al. 2016; Bardakci et al. 2019). Abuashwashi et al. (2016) reported that in recent

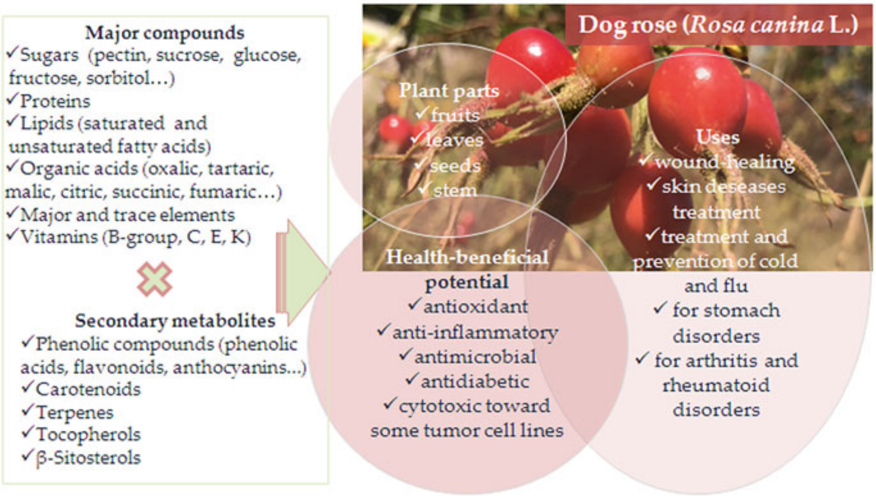


Fig. 3.11 Chemical composition and health-promoting properties of dog rose

years arose a novel use of hawthorn in the treatment of temporary nervous cardiac complaint symptoms.

Besides hawthorn's cardioprotective properties, it also finds a role in the treatment of arthritis, insomnia, gall bladder disease, and diarrhea (Barros et al. 2011; Pawlaczyk-Graja 2018). The hawthorn was traditionally used in the treatment of respiratory tract disorders as well as for relieving the symptoms of menopause (Barros et al. 2011).

The nutritious benefits of hawthorn fruits (berries) made its use as a source of vitamins and minerals even more worthwhile for improving general health. In that sense, the berries were and still are the constituents of numerous food products, such as jams, jellies, different drinks, and wine, or in the form of canned fruit (Barros et al. 2011; Nabavi et al. 2015).

The fruits are also used in the treatment of gout, depression, kidney stones, and intestinal problems, as a diuretic, and for the stimulation of digestion (Keating et al. 2014; Nabavi et al. 2015; Bardakci et al. 2019).

The exact active compounds in *C. monogyna* have not been elucidated with certainty so far. Most likely it could be simultaneous beneficial effects on cardiovascular system of the mixture of many different phytochemicals, including polyphenolic compounds (flavonoids) and triterpenoids from hawthorn leaves and flowers (Momekov and Benbassat 2013). The studies revealed that hawthorn possesses a positive inotropic effect, cardioprotective effects, and an antiarrhythmic effect; it increases coronary blood flow, etc. Many of these pharmacologically important activities are directly dependent on the action of flavonoids, particularly the inhibition of Na^+/K^+ adenosine triphosphatase (Na^+/K^+ pump) that leads to the positive inotropic effect (Momekov and Benbassat 2013). Moreover, the antioxidant activity of hawthorn's constituents can have profound impact on the body and vital effect on the cardiovascular system.

Barros et al. (2011) explored the antioxidant properties of *C. monogyna* flower buds, flowers, and fruits (unripe, ripened, and overripened) by radical scavenging effects (DPPH), reducing power, and inhibition of lipid peroxidation assays. The unripe fruits were the most active compared to other extracts, especially compared to the overripened fruits with the lowest antioxidant potential. Generally, the higher production of phenolic compounds in unripe fruits due to plant stress response can be connected to the higher bioactivity of the fruit extract in this stage of fruit maturity (Barros et al. 2011).

The methanolic extract of *C. monogyna* aerial parts and its fractions were tested in DPPH, and β-carotene bleaching assay showed strong antioxidant activity (Coimbra et al. 2020). The detection of quercetin and vitexin derivatives, along with phenolic acids and procyanidin polymers with high antioxidant level in tested material, contributed to overall bioactivity. Leaves of *C. monogyna* also showed significant ORAC, TRAP, and HORAC antioxidant activity (1405 μmol TE/g, 1301 μmol TE/g, and 882 μmol GAE/g, respectively), but lower compared to other Rosacea plants tested in this study (Denev et al. 2014).

The *C. monogyna* fruit extracts showed the highest antioxidant activity (using DPPH, FRAP, CUPRAC, and total antioxidant capacity tests) compared to four other *Crataegus* species from Turkey (*C. rhipidophylla*, *C. pontica*, *C. orientalis*, and *C. turcicus*) (Bardakci et al. 2019). Since the *C. monogyna* contained the highest total proanthocyanidin, hyperoside, and chlorogenic acid content, all polyphenolic compounds with various mechanisms of reaction with free radicals, no wonder it has pronounced antioxidant potential. Abuashwashi et al. (2016) showed that samples of *C. monogyna* from different geographical origins in Spain demonstrated significant antioxidant activity in ORAC (1.32–2.76 μmol TE/mg) and DPPH (IC_{50} 2.67–3.76 μg/mL) assays with positive correlation between antioxidant activity and phenolic content. Flavonoids and phenolic acids detected in *C. monogyna*, like kaempferol, quercitrin, rutin, hesperetin, arbutin gentisic acid, and chlorogenic acid, significantly contributed to general antioxidant potential of *C. monogyna* aerial part extracts from Spain (Abuashwashi et al. 2016). The extracts from buds and sprouts of *C. monogyna* from various locations in Italy also exerted antioxidant activity in ABTS radical cation assay with values ranging from 31.48 to 147.25 mg Trolox eq./kg (Ferioli et al. 2020). The phenolic composition of those hawthorn bud and sprout samples showed high content of phenolic acids, with prevalence of caffeic and neochlorogenic acid, and flavonoids, of which dominant were vitexin and its derivative vitexin-2″-*O*-(4‴-*O*-acetyl)-rhamnoside, and lower amount of flavonols where the most abundant were hyperoside and isoquercitrin (Ferioli et al. 2020); all of them are scientifically proven antioxidants with various modes of action (Heim et al. 2002; Taofiq et al. 2017).

In addition to the antioxidant activity of hawthorn, the effect of the digestive process on its antioxidant capacity was also reported. Keating et al. (2014) evaluated the influence of simulated digestion on the antioxidant activity of hawthorn preparations (infusion, decoction, berry tincture, and leaf and flower tincture) and phenolic standards commonly present in hawthorn. They showed that total phenolic content after exposure to simulated gastrointestinal fluid decreased only in infusions, but decoction showed the lowest antioxidant potential after digestion. In other preparations, activity decreased, but not significantly. On the other hand, based on the previous reports that hawthorn had extract poor bioavailability after oral administration regarding the low water solubility of flavonoids, Lucconi et al. (2014) had a goal to prepare the microparticulate delivery system of hawthorn methanolic extracts (fruits and flowering tops) with intestinal delivery for oral formations. The microencapsulated and microparticulated systems obtained from hawthorn flowers showed preservation of antioxidant activity during in vitro digestion and in intestinal conditions.

Hendrich et al. (2020) recently reported a high antioxidant potential of hawthorn fruit methanol and water extracts from Poland and showed their effectiveness in Trolox equivalent antioxidant activity (TEAA), DPPH, and ABTS assays, but also the inhibition of lipid peroxidation and lipoxygenase-1 (LOX-1) activity. The capacity of hawthorn to inhibit the process of lipid oxidation was used to prevent increasing of TBARS and volatile carbonyl concentration and odor in pork patties (Akcan et al. 2017). Also, the hawthorn extracts were added to prevent lipid oxidation and oxymyoglobin oxidation in bovine muscle homogenates (Shortle et al. 2014). In both cases, hawthorn showed significant potential against lipid oxidation in meat and proved its use as an antioxidant ingredient for the manufacturing of high-quality meat products with prolonged shelf life.

Another possible benefit of hawthorn extracts is their antimicrobial potential. Hawthorn leaves showed moderate antibacterial effects against *Staphylococcus aureus* (Denev et al. 2014), *Bacillus cereus*, and *Acinetobacter baumannii* (Coimbra et al. 2020), while it was much more efficient against different *Candida* spp. (Coimbra et al. 2020). Nunes et al. (2017), besides antioxidant properties of hawthorn extracts, evaluated also their antimicrobial and cytotoxic properties. The extracts were able to highly inhibit the growth of *Listeria monocytogenes* and in moderate manner *S. aureus*. Nevertheless, *C. monogyna* extracts had protective effect on normal fibroblasts, which can be associated with the high content of zinc along with the presence of phenolic compounds, like chlorogenic and neochlorogenic acids, quercetin, and vitexin. The effects of hawthorn flower buds and fruit extracts (unripened, ripened, and overripened) were tested on human tumor cell lines (MCF7, breast carcinoma; NCI-H460, non-small lung cancer; HeLa, cervical carcinoma; HepG$_2$, hepatocellular carcinoma) (Rodrigues et al. 2012). It was shown that the most active extracts on all cell lines were those of flower buds and unripened fruit of hawthorn, connecting their activity to the chemical composition. In flower buds, the most dominant were phenolic acids 3-*O*- and 5-*O*-caffeoylquinic acids, derivatives of quercetin and apigenin, and (-)-epicatechin, while in fruits, besides mentioned compounds, procyanidin polymers were the most abundant. Sahin-Yaglıoglu et al. (2016) also confirmed the antiproliferative effects of hawthorn flowers against rat brain tumor (C6) and human cervical cancer (HeLa) cell lines. The silver and gold nanoparticles were synthesized with *C. monogyna* leaf extract, and particularly AgNP showed significant antimicrobial activity against a panel of pathogenic microorganisms, e.g., *E. faecalis*, *A. baumannii*, *P. aeruginosa*, *P. mirabilis*, *S. aureus*, *E. coli*, and *K. pneumoniae* (Shirzadi-Ahodashti et al. 2020). Both AgNP and AuNP displayed cytotoxic properties against AGS and MCF-7 cells via apoptotic mechanism with increased ROS production.

The anticoagulant activity of hawthorn flower and fruit extracts was analyzed using activated partial thromboplastin time (aPTT) and prothrombin time (PT) bioassays in vitro. The extracts showed quite high anticoagulant activity with prolongation of the plasma coagulation process (Pawlaczyk-Graja 2018). Those results were another proof that hawthorn can be recommended for the prevention and treatment of cardiovascular diseases. The antithrombotic activity of hawthorn ethanolic extract was demonstrated in vivo in carrageenan-induced tail thrombosis model by Arslan et al. (2015). The activity was ascribed to the high content of proanthocyanidins which exert antithrombotic effects and promote vascular function. Another in vivo experiment showed hyperglycemic activity of hawthorn fruit extract with alleviation of oxidative stress and protection of pancreatic tissue in streptozotocin-induced diabetic rats (Chahardahcharic and Setorki 2018). The immunomodulatory effects of *C. monogyna* extract were also demonstrated in vivo in BALB/c mice (Lis et al. 2020), whereby the authors came to a conclusion that hawthorn modulates the lymphocyte subsets and stimulates the humoral immune response so it can be used as an immunomodulator. The predominant compounds and bioactivity of hawthorn are summarized in Fig. 3.12.

Wild fruits are part of both tradition and religion and are closely connected with the customs of many people. Ever-increasing interest for high-quality food products associated with health-beneficial effects highly encourages researchers to intensively study natural products. Wild-growing fruit plants contain a wide assortment of

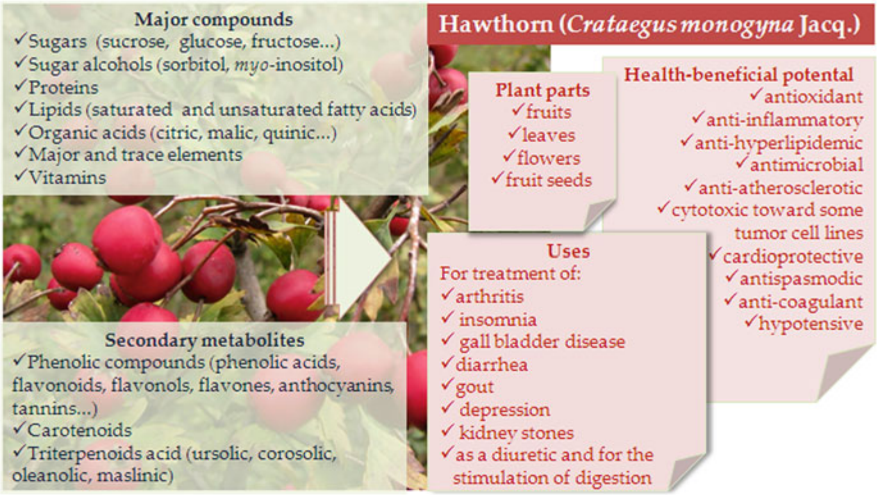


Fig. 3.12 Chemical composition and health-promoting properties of hawthorn

nutritional and health-promoting compounds that are important in the pharmaceutical industry. Local, traditional, healthy, and functional foods are just some attributes that attract consumers when purchasing food. Recently, originality and authenticity of food products became highly important categories to consumers as well as producers. In addition, the interest in collection of the fruits of wild plants is gaining attention from the economic aspects.

3.5 Conclusion

Due to the powerful health-promoting properties of wild fruits, blackthorn, Cornelian cherry, dog rose, and hawthorn are well-known in traditional medicine of many European and Asian countries. The plants are a good source of nutritionally valuable and health-beneficial compounds. Among them, secondary metabolites, especially polyphenolic compounds, mostly contribute to the bioactivity of fruits, leaves, flowers, and twig extracts of these plants, which is reflected in the anti-inflammatory, antimicrobial, antioxidant, antidiabetic, neuroprotective, and other activities. In traditional folk medicine, these plants are used as remedies for the prevention of colds and the flu, as well as for the treatment of various health disorders. But, in the light of climate change, wild fruits will be endangered in the future, because, first of all, their habitat will experience a decrease. Climate change can diminish wild fruit fields or shift them to other locations which can influence local populations. Changing flora can lead to the appearance of invasive species which can influence the pollination of the native wild fruit-producing plants.

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Chapter 4
Favorable Impacts of Drought Stress on the Quality of Medicinal Plants: Improvement of Composition and Content of Their Natural Products

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Abstract This review deals with 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, grown and cultivated in moderate climates. Basic biochemical reflections display that drought stress and the related metabolic changes are responsible for the higher natural product accumulation in plants grown in semi-arid regions (Selmar et al., Environmental challenges and medicinal plants. Springer, New York, 2017). Related data from the literature on the effect of drought on the concentration of natural products are compiled, and the relevant aspects are also outlined.

A thorough reflection on this issue emphasizes the necessity to differentiate decidedly between "concentration" and "content" of natural products (Paulsen and Selmar, J Appl Bot Food Qual 89:287–289, 2016). Next, basic plant physiological coherences expound that in principle there are three causes for the observed changes

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in the concentration of natural products (Yahyazadeh et al., Phytochemistry 152: 204–212, 2018).

- **Changes in the reference values**: Drought stress-induced enhancement of the natural products' concentration is due to a reduced production of biomass in the stressed plants.
- **Passive shift**: Under drought stress, the stomata are closed. The shortage of CO_2 evokes a strongly elevated level of $NADPH+H^+$. Accordingly, all processes consuming $NADPH+H^+$ are boosted, although enzyme activities were not changed.
- **Active upregulation**: The activity of enzymes responsible for the biosynthesis of natural products is enhanced by increased gene expression.

Apart from these quantitative changes, in some cases, also the spectrum of specialized metabolites is altered in response to the stress situation (Abouzeid et al., J Nat Prod 80:2905–2909, 2017).

Based on these physiological considerations, practical aspects for the application of drought stress to deliberately improve the quality of spice and medicinal plants are displayed (Kleinwächter and Selmar, Physiological mechanisms and adaptation strategies in plants under changing environment. Springer, New York, 2014; Kleinwächter and Selmar, Agron Sustain Dev 35:121–131, 2015).

Keywords Drought stress · Specialized metabolites · Over-reduced state · Spice plants · Medicinal plants · Phytomodificines

4.1 Introduction

The lack of irrigation water represents the most severe problem in agriculture. The resulting water deficiency provokes aridity, which manifests various drought stress symptoms, i.e., retardation of plant growth and a severe decrease in biomass production. In consequence, the yield of crop plants is massively reduced (Jaleel et al. 2009). However, in contrast to such adverse and unfavorable effects of water shortage, in some cases, aridity might likewise be advantageous for the production of some plant-derived commodities, i.e., the cultivation of spice and medicinal plants (Kleinwächter et al. 2015). We all are aware that aromatic plants grown in semi-arid areas such as the Mediterranean regions generally are much more aroma-intensive than equivalent plants, which had been cultivated in moderate climates (e.g., Selmar et al. 2017). Actually, in daily life, this well-known phenomenon is explained by the simple but non-scientific statement: "In Southern Europe, the plants are exposed to far more sunlight, resulting in increased rates of biosynthesis." Although, at first sight, this misinterpretation might appear to be consistent, plant biologists are aware that even in Central Europe, for plants growing in open areas without any shade, sunlight generally is not a limiting factor for their growth (Wilhelm and Selmar 2011). On the contrary, most plants absorb much more light energy by their leaves than they require for their photosynthetic CO_2 fixation (Wilhelm and Selmar 2011).

In consequence, there must be another explanation for the phenomenon of enhanced accumulation of natural products in plants growing in Southern Europe. In this context, drought stress is of particular interest (Selmar and Kleinwächter 2013a). This chapter outlines the various levels of pertinence and displays the basic scientific background of the entire syndrome. Indeed, in the past, these coherences have not been considered adequately, while related contemplations are well established (e.g., Kleinwächter and Selmar 2014, 2015).

4.2 Drought Stress Frequently Entails an Enhanced Concentration of Natural Products

We all are aware that environmental conditions strongly influence the growth and development of plants by affecting their metabolism and their metabolic capacity, respectively (Bohnert et al. 1995). These coherences also apply to the biosynthesis and accumulation of natural products. In this context, numerous studies on the impact of various factors like temperature, light regime, nutrient supply, etc. on secondary metabolism have been conducted (for review, see Gershenzon 1984; Falk et al. 2007; Das and Bhattacharya 2016). Accordingly, there is no doubt that quite severe environmental conditions, such as various stress situations, which strongly impact the entire general metabolism (Sampaio et al. 2016; Bohnert et al. 1995), also alter the secondary metabolism.

With respect to biological stress, lots of papers had been published which describe the elicitation of natural products' synthesis in response to pathogen or herbivore attack (for review, see, e.g., Harborne 1988; Hahlbrock et al. 2003; Hartmann 2007; Namdeo 2007; Wink 2010). In this context, many molecular responses to these biotic stresses had been elucidated (e.g., Davies and Schwinn 2003; Zhao et al. 2005; Nascimento and Fett-Neto 2010; De Geyter et al. 2012). In the same manner, the molecular background for the stress-induced impact on general metabolism and, thus, on plant growth and development had been also studied intensively (Zhu 2002; Shinozaki and Yamaguchi-Shinozaki 2007; Baldoni et al. 2015). Additionally, a tremendous lot of papers deal with the impact of abiotic stresses on the secondary metabolism. However, the profound knowledge of the corresponding biological background is still limited (for review, see, e.g., Ramakrishna and Ravishankar 2011; Selmar and Kleinwächter 2013a, b; Kleinwächter and Selmar 2014).

Up to now, a large number of studies revealed that plants exposed to drought stress indeed accumulate higher concentrations of secondary metabolites than those cultivated under well-watered conditions (Table 4.1). These data clearly expound that the drought stress-related enhancement in the concentration of natural products is a quite common feature. These coherences obviously account more or less for all the different classes of natural products. In stressed plants, the enhanced concentration of simple as well as complex phenols is described. In the same manner, the

Table 4.1 Enhanced concentrations of natural products in drought-stressed plants

Phenolic compounds			
Helianthus annuus	Chlorogenic acid	Massive increase	del Moral (1972)
Prunus persica	Total phenols	Higher contents	Kubota et al. (1988)
Thymus capitatus	Total phenols	Higher contents	Delitala et al. (1986)
Pisum sativum	Flavonoids	Strong increase	Nogués et al. (1998)
Pisum sativum	Anthocyanins	Strong increase	Nogués et al. (1998)
Echinacea purpurea	Total phenols	Strong increase	Gray et al. (2003a)
Hypericum perforatum	Total phenols	Significant increase	Gray et al. (2003b)
Hypericum perforatum	Hyperoside	Slight increase	Gray et al. (2003b)
Hypericum perforatum	Rutin	Strong increase	Gray et al. (2003b)
Crataegus spp.	Chlorogenic acid	Massive increase	Kirakosyan et al. (2004)
Hypericum brasiliense	Total phenols	Strong increase	de Abreu and Mazzafera (2005)
Crataegus spp.	Catechins/epicatechins	Massive increase	Kirakosyan et al. (2004)
Hypericum brasiliense	Rutin, quercetin	Massive increase	de Abreu and Mazzafera (2005)
Hypericum brasiliense	Xanthones	Strong increase	de Abreu and Mazzafera (2005)
Camellia sinensis	Epicatechins	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)
Trachyspermum ammi	Total phenols	Strong increase	Azhar et al. (2011)
Labisia pumila	Total phenols	Significant increase	Jaafar et al. (2012)
Labisia pumila	Anthocyanin/flavonoids	Significant increase	Jaafar et al. (2012)
Triticum aestivum	Total phenols	Significant increase	Ma et al. (2014)
Ocimum basilicum	Methyleugenol	Significant increase	Abdollahi Mandoulakani et al. (2017)
Ocimum basilicum	Methylchavicol	Significant increase	Abdollahi Mandoulakani et al. (2017)
Scutellaria baicalensis	Baicalin	Strong increase	Cheng et al. (2018)
Achillea pachycephala	Various phenols	Significant increase	Gharibi et al. (2019)
Carica papaya	Various phenols	Significant increase	Espadas et al. (2019)
Melissa officinalis	Total phenols	Significant increase	Ahmadi et al. (2020)

(continued)

Table 4.1 (continued)

Olea europaea	Various flavanoids	Massive increase	Mechri et al. (2020)
Ocimum basilicum	Rosmarinic, caffeic acid	Significant increase	Zare et al. (2021)
Isoprenoids/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-Pinene, carene	Strong increase	Llusià and Peňuelas (1998)
Cistus monspeliensis	Caryophyllene	Enormous increase	Llusià and Peňuelas (1998)
Solanum tuberosum	Steroid alkaloids	Strong increase	Bejarano et al. (2000)
Satureja hortensis	Essential oils	Increase	Baher et al. (2002)
Picea abies	Monoterpenes	Strong increase	Turtola et al. (2003)
Pinus sylvestris	Monoterpenes	Strong increase	Turtola et al. (2003)
Hypericum brasiliense	Betulinic acid	Strong increase	de Abreu and Mazzafera (2005)
Petroselinum crispum	Essential oils	Strong increase	Petropoulos et al. (2008)
Salvia officinalis	Essential oils	Massive increase	Bettaieb et al. (2009)
Bupleurum chinense	Saikosaponin	Significant increase	Zhu et al. (2009)
Salvia officinalis	Monoterpenes	Strong increase	Nowak et al. (2010)
Scrophularia ningpoensis	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)
Prunella vulgaris	Triterpenes	Slight increase	Chen et al. (2011)
Glycyrrhiza glabra	Glycyrrhizin	Massive increase	Nasrollahi et al. (2014)
Thymus vulgaris	Monoterpenes	Increase	Kleinwächter et al. (2015)
Petroselinum crispum	Essential oils	Massive increase	Kleinwächter et al. (2015)
Thymus citriodorus	Geraniol	Massive increase	Tátrai et al. (2016)
Thymus citriodorus	Thymol	Massive increase	Tátrai et al. (2016)
Thymus citriodorus	Carvacrol	Massive increase	Tátrai et al. (2016)
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)
Ocimum basilicum	β-Myrcene	Significant increase	Abdollahi Mandoulakani et al. (2017)

(continued)

Table 4.1 (continued)

Ocimum basilicum	α-Bergamotene	Significant increase	Abdollahi Mandoulakani et al. (2017)
Salvia nemorosa L.	Essential oils	Significant increase	Bidabadi et al. (2020)
Andrographis paniculata	Andrographolides	Significant increase	Chen et al. (2020)
Bupleurum chinense	Saikosaponin	Significant increase	Yang et al. (2020)
Alkaloids			
Senecio longilobus	Pyrrolizidine alkaloids	Strong increase	Briske and Camp (1982)
Lupinus angustifolius	Quinolizidine 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 and Çebi (2010)
Capsicum spp.	Capsaicinoids	Massive increase	Phimchan et al. (2012)
Catharanthus roseus	Total alkaloids	Massive increase	Amirjani (2013)
Catharanthus roseus	Vincristine	Massive increase	Amirjani (2013)
Catharanthus roseus	Vinblastine	Massive increase	Amirjani (2013)
Chelidonium majus	Benzylisoquinolines	Increase	Kleinwächter et al. (2015)
Papaver somniferum	Benzylisoquinolines	Strong increase	Behnam et al. (2017)
Papaver bracteatum	Benzylisoquinolines	Significant increase	Behnam et al. (2017)
Papaver armeniacum	Benzylisoquinolines	Significant increase	Behnam et al. (2017)
Papaver argemone	Benzylisoquinolines	Strong increase	Behnam et al. (2017)
Chelidonium majus	Coptisine	Significant increase	Yahyazadeh et al. (2018)
Lupinus angustifolius	Quinolizidine alkaloids	Massive increase	Frick et al. (2018)
Lycoris aurea	Galanthamine, lycorine	Significant increase	Xiang and Cao (2020)
Catharanthus roseus	Total alkaloids	Significant increase	Yahyazadeh et al. (2021)

(continued)

Table 4.1 (continued)

Various classes			
Manihot esculenta	Cyanogenic glucosides	Strong increase	de Bruijn (1973)
Triglochin maritima	Cyanogenic glucosides	Strong increase	Majak et al. (1980)
Brassica napus	Glucosinolates	Massive increase	Jensen et al. (1996)
Coffea arabica	γ-Aminobutyric acid	Massive increase	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	Ballhorn et al. (2011)
Tropaeolum majus	Glucotropaeolin	Higher content	Bloem et al. (2014)
Brassica rapa	Glucosinolates	Significant increase	Park et al. (2021)

concentration of terpenes is also described to be higher in stressed plants than in the controls (Table 4.1). Furthermore, the concentrations of nitrogen-containing substances, such as alkaloids, cyanogenic glucosides, and glucosinolates, are also positively impacted by drought stress (Table 4.1). In consequence, there is no doubt that drought stress consistently enhances the concentration of secondary plant products (Selmar and Kleinwächter 2013a; Kleinwächter and Selmar 2014, 2015).

4.3 Why Is the Concentration of Natural Products Enhanced in Drought-Stressed Plants?

When comparing the concentrations of natural products in stressed and control plants, we always have to consider that drought severely reduces plant growth (Jaleel et al. 2009). Accordingly, stressed plants reveal far lesser biomass than the well-watered controls. Consequently, even when the rate of biosynthesis of natural products would not be affected, i.e., it is the same in stressed and control plants, their concentrations (on dry or fresh weight basis) indeed are elevated, just due to the lesser biomass of the stressed plants (Paulsen and Selmar 2016; Yahyazadeh et al. 2018). Accordingly, even when no change in the rate of biosynthesis takes place, due to the lower reference values, the concentration of natural products is enhanced. This effect is classified as “indirect” (Fig. 4.1; Yahyazadeh et al. 2018). However, to evaluate whether or not—in addition to this indirect effect—also the biosynthesis of

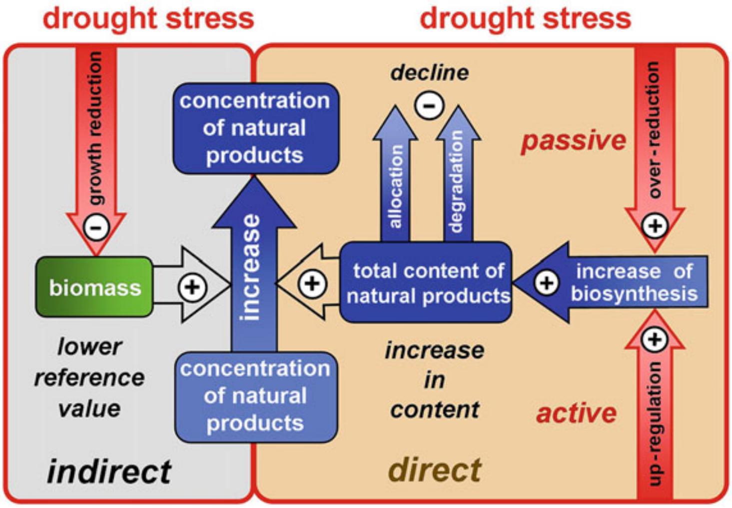

Fig. 4.1 The stress-related increase of natural products' concentration results from the interaction of various processes and factors. When thoroughly investigating any stress-related increase of natural product concentration, it is indispensable to differentiate decidedly between "concentration" and "content" natural products (Paulsen and Selmar 2016). In principle, there are three reasons for the observed phenomena: an indirect effect due to the reduced production of biomass in the stressed plants (Yahyazadeh et al. 2018). In this case, the increased concentration results just from changes in the reference values without any enhancements of the content of natural products. Alternatively, the concentration increase could be due to a higher content of specialized metabolites, denoted as direct effect (Yahyazadeh et al. 2018). In principle, there are two options for such direct enhancement. It could be caused either by a passive shift, as a consequence of the strongly elevated concentration of NADPH+H$^+$, which boosts the biosynthesis of highly reduced specialized metabolites (Selmar and Kleinwächter 2013b), or by an active upregulation of the enzymes responsible for the biosynthesis of natural products (Yahyazadeh et al. 2021)

natural products is directly influenced by the stress situation, it becomes necessary to determine the entire amount of a certain specialized metabolite per plant. Actually, various analyses expounded that stress enhances indeed the rate of biosynthesis (see next paragraph). In consequence, this "direct" impact is contraposed to the "indirect" enhancement of the concentration due to the lower reference values (Fig. 4.1; Yahyazadeh et al. 2018). Indeed, in all cases where the total amount of a certain specialized metabolite (the product of biomass and concentration) is higher in the stressed plants in comparison to that of the well-watered controls, such direct effect (i.e., an enhanced rate of biosynthesis) can be verified easily. However, when the duration of the stress is quite long and the massive growth reduction provoked a far lesser biomass of the stressed plants, a solid evaluation of direct effect might sometimes be quite problematic. In particular, the putative stress-related increase in the rate biosynthesis does not inevitably always result in a higher amount of a certain specialized metabolite, since it is over-compensated by the concurrently growth reduction. In other words, the product of biomass and concentration is lower in stressed plants although the concentration is enhanced (Paulsen and Selmar

2016). Albeit, the situation becomes even more complex, when considering that the newly synthesized natural products are produced in a far lesser quantum of biomass. A detailed discourse on this set of problems is given by Paulsen and Selmar (2016) and Yahyazadeh et al. (2021). Unfortunately, these coherences frequently are ignored in the literature, and a stress-related increase in the concentration of natural products is abundantly misinterpreted as sound verification of stress-induced increase of biosynthesis. Notwithstanding, many examples clearly expound that the overall content of natural products, i.e., the amount per entire plant, indeed is higher in plants exposed to drought in comparison to non-stressed individuals (see next chapter).

4.4 The Overall Content of Natural Products Is Increased by Drought Stress

As outlined above, most studies on the stress-related impact on the accumulation of natural products focus on the concentration of the active compounds and do not consider the overall plant growth (Kleinwächter and Selmar 2015). Since, in most of these studies, data on the overall biomass per plant are lacking, a reliable estimation of the entire amount of natural products per plant is not possible (Kleinwächter and Selmar 2014). The major reason for this deficit of information seems to be due to the fact that mostly only one certain plant part or plant organ, respectively, was studied, e.g., roots, leaves, flowers, or seeds. Nonetheless, in some papers, the total contents of natural products per entire plants are outlined or could be calculated from the published data, respectively (Kleinwächter and Selmar 2015).

In *Hypericum brasiliense* plants, both concentration and the total amount of phenolic compounds are drastically enhanced when grown under drought stress (de Abreu and Mazzafera 2005). Despite the fact that the stressed *H. brasiliense* plants were quite smaller than the well-watered controls, their overall content (product of biomass and concentration) was significantly enhanced, because of their extremely higher concentration of phenolic compounds (de Abreu and Mazzafera 2005). This clearly verifies that—apart from an indirect influence on the concentration caused by a lesser biomass gain—the biosynthesis of phenolic products was also directly increased by drought stress. A related situation was also reported for drought-stressed pea plants (*Pisum sativum*): the overall content of anthocyanins was about 25% higher in plants exposed to drought stress in comparison to well-watered controls (Nogués et al. 1998). In the same manner, Jaafar et al. (2012) showed that in *Labisia pumila* plants—in addition to the concentration—also the overall content of total phenolics and flavonoids per plant was significantly elevated when plants suffered drought stress.

As outlined for phenolic natural products, also the biosynthesis of various terpenoids is enhanced in response to drought stress. Nowak et al. (2010) evinced that in young sage plants (*Salvia officinalis*), the entire amount of monoterpenes per plant is significantly higher in the drought-stressed individuals than in the well-watered controls. Accordingly, the lower biomass gain of the stressed sage plants is over-compensated by a massive increase in biosynthesis.

Thus, both phenomena, i.e., indirect and direct stress-related enhancement (Fig. 4.1), contribute to the marked stress-related increase in the concentration of monoterpenes. However, the situation is not always that unambiguous. Although the concentration of monoterpenes in parsley (*Petroselinum crispum*) is strongly increased by drought stress, its overall contents are quite similar in stressed and in well-watered plants (Petropoulos et al. 2008). Accordingly, the drought stress-related concentration enhancement of monoterpenes in the leaves is more or less completely compensated by the accompanying lesser biomass gain. Similar results have been reported by Ninou et al. (2017): the overall content of essential oils per plant is nearly the same in drought-stressed and well-watered oregano plants (*Origanum vulgare*), although the concentrations of the essential oils were significantly increased. At the first sight, these data seem to illustrate that drought does not impact the rate of monoterpene biosynthesis at all. However, it has to be considered that in these plants, an equal amount of monoterpenes is produced by a far lower biomass (Paulsen and Selmar 2016). Thus, the biosynthetic activity per dry weight is strongly enhanced (Paulsen and Selmar 2016). This vividly displays the problem of not applying the appropriate reference value when comparing a stress-related increase of natural product biosynthesis. These coherences are discussed explicitly in a related case study by Paulsen and Selmar (2016) which outlined exemplarily the various conjunctures for reliably calculating a putative increase of essential oils in thyme plants exposed to drought stress. The authors expound that the impact of drought stress on rate of biosynthesis based on dry weight may change in the course of the treatment (Paulsen and Selmar 2016). In the first phase of the experiment, the biosynthetic activity (on dry weight basis) was much higher in the stressed plants than in the well-watered controls. However, when the stress persisted, the situation was reversed (Paulsen and Selmar 2016). These coherences vividly outline the complexity of the situation and the requirement to employ appropriate reference values.

Nonetheless, although only a limited number of data are available, it could be stated that drought stress indeed induces an increase in the biosynthesis of natural products, verified either by an enhancement of their total content per plant or by an augmentation of the biosynthetic activity on dry weight basis. In consequence, the question arises how stress—on a metabolic level—impacts the biosynthesis of natural products.

Since many specialized metabolites are not synthesized in the organ, in which they are mainly accumulated (e.g., Hartmann et al. 1989; Kajikawa et al. 2011; Nowak and Selmar 2016), the coherences related to the impact of drought stress on concentration and content of specialized metabolites become even more complex, i.e., transport processes have to be considered (Fig. 4.1; Kleinwächter and Selmar 2015). In this context, it has to be taken into account that the allocation of compounds is also strongly influenced by the actual stress situation (e.g., Rötzer et al. 2012). Unfortunately, up to now, no information is available how stress-related changes in the *source-sink* character of the various organs alter the allocation of specialized metabolites. Moreover, degradation processes have to be considered. It is well known that the actual concentration of various alkaloids is altered because of their degradation within the plants (Robinson 1974; Ashihara 2006), but up to now, reliable investigations on this issue still are lacking.

4.5 Metabolic Background of Enhanced Natural Product Biosynthesis

As outlined, two different stress-related effects influence the concentration of natural products, i.e., the indirect impact due to a lesser biomass gain entailing lower reference values and a direct effect caused by an enhanced biosynthesis. The latter one, i.e., the stress-related increase of biosynthesis could be either "active" or "passive" (Fig. 4.1; Selmar et al. 2017, Yahyazadeh et al. 2021). The underlying metabolic background is described in this section.

Energy saving represents one of the most important issues in our subsistence (Kleinwächter and Selmar 2014). This paradigm is omnipresent in our daily life—and indeed, at first sight, it seems reasonable to transfer this assertion also into plant biology (Kleinwächter and Selmar 2015). However, when thoroughly reflecting on this issue and considering that plants are autotrophic organisms, it becomes obvious that plants have no problem at all to cover their energy requirements. In contrast, generally, plants absorb much more energy than being required for their photosynthetic CO_2 fixation (Wilhelm and Selmar 2011). This fundamental claim easily can be verified by a conjuncture, with which we all are familiar: when the ambient CO_2 concentration is elevated, the rate of photosynthesis increases drastically (Wilhelm and Selmar 2011). Accordingly, under standard conditions, far more reduction equivalents (NADPH + H^+) are provided by the photosynthetic electron transport chain than the plants expend for the actual CO_2 fixation (Selmar et al. 2017). In other words, in general, plants are subjected to a massive oversupply of energy. In order to avoid an overflow of the electron transport chain, and thus the generation of oxygen radicals (Reddy et al. 2004; Szabó et al. 2005), this surplus of energy has to be dissipated efficiently, i.e., by non-photochemical quenching, photorespiration, or xanthophyll cycle (Fig. 4.2a; Kleinwächter and Selmar 2015). Indeed, under regular environmental conditions, this protective system operates properly. However, any stress situation, in particular drought stress, causes major imbalances. In response to water shortage, stomata are closed, and the CO_2 influx into the leaves is massively diminished (Fig. 4.2b, Selmar et al. 2017). As a consequence, the consumption of NADPH + H^+ in the Calvin cycle is strongly reduced. Although the various energy dissipating mechanisms are upregulated, the reduction status of the chloroplasts increases massively (Selmar and Kleinwächter 2013a, b; Kleinwächter and Selmar 2015). The surplus of electrons in the photosynthetic electron transport chain is directly transferred to oxygen, generating a huge number of superoxide radicals,

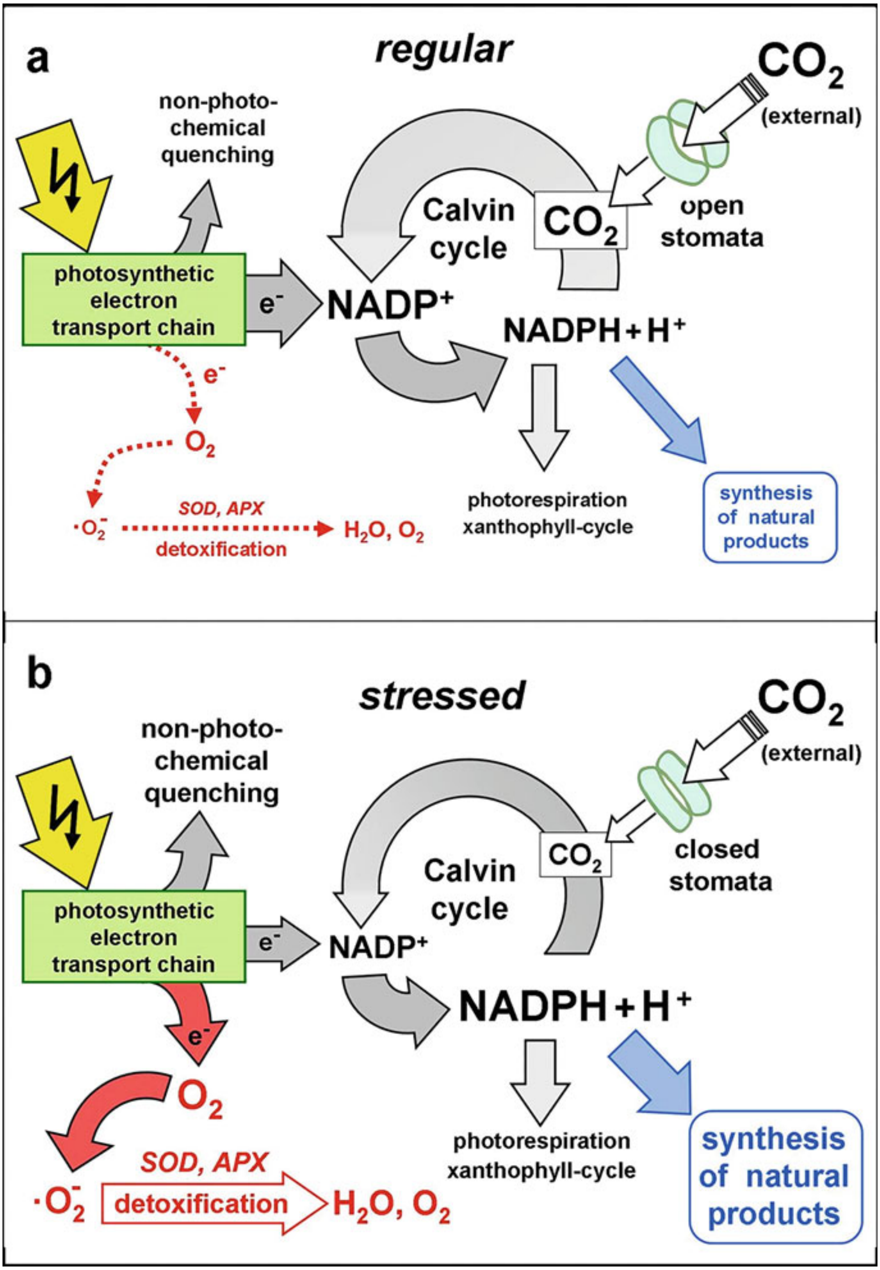

Fig. 4.2 Energy dissipation in plants according to Selmar and Kleinwächter (2013b). As the light energy absorbed by the photosynthetic apparatus is much higher than the energy required for CO_2 fixation, large amounts of energy are dissipated via non-photochemical quenching and effective re-oxidation of NADPH $+H^+$ by photorespiration and xanthophyll cycle. In consequence, the surplus of reduction capacity does not result in the generation of oxygen radicals as a result of electron transfer to molecular oxygen. In contrast to such well-watered conditions, energy fluxes in stressed plants are markedly shifted. Due to an enhanced diffusion resistance of closed stomata, the

which subsequently are detoxified by superoxide dismutase (SOD) and ascorbate peroxidase (APX). In consequence of this over-reduced status in the chloroplasts, the ratio of NADPH + H^+ to $NADP^+$ is strongly enhanced (Fig. 4.2b; Selmar et al. 2017), and, according to the law of mass action, all processes consuming NADPH + H^+ are increased (Kleinwächter and Selmar 2015). This decidedly also applies to the related processes involved in the biosyntheses of highly reduced secondary plant products, such as isoprenoids, phenols, or alkaloids (Selmar and Kleinwächter 2013b; Kleinwächter and Selmar 2015). When the biosynthesis of these compounds is increased solely by the over-reduced status without changing any enzyme activity, it is denoted as “passive shift” (Fig. 4.1; Selmar et al. 2017; Yahyazadeh et al. 2021).

In addition to the enhancement of natural product biosynthesis due to the passive shift mentioned above (Fig. 4.1), the rate of biosynthesis could also be increased by a stress-related upregulation of genes encoding the corresponding biosynthetic enzymes (Selmar et al. 2017; Yahyazadeh et al. 2021). Yet, whereas a tremendous lot of information is available on the impact of biological stresses, i.e., pathogen or herbivore attack, related investigations with respect to drought stress on the expression of enzymes involved in secondary metabolism were deferred (Selmar et al. 2017). Apart from some exceptions (e.g., Dixon 1986; Kurusu et al. 2010), most of the related studies have been performed within the last few years (Selmar et al. 2017).

In wheat leaves, the drought-induced accumulation of flavonoids is correlated with an increase of the expression level of genes encoding various enzymes of flavonoid biosynthesis (Ma et al. 2014). In the same manner, in *Scutellaria baicalensis*, the enhanced accumulation of baicalin coincides with the increase of the expression level and the activities of various enzymes of flavonoid biosynthesis, i.e., the phenylalanine ammonia-lyase and the chalcone synthase (Cheng et al. 2018). A similar correlation is reported for the drought-induced enhancement of flavonoid biosynthesis in *Bupleurum chinense* (Yang et al. 2020). Furthermore, Abdollahi Mandoulakani et al. (2017) reported a significant coincidence of the drought stress-related increase in the concentration of the two phenylpropanoidal compounds present in the essential oils of basil, i.e., methylchavicol and methyleugenol, with an intensification of the expression of genes encoding the key enzymes involved in the biosynthesis of phenylpropanoids. These coherences impressively outlined for phenolic compounds seem to apply in equal measure for terpenoids, too. Radwan et al. (2017) reported that in sage, the drought stress-related increase in the monoterpene content is correlated with an enhancement in the expression of several

Fig. 4.2 (continued) internal CO_2 concentration is strongly reduced in the stressed leaves. Accordingly, much less NADPH + H^+ is consumed within the Calvin cycle, and a much higher share of the energy has to be dissipated. Although non-photochemical quenching and photorespiration are enhanced, many electrons are transferred to molecular oxygen. The resulting superoxide radicals are detoxified by the stress-related induction of superoxide dismutase (SOD) and ascorbate peroxidase (APX). According to the law of mass action, the highly elevated reduction potential (ratio of NADPH + H^+ to $NADP^+$) passively enhances the synthesis of highly reduced natural products.

monoterpene synthases and concluded that in addition to a passive shift, the observed stress-related rise in monoterpenes is—at least in part—also due to elevated enzymatic activities. This is underlined by the finding of Palesh and Abdollahi Mandoulakani (2020), who demonstrated that in basil (*Ocimum basilicum*), the genes responsible for the monoterpene biosynthesis are upregulated under drought conditions. Furthermore, corresponding data are also available for triterpenoids: in *Glycyrrhiza glabra*, the drought-induced increase in the glycyrrhizin concentration coincides with an upregulation of genes encoding major enzymes responsible for the triterpenoid biosynthesis, i.e., the squalene synthase and the β-amyrin synthase (Nasrollahi et al. 2014). Yet, in this context, it has to be noted that different genotypes of a certain plant species might behave differently under drought stress. This vividly was demonstrated by Morshedloo et al. (2017) who showed that the drought stress-related impacts on essential oil content as well as on the related gene expression of two native subspecies of *Origanum vulgare* (subsp. virens and subsp. gracile) were quite significant.

Unfortunately, with respect to the impact of drought stress on the expression of enzymes involved in alkaloid biosynthesis, much lesser information is available. Yahyazadeh et al. (2018) showed that in *Chelidonium majus*, the drought stress-related enhancement of the major isoquinoline alkaloid coptisine is correlated with a corresponding increase in the expression of stylopine synthase, the key enzyme in the biosynthesis of isoquinoline alkaloids. Many further investigations are required to elucidate, whether or not these findings represent a quite general issue, and to confirm that—in addition to the passive shift—a stress-related increase in the contents of the natural products is, at least in part, also due to a stress-related upregulation of related genes (Selmar et al. 2017). Nonetheless, in forthcoming studies, the question on the biological significance of this effect will frequently arise. Indeed, ecological biochemistry taught us that the relevance of natural products is based on their functions within the interactions of the plants with their environment (Harborne 1988). Such specialized metabolites (traditionally called "secondary metabolites") repel herbivores, protect the plants against pathogens, or attract pollinators (Wink 2010; Hartmann 2007). However, any contemplation of the stress-related active upregulation of those substances, whose relevance is based on the various interactions with living organisms, exhibits a serious problem in understanding the biological background: most of the interactions with pathogens or herbivores are not positively affected by drought (Selmar et al. 2017). On the contrary, drought will massively reduce pathogen attack due to low humidity. However, the stress-related enhancement of natural product biosynthesis by the upregulation of the related genes has been manifested in the course of evolution. In consequence, apart from their protective role, there has to be a different and additional advantage by the observed upregulation of phenols, terpenes, and alkaloids (Kleinwächter and Selmar 2014; Selmar et al. 2017; Yahyazadeh et al. 2021).

Apart from the interactions with living organisms, various specialized metabolites are also relevant with respect to fending various abiotic stresses, e.g., by protecting the plants against UV light or too high light intensities, by reducing the transpiration, or by acting as compatible solutes or radical scavengers (Wink 2010; Edreval et al.

2008). Accordingly, upregulation of genes encoding enzymes responsible for the synthesis of metabolites protecting the plants against abiotic stresses is beneficial. However, most of the reported cases concern specialized metabolites relevant for the plants' interactions with other organisms and not for protecting them against abiotic stresses (Kleinwächter and Selmar 2014). In consequence, a sound explanation for most of the observed drought stress-related upregulation of specialized metabolism is lacking. When seeking a clue for a related significance, the strong isoprene emission of numerous plants (e.g., Fall 1999; Sharkey and Yeh 2001) is of special interest. The authors showed that under standard conditions, the emission of isoprene is neglectable and the entire energy consumption for the biosynthesis of isoprene accounts for less than 1% of the entire photosynthetic energy. By contrast, when the plants suffer stress, isoprene synthesis and emission increase drastically. Magel et al. (2006) displayed that at elevated temperatures, the amount of energy, which is dissipated by the strongly enhanced isoprene emission, could account for more than 25% of the energy used for net photosynthesis and deduced that the massive re-oxidation of NADPH + H^+ by the isoprene biosynthesis and the energy requirement significantly contribute to the dissipation of the excess of photosynthetic energy. In addition, the significance for dissipation of a surplus of energy, the emission of isoprene, contributes to cool down the stressed leaves (Behnke et al. 2007). Apart from isoprene, various other volatile organic compounds (VOCs), e.g., terpenes, alkanes, and alkenes, are described to be emitted by stressed plants (Kesselmeier and Staudt 1999).

We have to realize that—apart from all the well-established and relevant ecological functions—the drought stress-related increase in the biosynthesis of highly reduced natural products might also contribute to dissipate a surplus of energy (Wilhelm and Selmar 2011; Kleinwächter and Selmar 2015; Yahyazadeh et al. 2021). In consequence, the stress-related increase of natural product biosynthesis might exhibit an additional relevance by impairing the stress-related over-reduced status and thereby reducing the generation of toxic oxygen radicals. In other words, not only the accumulation of highly reduced natural compounds, which could act as radical scavengers, protects the plant against stress-related damage (e.g., Grace and Logan 2000) but also the related biosynthesis (Selmar et al. 2017; Yahyazadeh et al. 2021).

4.6 How to Induce Drought Stress

Based on the coherences outlined above, it seems obvious to exploit the stress-induced increase in the biosynthesis of natural products to deliberately increase their content in medicinal and spice plants, thereby increasing the product quality (e.g., Selmar and Kleinwächter 2013a; Kleinwächter and Selmar 2015; Selmar et al. 2017). Indeed, the simplest approach to create drought stress is altering the irrigation regime (e.g., Radovich et al. 2005; Kleinwächter and Selmar 2015). Such a technique, however, is restricted to semi-arid regions, where supplemental watering is required, which could be reduced. In contrast, in moderate climates, the water supply by rainfall cannot be influenced directly (Kleinwächter and Selmar 2014). Nonetheless, also in these areas, the moisture content of the soil can be altered. In this context, the choice of the cultivation area can be advantageous, e.g., to choose fields with slopes, which will retain water markedly less than flat plains (Kleinwächter and Selmar 2014). However, even in plains, the drainage capability can also be enhanced by the establishment of furrows or ridges, whose design and shaping directly impact the drainage properties. Moreover, drainage alternatively could be also achieved by increasing the proportion of sand in the soil. This however would irreversibly change the character of the soil and should only be applied in exceptional cases (Kleinwächter and Selmar 2014).

Instead of reducing soil moisture, stress can be induced by applying growth regulators, which are involved in the relevant signal transduction chains. Jasmonic acid is known as a potent regulator of genes involved in most biotic and abiotic stress responses, including drought stress (Turner et al. 2002: Kazan and Manners 2008). Methyl jasmonate (MeJA) is a volatile ester of jasmonic acid (JA), which first was successfully employed in numerous tissue and cell culture systems to enhance the concentration of secondary metabolites (for review, see Namdeo 2007). After its uptake into the cells, MeJA is hydrolyzed, and the active growth regulator is generated. However, it always has to be considered that higher concentrations of JA are known to induce senescence, too (Cree and Mullet 1997). Thus, it is crucial to apply MeJA in suitable concentrations, which do not yet promote senescence, but which are sufficient to mimic drought stress. Up to now, various corresponding approaches are reported which employed successfully MeJA to impact specialized metabolism. In this context, the application of MeJA enhanced the concentration of phenols and monoterpenes in *Ocimum basilicum* (Kim et al. 2006). In the same manner, in *Brassica rapa* and in *Tropaeolum majus*, the concentrations of glucosinolates increased after MeJA treatment (Loivamäki et al. 2004; Bloem et al. 2014). Analogously, the concentration of flavones in parsley and of alkaloids in *Chelidonium majus* was enhanced in response to MeJA treatments (Kleinwächter et al. 2015). In contrast, no effect could be determined for the monoterpenes in thyme, although classical drought stress indeed resulted in a significant enhanced monoterpene concentration in comparison to the well-watered controls (Kleinwächter et al. 2015). Apparently, the efficiency of MeJA treatments depends on an appropriate concentration, which obviously is different for various plant species. MeJA treatment of *Catharanthus roseus* not only does impact the alkaloid content but also massively alters the composition of indole alkaloids (Aerts et al. 1994; Abouzeid et al. 2017). The specific background of this phenomenon is presented in detail in the next section. In conclusion, these promising approaches outline that the application of MeJA indeed is an alternative for a direct drought treatment in order to improve the quality of medicinal and spice plants.

Apart from MeJA, also salicylic acid (SA)—the key signal substance, responsible for systemic resistance (for review, see Durrant and Dong 2004)—has been employed to influence the synthesis and accumulation of secondary metabolites. SA treatment resulted in a strong increase in the concentration of phenolic natural products in *Echinacea purpurea* leaves (Kuzel et al. 2009) as well as in an enhanced accumulation of glucosinolates in oilseed rape (Kiddle et al. 1994). Unfortunately, as already mentioned for JA, SA also impacts on primary metabolism and developmental processes. In this context, the induction of senescence and retardation of growth are of special concern, when these growth regulators are applied to increase product quality (Kleinwächter and Selmar 2014). Accordingly, just in the same manner as outlined for the deliberate induction of drought stress, also the application of salicylic acid and methyl jasmonate requires a thorough balancing of pros and cons (Kleinwächter and Selmar 2014). This, however, necessitates a sound and comprehensive knowledge of the related scientific background, in particular, the knowledge of the optimal concentration of the growth regulators and of the best time for their application (Kleinwächter and Selmar 2014).

4.7 Stress-Induced Changes in the Spectrum of Specialized Metabolites: Quantitative Changes

When Abouzeid et al. (2017) sprayed lesser periwinkle (*Vinca minor*) with MeJA to deliberately enhance the contents of indole alkaloids, the outcome was really surprising (Selmar et al. 2017). Indeed, the alkaloid spectrum of the control plants was in accordance with the literature, and vincamine and vincadifformine represent the major components (Proksa and Grossmann 1991; D'Amelio et al. 2012). However, the alkaloid composition of the MeJA-treated plants was drastically different. In the stressed plants, only small amounts of vincamine and vincadifformine were detectable. Obviously, in response to stress induction, the contents of these both alkaloids were massively decreased. In contrast, the contents of minovincinine, minovincine, and 9-methoxyvincamine drastically increased (Abouzeid et al. 2017, 2019). Based on the high structural similarities of vincamine and 9-methoxyvincamine and their inverse changes in concentrations, it was postulated that—as a result of the stress induction—vincamine was converted to 9-methoxyvincamine (Fig. 4.3, Abouzeid et al. 2019). In the same manner, due to the opposed changes in the concentrations of vincadifformine on the one side and of minovincinine and minovincine on the other side, it is assumed that vincadifformine was converted first to minovincinine and finally to minovincine (Abouzeid et al. 2019). Since the required hydroxylations are frequently catalyzed by cytochrome P450 enzymes, which are reported to be induced by stress (e.g., Narusaka et al. 2004; Pandian et al. 2020), it is very likely that the conversion of vincadifformine first to minovincinine and finally to minovincine is also catalyzed by such stress-induced cytochrome P450 enzymes (Abouzeid et al. 2019). Indeed, this conjecture was verified by the application of naproxen, a well-known inhibitor for cytochrome P450 enzymes (Abouzeid et al. 2019). In plants treated simultaneously with MeJA and naproxen, the conversion of the major alkaloids present in *V. minor* plants effectively was suppressed.

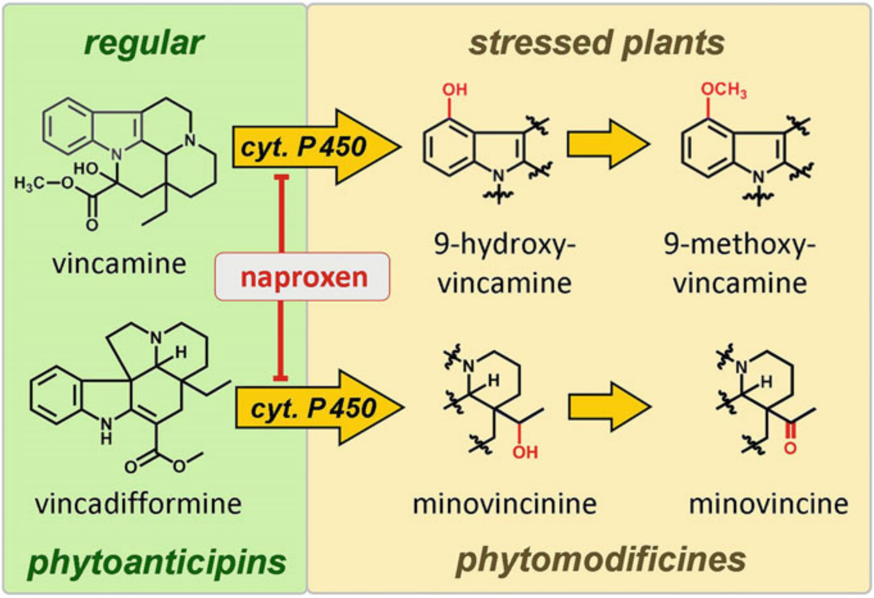

Fig. 4.3 Conversion of indole alkaloids in *Vinca minor*. According to Abouzeid et al. (2019), in stressed *Vinca* plants, the typical phytoanticipins vincamine and vincadifformine are modified by cytochrome P450 enzymes. The resulting derivatives represent a new category of specialized metabolites, denoted as phytomodificines

The coherences outlined above verify that alkaloids genuinely accumulated in *V. minor* plants are modified in response to the stress induction. This, however, signifies that specialized metabolites—which constitutively are accumulated in a plant and which accordingly are denoted as phytoanticipins—are modified in response to a stress situation (Abouzeid et al. 2019). Indeed, the biosynthesis of novel substances in response to stress is well known as a result of pathogen or herbivore attack (Wink 2010). But in all these cases, the relevant specialized metabolites, denoted as phytoalexins, are synthesized de novo from primary metabolites (Pedras et al. 2011). In consequence, in addition to phytoanticipins and phytoalexins, a third category of specialized metabolites, i.e., phytomodificines, had to be introduced. When substances are generated as a result of the stress-induced modification of previously accumulated phytoanticipins, they are denoted as phytomodificines (Abouzeid et al. 2019). This vividly outlines 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. Indeed, there is an enormous potential to exploit the stress-related changes in the spectrum of specialized metabolites (Selmar et al. 2017). In particular, the emergence of new, hitherto unknown compounds as well as the changes in the overall pharmacological activity of related extracts impressively outlines the advantages of applying growth regulators to medicinal plants.

4.8 Practical Deliberations

In general, drought is a negative factor for plant cultivation and is responsible for severe yield losses in agriculture. However, with respect to spice and medicinal plants, the situation is different (Kleinwächter and Selmar 2015). Since their content of relevant natural products determines the quality of the related commodities, any approaches to increase this quality could be favorable. Based on the insights displayed in this review, we have to realize that the concentration of natural products and, thus, the product quality of spice and medicinal plants can be deliberately enhanced by applying moderate drought stress during their cultivation (Kleinwächter and Selmar 2015). In the recent past, several successful approaches were reported (Bloem et al. 2014; Kleinwächter et al. 2015). Nonetheless, we always have to consider that drought stress also reduces the biomass production of spice and medicinal plants (Jaleel et al. 2009). Accordingly, the interference of these two stress-related effects, i.e., lesser biomass production and enhanced concentrations of relevant compounds, has to be considered (Kleinwächter and Selmar 2015). Thus, before any employment of moderate drought stress to deliberately modulate the biosynthesis of natural products, the question has to be answered what is required, a high concentration of relevant compounds or a large overall yield. When the quality of the commodity is strongly determined by a high concentration of natural products, putative wastages of biomass and thus of the overall amount of relevant compounds are acceptable. On the other hand, when the total yield of a certain substance is in the focus, detriments in biomass production should be minimized. These considerations are valid for both alternatives, a classical induction of stress by reducing the watering or by applying growth regulators such as MeJA.

Apart from the interaction of the stress-induced growth retardation and specialized metabolism, we have to consider that stress also influences the general metabolic status of a plant and thereby alters the ratio between generative and vegetative characteristics (e.g., Nederhoff and Houter 2009). Furthermore, drought stress may change the *source-sink* properties of the entire plant and, thus, the allocation of natural products from one organ into another. This aspect is of special interest when the related plant-derived commodity comprises only one certain organ, e.g., roots, leaves, seeds, or flowers.

A further promising aspect concerns the phytomodificines. In all cases, in which the employment of stress results in the emergence of new hitherto unknown compounds (or in massive changes of the spectrum of natural compounds), this approach will facilitate the search for new, previously unknown bioactive natural products and thereby accelerate the development of novel, highly needed drugs.

In addition to the well-known quality enhancement of medicinal plants grown under semi-arid conditions, whose scientific background is outlined above, there are various further examples that illustrate the relevance of stress induction for the quality of plant-derived commodities (Kleinwächter and Selmar 2015). However, sometimes the opposite, i.e., the omission of stress, is required for an enhancement of the quality of plant-derived commodities (Kleinwächter and Selmar 2015). These coherences are valid for those products, whose higher quality is attributed to a lower content of specialized compounds. To generate the highest quality of Japanese green teas, which exhibit only low concentrations of unpreferred phenolic compounds, the *Camellia sinensis* plants frequently are grown under artificial shading (Wang et al. 2012): plants grown under lower light intensities are subjected to far less oxidative stress (Fig. 4.2) than plants, which are grown in full sunlight. In consequence, in the shaded tea plants—as a result of a significantly decreased over-reduced status—the synthesis of secondary plant products should be reduced (Kleinwächter and Selmar 2015). This assumption was verified by related analysis, which revealed that the content of specialized metabolites, i.e., monoterpenes and coumarins, is significantly lower in the shaded, non-stressed tea plants than in the related controls exposed to full sunlight (Shimoda et al. 1995).

Further impressive examples for the impairment of specialized plant products by reducing the stress level are the differences in the color of tobacco leaves (Kleinwächter and Selmar 2015). Whereas the tobacco leaves from *Nicotiana* plants grown in full sunlight in Southern and Middle America display the typical dark colors of the so-called Brazil quality (Andersen et al. 1985), tobacco grown in Sumatra exhibits much brighter hues. This is caused by the far lower solar irradiance due to the foggy Sumatran climate. Corresponding phytochemical analysis indeed revealed that the concentrations of total phenols and alkaloids are quite lower in the tobacco leaves grown under low irradiance (Andersen et al. 1985). This context is exploited by artificial shading of tobacco plants. In doing so, even when grown in Brazil, the typical Sumatra qualities are generated. These coherences confirmed once more the significant impact of stress on the synthesis of specialized plants' products.

These reflections clearly display that the effects of drought stress on the accumulation of natural products and thus on the quality of medicinal plants are multilayered and very complex. Its basic enhancing influence on the biosynthesis of specialized metabolites could be compensated or even over-compensated by other stress-induced processes. As a consequence, we always have to weigh up advantages and drawbacks of a certain approach (Kleinwächter and Selmar 2014). To facilitate corresponding assessments, Kleinwächter and Selmar proposed to answer some ensuing questions (Selmar and Kleinwächter 2013a; Kleinwächter and Selmar 2014, 2015):

- What is required, a high concentration of natural products or a large bulk (total amount versus high concentrations in the commodity)?
- What kind and which level of stress enhance the accumulation of the desired compounds without causing too high losses in biomass?
- Can the accumulation of the required natural products be increased by the application of signal transducers or growth regulators (e.g., MeJA, salicylic acid)?

- Are the substances synthesized and accumulated in source or in sink tissues?
- Are the substances synthesized and accumulated in generative or vegetative organs?
- Should the stress be applied only within a certain phase of cultivation or while a special developmental phase in order to obtain maximal quality?

4.9 Conclusion

Spice and medicinal plants grown under semi-arid conditions reveal significantly higher concentrations of relevant natural products than identical plants, grown and cultivated in moderate climates (Kleinwächter and Selmar 2015). Basic biochemical reflections expound that three causes for the observed changes could be responsible for the stress-related increase in the concentration of natural products (Selmar and Kleinwächter 2013b). Since the biomass of the stressed plants is quite lower than that of the well-watered controls, even without any difference in the extent of biosynthesis, the concentration (on fresh or dry weight basis) on the stressed plants is enhanced (Paulsen and Selmar 2016; Yahyazadeh et al. 2021). In contrast to this indirect effect, indeed the entire amount of specialized metabolites could be higher. This direct effect could be due to either an active upregulation of the biosynthetic enzymes or to a passive shift, evoked by an elevated level of $NADPH+H^+$, caused by the shortage of CO_2 as a result of stomata closing (Yahyazadeh et al. 2018). Apart from these quantitative changes, also the spectrum of specialized metabolites could be altered in response to the stress situation (Abouzeid et al. 2017). Based on these physiological considerations, practical aspects for the application of drought stress to deliberately improve the quality of spice and medicinal plants are outlined.

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Chapter 5

Adaptation Strategies of Medicinal Plants in Response to Environmental Stresses

Muatasim Jan, Tawseef Ahmad Mir, Rakesh Kumar Khare, and Neha Saini

Abstract During the complete life phase, medicinal plant species encounter varied environmental problems, which have adverse impacts on their growth, productivity, reproductive ability, and survival. These plants develop effective strategies for preventing or tolerating all of these stresses, which helps them to adjust to stressful circumstances. Such types of strategies of adaptation are found at structural, anatomical, hormonal, molecular, and biochemical stages. To adapt and protect themselves from the environment, these plants use different mechanisms including epigenetic memory, molecular crosstalk, ROS (reactive oxygen species) signaling, plant hormone accumulation (such as abscisic acid, jasmonates, ethylene, and salicylic acid), redox status and inorganic ion flux changes, resistance of R-gene, and systemic acquired resistance. A detailed understanding of various strategies used by the plant species to the stress in the environment is needed to enhance the production of crops in stress-like situations. Analyzing plant response to an environmental stress exposes metabolism pathways and other cascades that are activated following a stressful situation. Furthermore, understanding the anatomical and molecular mechanisms of plant species stress response would provide new insights toward the production of genetically engineered species that have high resistance to various stresses.

Keywords Medicinal plants · Adaptation strategies · Environmental stress

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5.1 Introduction

In the survival of mankind and the health of natural ecosystems, biodiversity plays a pivotal part. Medicinal plants, which are a component of this biodiversity, constitute the backbone of the global healthcare system and economy. Medicinal plants are a significant natural resource and an important aspect of the healthcare industry as well as the environment (Mir et al. 2021a, b). However, the survival and the development of medicinal herbs are hampered due to various environmental stresses (Osakabe et al. 2012; Stella et al. 2013; Niinemets 2010). Medicinal plants which are open to diverse stresses (biotic and abiotic) experience a disturbance and physiological imbalance, resulting in decreased growth, development, and productivity (Atkinson and Urwin 2012).

Drought, salt, severe temperature, insufficient or extreme conditions of light, pollution, and radioactivity are all examples of abiotic factors that can cause environmental stress. Some of the important abiotic stresses including drought, cold, and salinity create substantial issues for medicinal herbs (Krasensky and Jonak 2012), including insufficient availability of water and nutrients and harmful salt ion concentration like the deficiency of Na, Cl, and Ca (Marschner 1995). Environmental pollution results due to growing urbanization, industrialization, and agricultural intensification, with some pollutants reaching lethal levels for plants. Abiotic stresses could cause serious damage to the stems of plants. Strong winds have the potential to snap twigs and cause flower buds to fall (Chelli-Chaabouni 2014). Intense sun radiations that occur over a lengthy period of time can burn the epidermis of freshly produced shoots. Under the salt stress, the decreased differentiation of xylem leads to decreased vessel lamina (Chelli-Chaabouni 2014; Escalante-Pérez et al. 2009). This is due to low nutrition concentration to cambium and reduced K+ concentration in salt-sensitive plant species. The biotic factors arise due to the interaction of plants with fungi, virus, bacteria, insects, and competition (Fig. 5.1). Besides their immediate impacts, the physiochemical features of the environment pose an indirect effect on the proliferation and development of plant parasites through their interaction with other organisms. The severity of the stresses is evaluated by the interactions (interspecific and intraspecific) of these organisms (Valdés-Gómez et al. 2011). The secretion of allelochemical substances by the plant may pose a number of effects on individual surrounding plant species and, as a result, on the distribution of organism ecosystems. Ultimately, these products show their impact on growth, germination of seeds, plant physiology, cellular system, metabolism, water and nutrient uptake, transport of mineral and food elements, and hormones (Lodhi 1976; Blanco 2007). Long-term abiotic stress can damage plant tissues and organs, making them more susceptible to disease (Chelli-Chaabouni 2014). Long-time biotic stresses due to insects and pathogens worsen the effects of abiotic stresses including deficiency of water (Englishloeb 1990; Khan and Khan 1996). By the time medicinal plants have developed most effective mechanisms to tolerate the number of environmental stresses in the nature (Table 5.1). Besides the fact that medicinal plants vary in their response and susceptibility to stresses, they

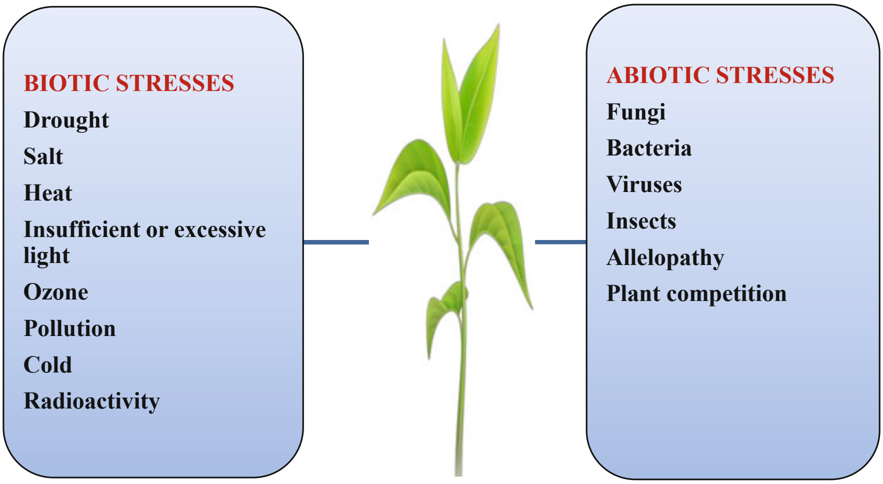

Fig. 5.1 Types of stresses affecting the plant

have evolved a variety of adaption strategies to translate cascade signaling and networks of a gene transcription to get adapted and to survive in harsh environment (Sanghera et al. 2011; Joshi et al. 2016). To adapt and protect themselves from the environment, these plants use different mechanisms including epigenetic memory, molecular crosstalk, ROS (reactive oxygen species) signaling, plant hormone accumulation, inorganic ion flux changes and redox status, resistant R-gene, and (SAR) systemic acquired resistance as well as other mechanisms (Kissoudis et al. 2014; Wani and Kumar 2015). To check the identification and entrance of pathogens at the earliest, the physical barriers including stomata, cuticle, and cell walls play a pivotal role (Asselbergh et al. 2007). Stress-prompted biochemical responses result in the accumulation of metabolites including quaternary amino acids, polyamines, proline, and hydroxyl substances including polyols, oligosaccharides, and sucrose (McCue and Hanson 1990).

5.2 Structural Adaptations of Medicinal Plants to Environmental Stresses

Because of the negative effect of different stressors (biotic and abiotic), medicinal plant productivity is decreasing. To preserve this production of plants from the changing climate and an increasing global population, minimizing these losses is a critical concern (Devi et al. 2017). The plant adapts its morphological structures in response to the harsh climate conditions in order to survive in the current habitat (Fig. 5.2). Drought, salinity, wounding, and disease attacks, as well as heavy metals and high salinity, all adversely affect the plant growth and productivity (Wani et al.

Table 5.1 Adaptation strategies of some medicinal plants in response to environmental stresses

Adaptation	Stresses	Plant species	Citations
Thickening of the cuticle of leaf	Droughts	*Olea europaea*	Bosabalidis and Kofidis (2002)
Thickening of palisade layers of the mesophyll	Salinity	*Olea europaea*	Devi et al. (2017)
Thickening of leaf epidermis	Drought	*Ziziphus mauritiana*	Devi et al. (2017)
Lignification of cell wall	Pathogens	*Ziziphus mauritiana*	Ninemets et al. (1999)
Anatomical plasticity of leaves	Drought	*Quercus coccinea* *Quercus rubra*	Ashton and Berlyn (1994)
Movement of chloroplast	Restrictive light conditions	*Tradescantia albiflora*	Park et al. (1996)
Presence of lenticels	Flooding	*Acer campestre* *Fraxinus excelsior* *Alnus glutinosa*	Chelli-Chaabouni (2014)
Increase in the density of roots	Salinity	*Callistemon citrinus*	Alvarez and Sanchez-Blanco (2014)
Presence of adventurous roots	Flooding	*Sapium sebiferum* *Taxodium distichum*	Wang and Cao (2012)
Increase in root diameter	Salinity	*Portulaca oleracea* *Pinus banksiana*	Franco et al. (2011); Croser et al. (2001)
Decrease of vessel lumina and increase in IAA-amido conjugates in xylem	Salinity	*Populus euphratica*	Junghans et al. (2006)
Reduction in stomatal size	Pollution	*Sida acuta*	Ogunkunle et al. (2013)
Increase in endogenous level of salicylic acid	Drought	*Phillyrea angustifolia*	Munne-Bosch and Penuelas (2003)
Cuticular waxes and hairy texture	Higher levels of light	*Eucalyptus* spp. *Prunus* spp.	Cameron (1970); Holmes and Keiller (2002)
Accelerate fine root production	Water scarcity	*Fagus* spp.	Leuschner et al. (2001)
Cell wall flexibility	Water scarcity	*Vitis vinifera*	Patakas and Noitsakis (1997)
Accumulation of phenolics	Pollution	*Trifolium* spp.	Gostin (2009)
Increase in tissue flexibility and a reduction in osmotic potential	Water shortage	*Persea americana*	Chartzoulakis et al. (2002)
Accumulation of proline	Salinity	*Salvia officinalis* *Trachyspermum ammi* *Achillea fragrantissima*	Hendawy and Khalid (2005); Ashraf and Orooj (2006)

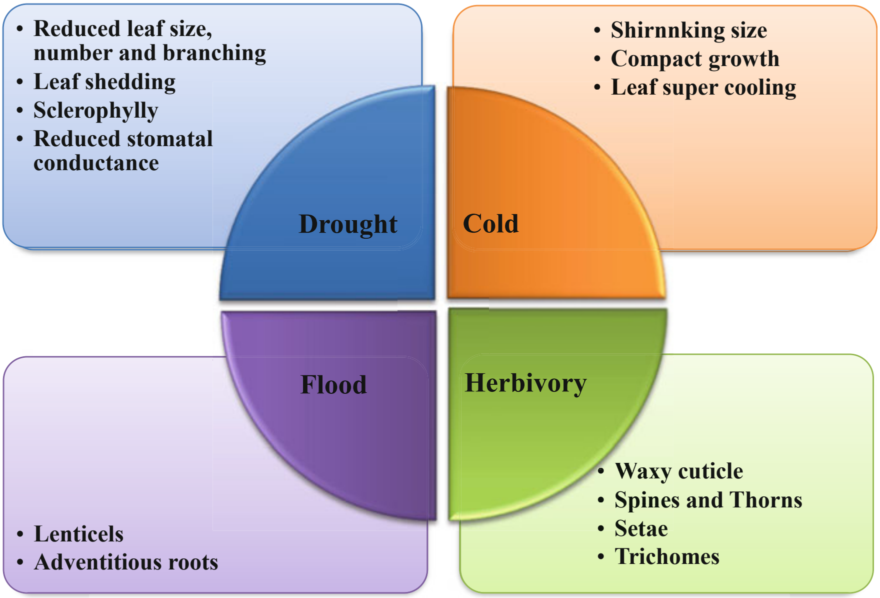

Fig. 5.2 Various morphological adaptations of medicinal plants to environmental stresses

2016; Hossain et al. 2016). The drought-related reductions in plant production may be due to the closure of stomata in reaction to the reduced levels of water in the soil, which lowered CO2 uptake and, hence, reduced the process of photosynthesis (Cornic 2000; Flexas et al. 2004). Plants that grow in arid environments have acquired xeromorphic characteristics such as leaf shedding and a reduction in size and branching and number of leaves, which is an adaptation of plant toward the drought conditions (Devi et al. 2017). Sclerophylly is the yet another adaptation strategy of plants against the drought stresses, in which plant species grow leaves that are stiff and are not permanently harmed by wilting process (Micco and Aronne 2002). Plants that exist in cold climates change the morphology to avoid the effects of moving air by shrinking their body structure and by adopting the compressed pattern of growth. Furthermore, several tropical alpine medicinal plant species have been reported to engage in leaf super-cooling, which involves the retention of water in a metastable gel form in freezing temperature (Devi et al. 2017). The tissues freeze and perish as soon as the super-cooling capability is depleted, approximately 12 °C. It has been observed that in cold climate plants, the water in the xylem is super-cooled to 40 °C (Korner 2016).

The primary security of a plant against herbivores is the morphological structures of the plant, which also acts as shield against insect resistance (Handley et al. 2005; Chamarthi et al. 2010). Physical barriers are considered as an adaptation against insect pests (He et al. 2011). Plants that are nutrient-deficient allot more photosynthates to their root system (Kozlowski and Pallardy 2002). Tolerant plants employ a variety of strategies to restore the function of root system and reimburse for damage of roots and mortality. During the dry mid-summer period, when there is deficiency of water, species of *Fagus* accelerate the production of fine roots to reimburse the losses of root biomass (Leuschner et al. 2001). By the development of adventitious roots, plants also save themselves from flood conditions.

In anaerobic environments, hypertrophied lenticels grow in plants which provide a channel for diffusing oxygen through the live cells of bark and release the hazardous chemicals associated with anaerobiosis (Glenz et al. 2006). Plants like *Acer campestre*, *Fraxinus excelsior*, *Salix alba*, *Alnus glutinosa*, and *Populus nigra* have been reported to have lenticels as a morphological adaptation to floods (Hook 1984; Siebel et al. 1998).

Plant species respond to biotic (herbivores, bacteria, virus, and fungi) and abiotic (droughts, UV radiations, salinity, higher temperature, and heavy metals) disturbances by rolling their leaves (Bosabalidis and Kofidis 2002; Kadioglu et al. 2012). In the plant species under osmotic tension, this physical movement, along with the closure of stomata, serves a critical function in reducing water loss and maintaining cell turgor. Stomata closure, on the other hand, reduces gaseous exchanges through the leaves, as well as plant photosynthetic activity, because CO_2 intake is reduced. Plant species try to change their crown construction manner and the leaf angle placement within the crown irradiance to maximize carbon absorption (Egea et al. 2012; Guàrdia et al. 2012). Photon flux density is regulated by the movement of chloroplast at cellular level (Way and Pearcy 2012). The overall size and grana stacking inside chloroplasts can fluctuate within 10 min in the exposure of heavy light (Rozak et al. 2002).

5.3 Anatomical Adaptation of Medicinal Plants to Environmental Stress

5.3.1 Water, Light, and Oxygen Stress

The water stress arises when there is a modest loss of water, which causes stomata to close and gas exchange to be limited. Water stress tolerance is influenced by changes at cellular level. It seems to be the result of a rapid osmotic stress-induced compatible protein and solute accumulation (Shao et al. 2005). Water stress affects the most important activities of a plant including cell division process, cell maturation, and differentiation, which impact plant growth quantity and quality (Correia et al. 2001; Cabuslay et al. 2002). Stiff, leathery leaves are a sought of adaptation strategy by plant species in response to stress of water. The sclerophyllous leaves have supporting tissues (like sclereids, epidermis of thick wall, etc.) that restricts the collapse of plant systems in the scarcity of water, hence lowering the chances of injuries to plant body (Correia et al. 2001). Due to the presence thick walled epidermis, sclerophyllous leaf loses its little volume during dry circumstances, while the thin mesophylls cells shrivel severely, making large gaps between the cells. By this, the process of photosynthesis continues even when other leaf types wilt due to extreme water stress (Shields 1950).

Stress tolerance of some plant species has been linked to variations in cell wall flexibility under stressful situations. However, tissue elasticity has been postulated as a mechanism of stress adaptation in positive as well as negative ways (Chartzoulakis et al. 2002; Patakas and Noitsakis 1997). The cell wall flexibility of *Vitis vinifera* has been recommended as a viable approach for overcoming water shortages (Patakas and Noitsakis 1997) and severe ultraviolet radiations (Lesniewska et al. 2004). In contrast, two most common mechanisms of adaptation of increase in tissue flexibility and decrease in the osmotic potential in *Persea americana* helps it to tolerate the various environmental stresses (Chartzoulakis et al. 2002).

Aerenchyma tissues are considered an adaptation to anaerobiosis of plant species which provide an enormous air gaps between the cells that allows oxygen to diffuse in the roots (Wang and Cao 2012). The increased palisade parenchyma, the gaps between cells, and a subsequent reduction in the spongy parenchyma are among the anatomical changes in leaves that aid CO2 transport in the presence of small stomatal openings (Acosta-Motos et al. 2017).

5.3.2 Heat Stress

Global climate change is currently raising temperatures, which is posing a significant effect on medicinal plants. Plant species subjected to heat stress on a frequent basis can inhibit survival and development, and by this, mortality is increased (Sayed 1996). Due to continuous increasing temperatures, the anatomical structures of the plant including tissues, cells, and subcellular systems are affected. Development of small cells, closure of stomatal guard cells, increased density of trichomes and stomata, and development of larger xylem vessels in roots and the shoot are general adaptations of plants in heat stress (Anon et al. 2004). Due to this, the severity of damage in mesophyll cells is increased (Zhang et al. 2005). In order to respond to higher temperatures, the plants use a number of mechanisms including the generation of polymorphic leaf so as to decrease the transpiration by the process of bimodal stomata to reduce the process of photosynthesis (Sayed 1996). Chloroplasts in the mesophyll cells and stroma lamellae in grape vine expand, and the compounds present in the vacuoles aggregate together, whereas the mitochondria becomes empty by the disruption of cristae (Zhang et al. 2005).

5.3.3 Salinity Stress

The most discussed and dangerous environmental stress which is involved in limiting the growth and development mostly in arid and semi-arid climatic conditions is none other than salinity (Wani and Gosal 2011; Wani and Hossain 2015).

The soil is said to be saline when the electric conductivity of the soil solution exceeds 4dS ml (40 mM NaCl) and roughly 0.2 MPa of osmotic pressure is formed, thereby severely lowering plant growth (Wani et al. 2010). Large amount of Na+ is accumulated by plant species in saline environments, thereby inhibiting K+ ions from being absorbed (Dang et al. 2010). In plants, chlorosis and necrosis are found due to saline soil, mostly owing to Na+ buildup, which interrupts many of the important functions of a plant (Munns 2002).

Plants that show resistance to NaCl adjust to saline environment by making some key adaptations including chlorophyll increase and leaf structure changes, which ultimately contribute to the prevention of leaf ion toxicity, limiting the loss of water and protecting the process of photosynthesis (Acosta-Motos et al. 2017; Franco et al. 2011). It has been reported that *Callistemon citrinus* plant if irrigated with salt water improves root breadth and density (Croser et al. 2001; Álvarez and Sánchez-Blanco 2014). The characteristics of cell wall alter under saline circumstances, and leaf turgor and photosynthetic rates drop, resulting in a decrease of the total area of a leaf (Rodríguez et al. 2005). To compensate the xylem hydraulic conductivity loss caused by the stress of salt, several woody plants reduce vessel lumen and increase wall strength in response to salinity. For example, the vessel lumen of *Populus euphratica*, which is resistant to salt stress, decreased less than *Populus canescens*, which is a salt stress sensitive plant (Junghans et al. 2006).

5.3.4 Heavy Metal Stress

Important transition elements (zinc, iron, copper, manganese, cobalt, molybdenum, and nickel) as well as elements which are not essential (lead, cadmium, mercury, and chromium) are included in heavy metals. These elements are dangerous for medicinal plants when their concentration is increased beyond the threshold level. Heavy metals which have toxic properties are important factors that harm metabolic and physiological processes of medicinal plants (Farid et al. 2013). In recent years, heavy metal concentrations have reached to dangerously high levels (Sainger et al. 2011). Heavy metals pose direct influence on the growth and development. Furthermore, soil microbes and animals get affected as well which is harmful for the mineralization of organic compounds; hence, nutrition hindrances are created in plants (Fernández et al. 2013; Tyler 1984). Heavy metals have a phytotoxic impact on plants causing a number of physiological, biochemical, and structural problems to the plant (Sanitá di Troppi and Gabbrielli 1999; Marques et al. 2000).

Accumulation of heavy metals like lead, mercury, and cadmium in *Bruguiera sexangula* made the vascular bundles deformed (Gupta and Chakrabarti 2013). Cd treatment caused the distortion of xylem and phloem, which finally resulted in apparent toxicity. The Cd-treated stem transverse section (TS) revealed the distribution of heavy metals even up to pith region (Zhao et al. 2000). Architectural alterations in *Arabidopsis thaliana* roots which are induced by certain abiotic stressors and Cu are thought to be due to metabolism of phytohormone and buildup of local auxin near the pericycle of root (Pasternak et al. 2005). In the root tissues of *Brachiaria decumbens*, larger cell layers of endodermis and exodermis have been discovered as well as thickness in xylem and cortical cell walls by the effect of heavy metals. The thickness of leaf blades was decreased with the increase in heavy metal contamination, resulting in a shift in the root development rate (Gomes et al. 2011).

5.3.5 Pollution Stress

Due to increasing urbanization, industrial growth, and agricultural intensification, the environmental pollution has increased dramatically, leading certain toxins to reach dangerous levels for plants. Because medicinal plants are so important, researchers are increasingly interested in the impact of phytotoxic chemicals on them.

A prevalent response of a plant to any stress is an increase in phenolics and lignin accumulation (Wild and Schmitt 1995). The presence of phenolic chemicals in *Trifolium* spp. reveals that the accumulation of these compounds occurs due to long-term exposure of the plant to air pollution (Gostin 2009). Cement dust has been found to affect epidermal structures such as trichomes in *Cajanus cajan* plants (Baralabai and Vivekanandan 1996). Verma et al. (2006) discovered a substantial drop in the density and index of stomata grown under varied levels of smoke of coal. Nevertheless, no morphological changes in *Pennisetum purpureum* were found as a result of cement factory pollution, which might imply that dosage response of plants has not been achieved (Ogunkunle et al. 2013). Growing medicinal trees in cities reduced the size and density of stomata and thinned the cuticle, but had no effect on other morphological features, designating the plan trees can tolerate the traffic conditions in megacities (Pourkhabbaz et al. 2010).

5.4 Hormonal and Enzymatic Adaptation Strategies

The hormonal physiology of the plants is affected by environmental stresses. The tissue growth of vessels and secondary development in plants are regulated by hormones (Osakabe et al. 2012). Auxins play a significant role in cell division, expansion of cells, apical dominance, and growth and development of roots and vessel tissues (Osakabe et al. 2012). Secondary development is also controlled by the hormones (Nilsson et al. 2008). Salt-tolerant plants can utilize the conjugates of auxin as auxin source to compensate the physiological imbalance of auxin caused due to salt stress conditions.

Because of its involvement in the plant leaf abscission, abscisic acid (ABA) gets its name. It is also known as a “stress hormone” because of its responsiveness and particular function in plant adaptation to abiotic stressors. Endogenous ABA levels rise fast in response to environmental stressors, activating particular signal pathways and expression levels of genes (O'Brien and Benkova 2013). According to Nemhauser et al. (2006), 10% of protein-encoding genes are controlled transcriptionally by ABA.

In plants, it also serves a function of internal signaling and allows them to survive in harsh environments (Keskin et al. 2010). Abscisic acid in plants is used to convey the message that the plant is having stress conditions near the roots when they are water-stressed, which leads to behavior of water-saving anti-transpiration (Wilkinson et al. 2012). Different abiotic conditions promote ABA accumulation (Escandón et al. 2016; Pashkovskiy et al. 2019). ABA is believed to have a function in controlling biomass of woody medicinal plant species, by influencing the area of leaf in the trade-off biomass allocation under stressful conditions (Yu et al. 2019).

During the drought stress conditions of a plant, salicylic acid plays a pivotal part (Miura et al. 2013). It also plays a vital role in chilling (Yang et al. 2012), salinity (Fahad and Bano 2012), and heat stress conditions. In *Phillyrea angustifolia*, salicylic acid was increased five times during drought conditions (Munne-Bosch and Penuelas 2003). Water scarcity increased salicylic acid levels in *Hordeum vulgare* roots by almost threefold (Bandurska and Stroinski 2005).

Defense reaction of a plant species to heat stress is likewise influenced by ethylene (Larkindale et al. 2005). Environmental stress causes ethylene buildup, which enhances the likelihood of plants surviving in these harsh conditions (Gamalero and Glick 2012). In response to hypoxic conditions, ethylene accumulation occurs in waterlogged soils. Ethylene serves as a regulator against anaerobiosis in waterlogged conditions (Sairam et al. 2008).

Homeostasis at cellular level and antioxidant defense are two plant defense systems against heavy metal toxicity. A number of enzymatic antioxidants, including peroxidase, superoxide dismutase, glutathione S-transferase, and catalase, are capable to change superoxides to hydrogen peroxide, which can be converted to water and oxygen, whereas ROS are directly detoxified by proline, glutathione, and ascorbic acid which are non-enzymatic antioxidants (Xu et al. 2009; Yadav et al. 2014; Singh et al. 2015). In the leaf portion of some medicinal plants like *Salvia officinalis*, *Trachyspermum ammi*, and *Achillea fragrantissima*, proline is accumulated in response to saline conditions (Hendawy and Khalid 2005; Ashraf and Orooj 2006).

5.5 Molecular and Biochemical Adaptation Strategies

Understanding the complicated nature of plant adaptation strategies would need a thorough examination to understand the stress-response genes and their function (Wani et al. 2012). Studies reveal that the genes involved in defense response get active by various pathogenic actions and a number of abiotic stresses. Individual genes of defense are activated in response to certain factors of environment, that is, a complex signaling network is at work, which provides a plant defense against a number of stresses such as viruses (Jaspers and Kangasjarvi 2010; Khan and Wani

2014) The "basic leucine zipper (bZIP)" domain transcription factors regulate a number of key activities of plant, including pathogen defense, regulation of osmosis, sugar and hormone signaling, signaling of abiotic stresses, metabolism of energy, and more (Perez-Clemente et al. 2013; dos Reis et al. 2016). ABA hypersensitivity, reduction in transpiration, and increased tolerance to drought are caused by the overexpression of AREB2/ABF4 or ABF3 genes in *Arabidopsis* (Fode et al. 2008a, b). In *Oryza sativa*, OsABF1 gene and S1AREB gene in *Solanum lycopersicum* have been shown to improve salt tolerance and drought tolerance in plants and might be used as possible agents for enhancing plant tolerance of salt (Amir et al. 2010; Hsieh et al. 2010).

Oryza sativa, *Arabidopsis*, and *Glycine max* (Le et al. 2011) have all been shown to contain plant-specific transcriptional factors of the NAC family (Hu et al. 2010). Response to stresses such as cold, salt, drought, bacteria, fungus, and oxygen stress is mediated by these transcription factors (Nuruzzaman et al. 2013).

To adapt to harsh environmental circumstances, the APETALA2 (AP2)/ethylene-responsive element binding factor (ERF) proteins work as either activators or repressors of gene transcription in response to both biotic and abiotic stressors (Fujimoto et al. 2000). The AP2/ERF genes' RNA levels are believed to be influenced by stresses like cold conditions, droughts, pathogen infestations, salt, injury, or the jasmonates and salicylic acid treatment (Onate-Sanchez and Singh 2002). In *Nicotiana tabacum*, the abovementioned genes led to increased resistance to pathogen infestation (Park et al. 2001) and have been reported in *Arabidopsis* (Kang et al. 2011) and *Malus* spp. (Zhao et al. 2012).

Proteins including MYB and MYC play a pivotal role toward adverse conditions of environment. Against the pathogen infestation, AtMYB30 had a hypersensitive cell death pathway (Raffaele et al. 2008). Seo and Park (2010) found that AtMYB96 regulates stress owing to water and resistance to disease via ABA signaling pathway and that AtMYB15 improves stress owing to cold (Agarwal et al. 2006). The protein family of MYC plays a pivotal part in the process of apoptosis and hypersensitive cell death in reaction to infection by pathogens (Agarwal and Jha 2010).

In the low-lying areas, most severe abiotic stresses are submersion/waterlogging. In deep water *Oryza sativa* cultivars, three QTLs are principally important for stem elongation. SNORKEL1 and SNORKEL2 ethylene response factors help *Oryza sativa* to adapt in profound waters (Hattori et al. 2009). SUB1A encoding for ethylene response factors is believed to play a part in plant resistance in waterlogged soils (Xu and Mackill 1996; Xu et al. 2006). Upon desubmergence, drought and oxidative stress promote SUB1A expression responsible for controlling the expression of genes responsible for ABA-mediated adaptation in drought. Furthermore, SUB1A stimulates the gene expression in the process of (ROS) detoxification and lowers the accumulation of ROS when exposed to oxidative stress (Fukao et al. 2011).

Phosphate (Pi) is considered a common restrictive factor in the output of plant despite being an important macronutrient for numerous metabolic activities. The complex physiological, biochemical, and morphological adaptations are started by Pi deficiency; this process is also called as 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).

5.5.1 Molecular Crosstalk and Epigenetic Memory for Stress and Adaptation

The stress tolerance of medicinal plant species can be increased by examining physiological and molecular crosstalk in more detail (Kissoudis et al. 2014). According to whole genome expression meta-analysis investigations, a considerable amount of gens are frequently controlled in biotic and abiotic stress conditions (Shaik and Ramakrishna 2014).

Plants have evolved sophisticated mechanisms regulated by gene transcription networks and signaling cascades responding to environmental stimuli in order to withstand diverse biotic and abiotic challenges (Fu and Dong 2013; Yamaguchi-Shinozaki and Shinozaki 2005). Since environmental disruptions occur regularly, plants have been shown to remember previous stress circumstances and utilize these memories to assist them act when the same pressures recure and to adapt to new difficulties. The epigenetic mechanisms in the regulation of expression of genes via short RNAs, changes in histone and methylation of DNA are the scenario behind such memories. Stress memory and adaptability in medicinal plant species rely on epigenetic processes like these (Kinoshita and Seki 2014).

One of the most well-known of these “memory” systems is the “defense priming” system, which regulates the reaction to a virus or herbivore assault (Pastor et al. 2013a). In this system, the plant species responds to second attack by the disease or herbivore with a faster and more powerful response than the first, increasing its chances of survival. Medicinal plants have been found to employ a number of priming techniques, including the deposition of intermediate molecules in cellular compartments, the activation of mitogen-triggered kinases, and epigenetic processes (Conrath 2011; Pastor et al. 2013b).

5.6 Conclusion and Future Prospects

Medicinal plants face a varied number of environmental stresses during their lives, which have an impact on productivity, survival, and species distribution. Plants utilize a range of morphological, anatomical, biochemical, and molecular adaptation strategies to cope with such stresses. It's critical to have a full understanding of the many processes by which plants adapt to environmental stresses, since this will aid in the discovery and, perhaps, production of genotypes that are more resistant to environmental stresses. With the fast advancement of genomic technology, widespread studies are being conducted to better understand plant stress responses. There are still numerous obstacles to overcome in order to find and comprehend the intricacy of stress signal-transduction pathways. Furthermore, the influence of expected climatic changes on plant stress sensitivity should be considered in order to avoid widespread damage to medicinal plant diversity. Various contemporary techniques (proteomics, genomics, and metabolomics) could help in the discovery of the genetics that underpin responses to stress, allowing for direct and impartial nursing factors that affect growth and productivity of the plant.

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Chapter 6
Physiological and Biochemical Responses of Medicinal Plants to Salt Stress

Kazem Ghassemi-Golezani and Soheila Abdoli

Abstract Salt stress is a major detrimental factor that can reduce growth, but may enhance the essential oil content of medicinal plants. Physiological performance of medicinal plants may be changed by salinity, depending on species and stress level. Extreme exposure of plants to salinity can enhance sodium accumulation in plant cells that induces ionic and oxidative stresses. Notable changes in the generation of reactive oxygen species and antioxidative defense system under salt stress have been reported for several plants. High Na^+ concentration of saline soils increases sodium and decreases essential nutrient uptakes by plants, leading to nutrient imbalance, cell membrane injury, and reduction in chlorophyll content and photosynthesis. This stress can also limit water availability to the plants, which retards growth and development. The stressed plants adjust the biosynthesis of osmolytes such as proline and soluble sugars to cope with water limitation caused by salinity. Essential oil, as the main secondary metabolite of most of the medicinal plants, is strongly influenced by salinity. Salinity can change essential oil quantity and may also induce synthesis of new constituents. This is also a way for improving antioxidant capacity of plants. Several new techniques have been proposed to promote salt tolerance and essential oil production of various medicinal plants under stress.

Keywords Essential oil · Medicinal plants · Salinity · Secondary metabolites

6.1 Introduction

The demands for producing perfumes, medicine, and natural cosmetics from medicinal and aromatic plants are increasing (Maisuthisakul et al. 2007; Baatour et al. 2010). The anti-inflammatory, antispasmodic, and anti-analgesic properties of medicinal plants are attributed to their secondary metabolites such as tannins, terpenoids, and saponins, with great medicinal value to combat and cure various diseases (Balasundram et al. 2006). Due to the antioxidant capacity of essential oil

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for the preservation of human health (Giorgi et al. 2009), today, medicinal plants are considered as popular cash crops. This popularity is increasing with the realization of harms and toxicity associated with the use of synthetic drugs and antibiotics. Production of these plants and essential oil could be influenced by various environmental stresses such as salinity.

Salinity is one of the most significant environmental stresses limiting plant production worldwide, through negative impacts on anatomical, physiological, and enzymatic properties of plants. Saline soil can be defined as soil having an electrical conductivity (EC) of the saturated paste extract of 4 dS m^{-1} (40 mM NaCl) or more (Shrivastava and Kumar 2015). Salt stress may affect medicinal plants at various developmental stages. The early events of seed germination could be inhibited by salinity. This is supported by previous reports on the germination of *Ocimum basilicum* (Miceli et al. 2003), *Origanum majorana* (Ali et al. 2007), *Thymus maroccanus* (Belaqziz et al. 2009), *Ochradenus baccatus* (Hashem et al. 2014), *Cichorium intybus*, and *Foeniculum vulgare* (Hokmalipour 2015) seeds under salt stress. Seedling growth of *Thymus maroccanus* (Belaqziz et al. 2009), *Ocimum basilicum* (Ramin 2006), *Chamomilla recutita*, and *Origanum majorana* (Ali et al. 2007) is also negatively affected by this stress. Retarding assimilate mobilization, limiting cell division, and injuring hypocotyls have been noted as the main causes for these effects (Said-Al Ahl and Omer 2011). Morphological traits related to leaf growth and weight have been reduced due to salinity in several medicinal plants such as *Thymus vulgaris* (Najafian et al. 2009), *Salvia officinalis* (Ben Taarit et al. 2009), and *Mentha pulegium* (Oueslati et al. 2010). Under saline condition, all the major processes such as photosynthesis, electron transport chain, and lipid and protein metabolism are affected (Acosta-Motos et al. 2017; Ghassemi-Golezani et al. 2020; Muhammad et al. 2021). To cope with salt stress, it is essential to enhance water retention despite the strong outer osmotic pressure and to maintain photosynthetic activity.

It is well established that accumulation of natural compounds in plants strongly depends on environmental stresses (Rioba et al. 2015; Kahveci et al. 2021). Although salt stress reduces plant growth and productivity, it may affect essential oil production either positively or negatively. The essential oil content of some medicinal plants such as mint (Aziz et al. 2008) and dill (Ghassemi-Golezani and Nikpour-Rashidabad 2017) plants was decreased by salinity. However, salt stress increased the essential oil of marjoram (Baghalian et al. 2008). It has been also proved that salt stress can alter the percentage of some constitutes in the essential oil of marigold (Khalid and da Silva 2010), sage (Ben Taarit et al. 2010), and rosemary (Tounekti et al. 2015). This chapter not only broadens the understanding of the physiological and biochemical mechanisms of medicinal plants in response to salinity but will also provide concise knowledge about various techniques that can enhance salt tolerance and productivity of these plants.

6.2 Physiological Responses to Salt Stress

Plants grown under salinity are subjected to physiological changes which cause stomatal closure and reduced photosynthetic rate, followed by lower plant growth and development and notable yield losses (Gengmao et al. 2015; Sharifi and Bidabadi 2020; Abdoli et al. 2020). Two main salt tolerance mechanisms are (1) limiting salt ion influx into the plant and cytoplasm and (2) minimizing the level of stress through physiological and molecular changes. Under salt stress, net Na^+ accumulation in plant cells is specified by the ion-exchange activity of Na^+ influx and efflux. The Na^+ influx occurs largely via ion channels such as the high-affinity K^+ transporter HKT and non-selective cation channels (*NSCC*), but the Na^+ efflux is linked to *SOS1*, a Na^+/H^+ antiporter (Wu 2018). Limitation of Na^+ entrance into the roots may be related to the downregulation of genes encoding the Na^+ influx transporters or channels (Assaha et al. 2017). For example, downregulation of *CNGCs*, which are penetrable to cations such as Na^+, K^+, and Ca^{2+} (Mian et al. 2011; Hanin et al. 2016), can prevent Na^+ absorption. To survive under saline condition, medicinal plants might employ various physiological responses toward salinity tolerance. The common responses are disruption of oxidative redox in plant cells, accumulation of osmolytes, and changes in nutrient homeostasis and photosynthetic pigments, which directly affect the growth and productivity of these plants.

6.2.1 *Oxidative Responses*

The usual consequence of salinity is excessive accumulation of reactive oxygen species (ROS) in plant cells particularly in chloroplasts and mitochondria, which results in lipid peroxidation, enzyme inactivation, protein and DNA damages, and interaction with other vital constituents of plant cells. Salt stress can lead to stomatal closure, which limits carbon dioxide availability in the leaves and prevents carbon fixation, resulting in an excessive excitation energy in chloroplasts, which in turn increases the generation of ROS such as superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^\bullet$), and singlet oxygen ($^1O_2$) (Parida and Das 2005; Ahmad and Sharma 2008; Ahmad et al. 2010, 2011). The membranes of plant cells are the primary sites of salt injury. Membrane destabilization is mostly associated with lipid peroxidation, which can be initiated by ROS or by lipoxygenase activity. Disruption of membrane structure and permeability, metabolic toxicity, and damage to ultra-structures due to ROS are the factors that may eventually lead to cell death (Petrov et al. 2015). In many studies, production of ROS is increased under saline conditions. The ROS-mediated membrane damage has been shown to be a major reason of the cellular toxicity by salinity in different medicinal plants such as rosemary (Tounekti et al. 2011), ajowan (Abdoli et al. 2020), and thyme (Zrig et al. 2021).

Since the production of ROS and induction of oxidative stress by salinity are the main causes of reduced plant growth and productivity, regulation of ROS is a crucial process to avoid cellular cytotoxicity and oxidative damage. This can be achieved by a balance between generation and scavenging of reactive oxygen species. To rectify damaging effects of ROS, different defense mechanisms can be employed by plants subjected to various levels of salt stress. In general, these defense systems might be enzymatic or non-enzymatic. Increasing the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and ascorbate peroxidase (APX), glutathione peroxidases (GPX), and glutathione sulfotransferases (GST) (Kusvuran 2015; Farhangi-Abriz and Ghassemi-Golezani 2018) under salt stress can effectively remove ROS. The SOD converts superoxide radicals to H_2O_2, while APX and CAT detoxify H_2O_2 (Apel and Hirt 2004) to H_2O and O_2. Apel and Hirt (2004) reported that changes in the balance of scavenging enzymes stimulate compensatory mechanisms in plants. For instance, decreasing CAT activity upregulated the APX and GPX. Abdoli et al. (2020) stated that the activities of antioxidant defense enzymes in *Trachyspermum ammi* L. plants changed in parallel with the increased H_2O_2 and $O_2^{\bullet-}$. Enhancing antioxidant enzyme expression in *Catharanthus roseus* in response to salt stress is an indication of altering antioxidant enzyme activity and isoenzymes by salt stress (Elkahoui et al. 2005). Augmentation in oxidative stress promoted the activities of superoxide dismutase, ascorbate peroxidase, glutathione peroxidase, and catalase in salt-stressed borage plants revealing the high antioxidant potential of this medicinal plant (Afkari 2018). Increasing the activities of SOD, CAT, GR, and APX due to salt stress has been also reported in *Sesbania sesban* (Abd-Allah et al. 2015).

Furthermore, non-enzymatic antioxidants including proline, carotenoids, ascorbic acid, α-tocopherols, phenolics, and flavonoids may also play a role in inducing salt tolerance by protecting sensitive macromolecules against free radicals. There is a strong relationship between salt tolerance and enhanced activity of antioxidant enzymes and high level of proline and α-tocopherol (Naliwajski and Skłodowska 2021). Yan et al. (2017) found that salt stress reinforces the synthesis of phenolic compounds in honeysuckle (*Lonicera japonica* Thunb.) leaves, leading to the suppression of oxidative stress. Elevated antioxidant activity has also been reported in *Sesuvium portulacastrum* (Slama et al. 2015) and *Trigonella foenum-graecum* (Abdelhameed et al. 2021) under salinity. High phenolic content and valuable essential oil constitutes of medicinal plants are the natural antioxidant sources for scavenging ROS (Trouillas et al. 2003). The antioxidant capacity of essential oil is strongly related to its components. Alteration in these components may enhance the scavenging potential of essential oil in medicinal plants.

6.2.2 *Osmotic Adjustment*

A decline in osmotic potential of water containing high concentrations of dissolved salt ions causes an increase in osmotic stress in plant cells. Excessive Na^+ content in the root medium poses limitation in water accessibility for plants, leading to a disruption of plant water status and many other processes (Ghassemi-Golezani et al. 2021). One of the common metabolic consequences of osmotic stress is the accumulation of low-molecular-weight organic compounds with protective function called osmolytes, osmo-protectants, or compatible solutes, which are highly soluble and do not interfere with normal metabolic reactions, because they are non-toxic even at high cellular concentrations (Slama et al. 2015). These organic compounds can be classified in four groups: N containing compounds such as glycine betaine and proline; sugars such as raffinose and sucrose; polyols such as sorbitol and mannitol; and cyclic polyols such as pinitol (Rhodes et al. 2002; Gupta and Huang 2014). The production of proper organic osmolytes is metabolically and energetically expensive and potentially restricts plant growth by consuming considerable quantities of carbon that could otherwise be used for plant growth and productivity. In salt-subjected plants, changes in metabolism of several osmolytes, namely, sugars, proline, and glycine betaine, have been confirmed. Enhancing proline production in plants can limit the disintegration of plasmalemma under water deficit, thereby maintaining membrane integrity. Proline accumulation in plant tissues could be occurred in response to water and salt stresses (Mattioli et al. 2009). The lower level of proline in the stress-acclimated plants may be from the result of its possible conversion to proline betaine and hydroxyproline betaine, with higher osmoprotectant potential than proline. Glycine betaine is another major non-toxic osmolyte that enhances the osmolarity of the cells in salt-stressed plants, which has an important role in stress mitigation through osmotic adjustment (Gadallah 1999), protein stabilization (Mäkelä et al. 2000), and protection of the photosynthetic pigments from oxidative damage (Ashraf and Foolad 2007; Chaum and Kirdmanee 2010; Saxena et al. 2013). Salt stress also increases starch and soluble sugars such as glucose, fructose, fructans, and trehalose (Parida et al. 2004). These are also involved in osmo-protection, carbon storage, and ROS scavenging. Sugar alcohols are a class of polyols known as compatible solutes with low-molecular-weight chaperones and antioxidative properties (Ashraf and Foolad 2007). These compatible solutes protect enzymes and membrane structures that are sensitive to stress (Gupta and Huang 2014).

An increase in salt concentration induced the accumulation of soluble carbohydrates, total amino acids, and proline in *Trigonella foenum-graecum* plants (Abdelhameed et al. 2021). Increasing salt stress significantly enhanced soluble carbohydrates in *Salvia officinalis* (Hendawy and Khalid 2005) and *Satureja hortensis* (Najafi and Khavari-Nejad 2010). Similar results were obtained on *Rosmarinus officinalis* (Chetouani et al. 2019), which shows that salt stress significantly increases proline and carbohydrate contents of leaves. Increased proline content under salt stress may be caused by the upregulation of proline biosynthesis through the stimulation of pyrroline-5-carboxylate reductase (Farhadi and Ghassemi-Golezani 2020). However, some investigations revealed that reduced activity of enzymes in proline oxidation pathway such as proline dehydrogenase under salinity may lead to the accumulation of proline (Szabados and Savouré 2010). It has been reported that deduction of leaf water potential in salt-stressed ajowan plants is directly related to the osmotic and ionic stresses (Abdoli et al. 2020). In an experiment, Kotagiri and Kolluru (2017) investigated five different *Coleus* species including *C. aromaticus*, *C. amboinicus*, *C. forskohlii*, *C. barbatus*, and *C. zeylanicus* under saline condition. They found that *Coleus aromaticus* and *Coleus amboinicus* were more tolerant to salinity than the other species. This tolerance was achieved through high accumulation of carbohydrates, maintaining water potential and reducing electrolyte leakage.

6.2.3 Nutrient Status

The excessive soluble salts in the soil compete with the uptake and metabolism of nutrients that are vital for growth and development. Increasing salt uptake induces specific ion toxicities such as Na^+ and Cl^-, which can decrease the uptake of essential nutrients like potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), iron (Fe^{2+}), manganese (Mn^{2+}), zinc (Zn^{2+}), phosphorus (P), and nitrogen (N), leading to nutritional imbalance (Ghassemi-Golezani and Abdoli 2021). At high salinity, the toxic level of Na^+ and Cl^- can disrupt membrane and cause nutrient deficiency (Qureshi et al. 2007). In addition, the availability of micronutrients in saline soils is dependent on the solubility of micronutrients, soil pH, soil redox potential, and the nature of binding sites on the surfaces of organic and inorganic particle (McCauley et al. 2009). The suitable ion ratio affects physiological processes related to plant growth and development. Ashraf and Orooj (2006) in a study on ajowan (*Trachyspermum ammi* L.) at different levels of salinities (0, 40, 80, and 120 mmol L^{-1} NaCl) found that the Na^+ and Cl^- in both shoots and roots were increased, whereas K^+ and Ca^{2+} were decreased consistently with the progressive increment in salt level of the growth medium. The authors reported that *T. ammi* plants maintained higher K^+/Na^+ and Ca^{2+}/Na^+ ratios in the shoots than in the roots and the former ratio was greater than 1 even at severe salinity (120 mmol L^{-1}). However, some other medicinal plants such as *Peucedanum japonicum* are able to regulate nutrient uptakes. Reduction in Na^+ and Cl^- and increment of K^+ and Ca^{2+} contents in leaves of *Peucedanum japonicum* were an indication of high salt tolerance of this plant (Liu et al. 2020).

Selective ion uptake, ion efflux, and accumulation of Na^+ in vacuoles can reduce salt toxicity in plants (Assaha et al. 2017). These processes are controlled by some especial genes and antiporters. Three major genes involved in salt stress responses are *SOS1*, *SOS2*, and *SOS3* (Martínez-Atienza et al. 2007). The first gene that is stimulated by the entry of Na^+ into plant cells is *SOS3*, which in turn activates *SOS2* and *SOS1*. The roles of these genes are well known under salt stress, particularly in activating antiporters in plasma membrane. The *SOS1* encodes for a plasma membrane Na^+/H^+ antiporter, responsible for the efflux of sodium to the apoplast (Shi et al. 2000). The *SOS2* gene encodes a serine/threonine-type protein kinase, which activates *SOS1* (Liu et al. 2000). The *SOS3* gene encodes an EF-hand-type calcium-binding protein (Mahajan et al. 2008). The *SOS2* physically interacts with and is activated by *SOS3* (Halfter et al. 2000). Therefore, *SOS2* and *SOS3* define a regulatory pathway for Na^+ and K^+ homeostasis and salt tolerance in plants. Besides being regulated by *SOS2*, *SOS1* activity may also be regulated by *SOS4*. The *SOS4* catalyzes the formation of pyridoxal-5-phosphate, a cofactor that may serve as a ligand for *SOS1*, because the latter contains a putative binding sequence for this cofactor (Quintero et al. 2002). The *SOS1* (located in plasma membrane) and *NHX1* (located in vacuolar membranes) are two Na^+/H^+ antiporters involved in the exclusion of Na^+ from cytosol and/or accumulation of Na^+ in vacuoles to prevent Na^+ toxicity in salt-tolerant plants. At the plasma membrane, H^+-ATPase serves as the primary pump that generates a proton motive force that takes Na^+ out of cytosol (Janicka-Russak and Kabała 2015). The proton motive force created by vacuolar H^+-ATPases and H^+-PPase (Bassil and Blumwald 2014) has an important role in transporting Na^+ into vacuoles, which ultimately enhances salt tolerance of plants. The translational activation of H^+-ATPase in plasma membrane of *Jatropha curcas* roots helped to maintain potassium, calcium, nitrogen, and phosphorus absorptions by roots (Chen et al. 2012). Low salinity (4 dS m^{-1}) enhanced vacuolar H^+-ATPase and H^+-PPase pump activities, while further increase in salt stress reduced the activity of these pumps, resulting in an increased sodium uptake and accumulation that reduced plant growth in *Trachyspermum ammi* (Ghassemi-Golezani and Abdoli 2021).

6.2.4 Photosynthesis

Photosynthesis is the most important biochemical pathway that converts solar energy to chemical products in plants. These products are essential for plant growth and development. Decrement of chlorophyll content under salt stress is a commonly confirmed phenomenon. In several studies, the content of this photosynthetic pigment has been introduced as a sensitive index of the cellular metabolic state (Chutipaijit et al. 2011). Photosynthetic machinery and its efficiency are mainly affected by negative impacts of salinity on pigments, stomata functioning and gas exchange, thylakoid membrane structure, electron transport, and maximum efficiency of photosystem II (F_v/F_m), leading to reduced plant growth and yield (Wu et al. 2010; Khoshbakht and Asgharei 2015; Ghassemi-Golezani et al. 2020). Reduction in photosynthetic rates in medicinal plants like other crops under salt stress is greatly related to decrement of water potential and induced ionic and oxidative stresses. Overproduction of ROS results in pigment loss, reduction in CO_2 assimilation, and protein synthesis (Mittler 2002; Shahzad et al. 2019). Photo-oxidation of photosynthetic pigments and degradation of chlorophyll are common consequences of oxidative stress. The carotenoids, as biological antioxidants, play a critical role in protecting plant tissues from oxidative damages (Pérez-Gálvez et al. 2020). Decrement of carotenoids may lead to intense photo-oxidation in the plant tissues. According to Afkari (2018), the chlorophylls a and b and carotenoids of salt-subjected borage (*Borago officinalis* L.) plants were remarkably decreased with rising salinity. The highest levels of chlorophyll a, chlorophyll b, and carotenoids were obtained from plants grown under non-saline condition, and the lowest contents of photosynthetic pigments were recorded for plants grown under severe salinity (12 ds m^{-1} NaCl). Similarly, negative impacts of salt stress on photosynthetic machinery have been also noticed on red sage (Gengmao et al. 2014), pennyroyal (Ghassemi-Golezani and Farhadi 2021), and mint (Hosseini et al. 2021) medicinal plants. Farahbakhsh et al. (2017) found that salt stress remarkably reduced photosynthetic pigments and quantum yield and enhanced F_0 and F_m of henna (*Lawsonia inermis* L.) leaves. A significant reduction in maximum efficiency of photosystem II occurred under salinity levels of 0.6% to 1.0% NaCl (Zhao et al. 2019).

6.3 Biochemical Responses to Salt Stress

6.3.1 Secondary Metabolites

The antioxidant potential of medicinal and aromatic plants is mainly associated with their main secondary metabolites such as phenolic compounds, flavonoids, and mainly essential oils (Wannes et al. 2010). One of the most important industrial purposes in medicinal plants is promoting the synthesis of bioactive compounds and secondary metabolites. The synthesis of major classes of secondary metabolites is occurred through shikimic or mevalonate pathways. These pathways can be controlled by genetic and environmental stresses (Valifard et al. 2014). The distribution of carbon can be modified under salt stress, resulting in changes in the biosynthesis of main secondary metabolites such as phenolics, flavonoids, or terpenes in stressed plants (Vafadar Shoshtari et al. 2017). The phenolics, flavonoids, tannins, saponins, alkaloids, and terpenes are the main secondary metabolites of medicinal plants with substantial medical values to cure various diseases (Nobori et al. 1994; Hodek et al. 2002; Balasundram et al. 2006).

Several investigations have revealed that salinity may affect the quantity and quality of phytochemicals in medicinal plants. Increment of saponin and decrement of alkaloids, flavonoids, and tannins in *Acalypha wilkesiana* plants under salinity have been reported by Odjegba and Alokolaro (2013). Similarly, salinity caused considerable decrease in total phenolic, flavonoid, caffeic acid, chicoric acid, and rosmarinic acid contents in *Ocimum basilicum* plants (Kahveci et al. 2021). However, alkaloid content of *Catharanthus roseus* was increased by salinity (Jaleel et al. 2008). Decrement of total alkaloids, flavonoids, and tannins was observed in plants grown under salinity, which could be due to ROS impacts on enzymes involved in the biosynthesis of these metabolites (Sharma et al. 2012). Elevating phenolic and flavonoid components in myrtle (*Myrtus communis* L.) leaves might be interpreted as an adapting mechanism of plants to environmental condition (Vafadar Shoshtari et al. 2017), due to phenolic acids' roles in lignin biosynthesis (Cheynier et al. 2013). Similar results were also found in *Anethum graveolens* (Mehr et al. 2012), *Olea* *europaea* (Petridis et al. 2012), *Lactuca sativa* (Ouhibi et al. 2014), *Lonicera japonica* (Yan et al. 2017), and *Mentha pulegium* (Farhadi and Ghassemi-Golezani 2020), suggesting that salinity plays an important role in the induction of phenolic biosynthesis and consequently improving the antioxidant capacity of plants.

According to Duc et al. (2021), salinity significantly altered the polyphenol profiles of *Eclipta prostrata* plants. Exposure to salinity for 8 weeks was led to a notable decline in the content of total polyphenols and some of the major phenolics, particularly under high saline condition. However, synthesis of another two identified flavonoids including luteolin and luteolin-glucoside was elevated by salt stress. High valerenic acid accumulation has been shown in *Valeriana officinalis* plants under moderate (5 dS m^{-1}) salinity by Amanifar and Toghranegar (2020). Salt stress, especially 150 mM NaCl, improved the leaf medicinal quality of honeysuckle plants by inducing the synthesis of phenolics, flavonoids, chlorogenic acid, and luteolosid through the stimulation of gene transcription and activity of phenylalanine ammonia-lyase. The chlorogenic acid and luteolosid contents were elevated by 67.43% and 54.26% after 15 days of exposure to 150 mM NaCl, respectively (Yan et al. 2017). Ben Taarit et al. (2010) reported that salinity (25 to 75 mM NaCl) can decrease the polyunsaturated fatty acids while increasing the monounsaturated acids through enhancing gadoleic acid in salt-stressed sage (*Salvia officinalis* L.) plants. The authors also concluded that reduction in plant growth and total fatty acid contents and increasing the essential oil content in this medicinal plant by salt stress may be the result of altering assimilate partitioning patterns in favor of biosynthesis and accumulation of terpenes. The interaction of salt stress and growth stage can also provide interesting results in phenolic and flavonoid compounds of myrtle (*Myrtus communis* L.) plants. The highest content of linalool was reported at spring and summer for plants grown under non-saline condition and at fall for plants grown under 4 dS m^{-1} NaCl. Furthermore, harvesting in fall was resulted in the reduction of the major essential oil constitutes and enhancement of phenolic acids (Vafadar Shoshtari et al. 2017).

6.3.2 Essential Oil Quantity and Quality

The major secondary metabolite in most of medicinal plants is essential oil. The effect of salt stress on essential oil and antioxidant activity has been reported in many medicinal plant species such as *Matricaria chamomilla* (Razmjoo et al. 2008), *Salvia mirzayanii* (Valifard et al. 2014), and *Anethum graveolens* (Ghassemi-Golezani and Nikpour-Rashidabad 2017). The negative effect of salt stress in essential oil yield was reported for various medicinal plants like *Mentha piperita* (Tabatabaie and Nazari 2007), *Thymus maroccanus* (Belaqziz et al. 2009), *Ocimum basilicum* (Said-Al Ahl and Mahmoud 2010), and *Rosmarinus officinalis* (Sarmoum et al. 2019). The essential oil content was strongly reduced in salt-stressed *Matricaria chamomilla* (Razmjoo et al. 2008), *Mentha canadensis* (Yu et al. 2015), and *Anethum graveolens* (Ghassemi-Golezani and Nikpour-Rashidabad

2017) plants. Contrariwise, it has been demonstrated that salt stress may positively affect the essential oil content in some medicinal plants. The essential oil content of *Calendula officinalis* (Khalid and da Silva 2010), *Thymus vulgaris* (Cordovilla et al. 2014), *Rosmarinus officinalis* (Dehghani Bidgoli et al. 2019), and *Trachyspermum ammi* (Ghassemi-Golezani and Abdoli 2021) was significantly enhanced by salt stress.

Essential oil constituents may vary, depending on plant phenological growth stage, plant organs, and environmental conditions (Singh and Guleria 2013; Sarmoum et al. 2019). Previous studies have shown that salinity has also perceptible effects on essential oil constitutes (Neffati and Marzouk 2008; Ben Taarit et al. 2012; Tounekti et al. 2011). These effects depend on the severity of salt stress and plant species. Moreover, the synthesis of some constitutes was only induced by environmental stresses. Sarmoum et al. (2019) in a study on rosemary (*Rosmarinus officinalis* L.) revealed that based on GC/MS analysis, 10 and 11 volatile compounds were identified in essential oil of unstressed and salt-stressed rosemary plants, respectively. However, 13 volatile compounds were identified in essential oil of water-stressed plants. According to the finding of this research, the essential oil of rosemary plants subjected to saline water was differentiated from other irrigational treatments by the synthesis of hydrocarbon sesquiterpenes and oxygenated sesquiterpenes including dodecane and caryophyllene. Furthermore, the synthesis of cemirene in the essential oil of *Aloysia citrodora* was only induced by 5.6 dS m^{-1} NaCl (Tabatabaie and Nazari 2007). In another study on *Rosmarinus officinalis* by Dehghani Bidgoli et al. (2019), increasing salinity up to 10 gL^{-1} NaCl was led to an enhancement in phellandrene content of essential oil. Khalid and da Silva (2010) have found that irrigation of marigold (*Calendula officinalis*) plants with saline water enhanced essential oil yield and the main volatile constitutes such as α-cadinol and γ-cadinene. The low (25 mM NaCl) and moderate (75 mM NaCl) salinities promoted the essential oil production and the major oxygenated monoterpenes such as 1,8-cineole and α-thujone in sage plants. Nevertheless, high salinity (100 mM NaCl) may significantly reduce the essential oil content of plants (Ben Taarit et al. 2009). Abd El-Wahab (2006) observed that anethole percentage in fennel was diminished under salinity. The 1,8-cineole content of *Rosmarinus officinalis* essential oil was also considerably decreased with rising salt stress (Tounekti et al. 2015). Ben Taarit et al. (2010) in a study on *Salvia officinalis* found that viridiflorol was the main essential oil compound in control and 25 mM NaCl, but 1,8-cineole was the predominant compound under 50 and 75 mM salinities, and manool prevailed under 100 mM salinity. Yu et al. (2015) indicated that salinity had no effect on the content of oxygenated monoterpenes, increased the menthone and pulegone contents, while decreased menthol under saline condition. These variations could be due to the induction of the specific enzymes involved in the biosynthesis of these compounds by salinity (Karray-Bouraoui et al. 2009).

The antioxidant capacity of essential oil is mainly associated with essential oil constitutes. Salt stress may influence the antioxidant activity through the alteration of major components. Earlier studies (Du et al. 2008; Ramak et al. 2013) revealed that carvacrol-rich essential oils have a significant antioxidant property. Terenina et al.

(2011) concluded that the phenolic monoterpenes, carvacrol and thymol, are the main antioxidants and most biologically effective compounds in oregano essential oil. The antioxidant potential of *Origanum vulgare* essential oil was also related to phenolic monoterpenoid carvacrol (Morshedloo et al. 2017). According to Andreani et al. (2013), the essential oils rich and poor in p-cymene were found in plants grown under high and low salinities, respectively. This result reveals that oxygenated constitutes mainly have antioxidant and scavenging capacity in *Limbarda crithmoides* essential oil. The highest antioxidant activity of myrtle essential oil was found in plants grown under 6 dS m^{-1} salinity, based on DPPH radical scavenging activity, reducing power (FTC) and β-carotene/linoleic acid model systems (Vafadar Shoshtari et al. 2017).

6.4 Treatments to Improve Plant Productivity Under Salt Stress

Salinity is one of the main threats to sustainable agriculture that globally decreases plant production by impairing major plant processes. However, several novel approaches have been used to alleviate the effects of salinity on plant growth and productivity through stimulating various physiological, biochemical, and molecular processes. Some of these beneficial techniques are related to plant growth medium, rhizosphere, and seeds. Improving soil physical properties and its nutrient status by addition of bio-stimulants including plant growth-promoting rhizobacteria (PGPRs) and arbuscular mycorrhizal fungi (AMF) can help plants to cope with salt stress (Mesquita et al. 2014; Barnawal et al. 2016; Amanifar et al. 2019; Dastogeer et al. 2020). On the other hand, using foliar treatments including hormonal and nutritional treatments might be effective in mitigating salt toxicity on medicinal plants (Chrysargyris et al. 2019; Farhadi and Ghassemi-Golezani 2020). Some of the valuable methods to ameliorate detrimental impacts of salinity on various medicinal plants are summarized in Table 6.1.

6.4.1 Hormonal Treatments

Phytohormones, also known as plant growth regulators, are usually involved in physiological and molecular responses of many stressed plants. These responses include activation of the plant defense system, accumulation of osmo-protectants (Abdoli et al. 2020), regulation of stomatal opening and closing (Acharya and Assmann 2009), expression of defense-related genes (Hu et al. 2017), and interactions with other plant hormones (Ku et al. 2018; Wang et al. 2020). It is indicated that plant growth regulators such as salicylic acid, indole acetic acid, gibberellins, and jasmonates play critical roles in plant salt tolerance (Egamberdieva and

Table 6.1 The useful treatments for reducing salt damages on medicinal plants

Class of treatment	Treatment	Plant species	Effects	References
Foliar treatments	Fe_2O_3-NPs	*Trachyspermum ammi* L.	Improvement of water and nutrient status, photosynthetic pigments, and soluble protein content and stimulation of ATPase and PPase activities	Abdoli et al. (2020)
	Fe_2O_3-NPs	*Mentha piperita* L.	Increasing plant growth through enhancing nutrient uptakes and decreasing lipid peroxidation and proline contents and antioxidant potential	Askary et al. (2017)
	ZnO-NPs, Fe_3O_4-NPs	*Moringa peregrina*	Reducing salt toxicity and enhancing photosynthetic pigments and proline, sugar, and antioxidant capacity, resulting in improving plant growth	Soliman et al. (2015)
	K, Zn, Si	*Lavandula angustifolia* Mill.	Enhancing antioxidant capacity and essential oil yield and inducing biosynthesis of major volatile oil constituents	Chrysargyris et al. (2018)
	Si	*Glycyrrhiza uralensis*	Regulating the endogenous hormones including indole-3-acetic acid, gibberellic acid, and abscisic acid and improving plant growth	Lang et al. (2019)
	Si	*Capsicum annuum*	Upregulating the synthesis of proteins involved in several metabolic processes and preventing oxidative damage by increasing the activities of antioxidant enzymes	Manivannan et al. (2016)
	Se	*Coriandrum sativum*	Supplementation of 50 mM Se mitigated salt toxicity via reducing Na^+/K^+ ratio in plant tissues	Ghazi (2018)
	Salicylic acid	*Mentha pulegium*	Enhanced proline content via increasing pyrroline-5-carboxylate reductase activity and decreasing proline oxidase activity and stimulated antioxidant potential	Farhadi and Ghassemi-Golezani (2020)

(continued)

Table 6.1 (continued)

Class of treatment	Treatment	Plant species	Effects	References
	Salicylic acid	*Achillea millefolium* L	Enhancing primary and secondary metabolisms and improving essential oil quantity and quality	Gorni et al. (2020)
	Salicylic acid	*Salvia coccinea*	Increment of chlorophyll, carotenoids, and polyphenols and antioxidant potential	Grzeszczuk et al. (2018)
	Salicylic acid	*Rosmarinus officinalis* L.	Decreasing sodium and chloride uptakes; increasing phenolic, chlorophyll, carbohydrate, and proline contents; and stimulating antioxidant enzyme activities and gene expression	El-Esawi et al. (2017)
	Salicylic acid	*Catharanthus roseus*	Improving total alkaloids, vincristine and vinblastine	Idrees et al. (2011)
	Methyl jasmonate	*Carthamus tinctorius*	Promotion of plant growth through increment of proline, sugar, and photosynthetic pigments	Chavoushi et al. (2019)
	Gibberellic acid	*Nigella sativa* L.	Improving water status, proline content, and CAT and POX activities	Rashed et al. (2017)
	γ-Amino butyric acid, salicylic acid, and vermicompost extract	Saffron	Minimizing the detrimental effects of salinity by improving nutrient contents and triggering antioxidant defense system	Feizi et al. (2021)
Soil treatments	PGPRs	*Coriandrum sativum* L.	Augmenting chlorophyll a and b contents and CAT activity and reducing Na^+ absorption, thereby improving plant productivity	Rabiei et al. (2020)
	PGPRs	*Chlorophytum borivilianum*	Amelioration of negative effects of salinity on plants through alteration of hormones including reduced ethylene and abscisic acid contents and enhanced indole acetic acid in inoculated plants	Barnawal et al. (2016)
	Mycorrhizal fungi	*Valeriana officinalis* L.	Promoting proline, soluble sugars, and total phenolic contents and augmenting secondary metabolite synthesis	Amanifar and Toghranegar (2020)

(continued)

Table 6.1 (continued)

Class of treatment	Treatment	Plant species	Effects	References
	Mycorrhizal fungi	*Osmium basilicum*	Improving gas exchange, photosynthetic pigments, proline accumulation, and nutritional balance	Elhindi et al. (2017b)
	Mycorrhizal fungi	*Glycyrrhiza glabra*	Increasing K^+ and P contents and proline accumulation and stimulating the expression of major genes participating in secondary metabolites	Amanifar et al. (2019)
	Gibberellic acid	*Hibiscus sabdariffa* L.	Increasing photosynthetic pigments and leaf water content and inducing the activity of carbonic anhydrase	Ali et al. (2012)
	Zeolite	*Rosmarinus officinalis* L.	Decrement of sodium and chloride uptakes and increment of photosynthetic pigments and essential nutrient contents	Helaly et al. (2018)
	Vermicompost	*Borago officinalis*	Promoting antioxidant enzymes and photosynthetic pigments	Afkari (2018)
Seed treatments	Salicylic acid, tryptophan, and β-carotene	*Ocimum basilicum* L.	Enhancing growth and yield through relieving the adverse impacts of salinity by enhancing phenolic and linalool and eugenol contents and decreasing methyl eugenol percentage	Kahveci et al. (2021)
	Salicylic acid and gibberellic acid	*Anethum graveolens*	Accumulating osmolytes such as proline, glycine betaine, and soluble sugars, enhancing antioxidant enzyme activities and essence production of dill organs	Ghassemi-Golezani and Nikpour-Rashidabad (2017)
	Salicylic acid and indole acetic acid	*Abelmoschus esculentus*	Enhancing plant defense potential through stimulating antioxidant activities and DPPH radical scavenging capacity	Esan et al. (2017)
	Aminolevulinic acid	*Cassia obtusifolia* L.	Enhancing chlorophyll, soluble sugar, soluble protein, and proline contents and photochemical efficiency of photosystem II	Zhang et al. (2013)

NPs nanoparticles; *PGPRs* plant growth-promoting rhizobacteria

Jabborova 2015; Esan et al. 2017; Ghassemi-Golezani and Farhangi-Abriz 2018; Chavoushi et al. 2019; Delgado et al. 2021). Salicylic acid (SA) as a phenolic phytohormone is recognized to mitigate the harmful effects of salt stress through improving water and nutrient status and photosynthetic pigments (Abdoli et al. 2020), modulating leaf gas exchange (Miura and Tada 2014; Sah et al. 2016), and inhibiting ethylene synthesis (Khan et al. 2014). The positive effects of SA in mitigating salt stress on various medicinal plants have been well documented (Elhindi et al. 2017a; Gorni et al. 2020). Improving physiological attributes such as starch, sugar, protein and proline contents, essential oil production, and defense system (catalase, peroxidase, and ascorbic peroxidase activities) by SA can help to ameliorate salt toxicity in feverfew (*Tanacetum parthenium*) plants (Mallahi et al. 2018). Exogenous SA promoted several metabolites in *Egletes viscosa* plants which may contribute to the distributing of Na^+ and K^+ among roots and shoots, thereby overcoming salt stress-induced photosynthesis disruption (Batista et al. 2019). Treating salt-stressed henna (*Lawsonia inermis* L.) plants with SA increased photosynthetic pigments, protein content, CAT activity, leaf water content, and photochemical efficiency of photosystem II while decreasing electrolyte leakage, F_0 and F_m. The positive effects of SA on promoting plant growth were documented by Farahbakhsh et al. (2017). Foliar application of SA alleviated the adverse effects of 100 mM NaCl on *Salvia coccinea*, through increasing chlorophyll, carotenoids, and polyphenols and antioxidant potential (Grzeszczuk et al. 2018). The SA can enhance total soluble phenolics and lignin contents via significant stimulation of phenylalanine ammonia-lyase (PAL) activity (Neelam et al. 2014). The bioactive compounds and antioxidant potential of salt-stressed *Achillea millefolium* were stimulated by SA treatment (Gorni et al. 2019).

Jasmonic acid (JA) as an endogenous plant growth regulator is also identified as a stress-related hormone in plants (Wang et al. 2020). Environmental stresses have been observed to enhance JA biosynthesis-related genes and endogenous jasmonic acid (JA) in plant tissues (Tani et al. 2008; Du et al. 2013). Similar to SA, applications of jasmonates have been reported to promote salt tolerance in plants. Using methyl jasmonate slightly improved *Carthamus tinctorius* plant growth via enhancing proline, sugar, and particularly photosynthetic pigments (Chavoushi et al. 2019). Niazian et al. (2021) found that SA was more effective than methyl jasmonate for the synthesis of essential oil constituents including γ-terpinene, p-cymene, and thymol in stressed ajowan plants. According to Ali et al. (2012), application of gibberellic acid to the saline soil reduced the harmful effects of salinity on plant photosynthetic pigments and growth via enhancing the activity of carbonic anhydrase. The harmful impacts of salinity on *Nigella sativa* plants were reversed by gibberellic acid through the stimulation of the defense system by increased proline accumulation and antioxidant enzymes (Rashed et al. 2017). The beneficial impacts of foliar spray of SA and GA_3 in *Mentha piperita* plants were associated with improved physiological and biochemical processes and enhanced essential oil yield and menthol content (Khanam and Mohammad 2018). Pre-treatment of okra seeds with salicylic acid and indole acetic acid was resulted in higher antioxidant activities and DPPH radical scavenging capacity in okra (*Abelmoschus esculentus*), indicating the ameliorative potential of these hormones in mitigating the destructive effects of reactive oxygen species under salinity (Esan et al. 2017).

6.4.2 Nutritional Treatments

One of the most important damages of salt stress in plants is ionic restriction and its negative effects on growth through disruption of major physiological and biochemical processes. Improving nutrient status of plants may be considered as a method of alleviating salinity stress. Nutrients can be applied as either soil fertilizer or foliar spray to induce major salt stress tolerance mechanisms in medicinal plants (Abdoli et al. 2020; Amiripour et al. 2021). According to Chrysargyris et al. (2019), foliar application of K, Zn, and Si was proved as a useful technique to alleviate the stress effects caused by high salinity in spearmint (*Mentha spicata* L.) plants. This alleviation was achieved via increased antioxidant activity and detoxified oxidative stress products. According to Chrysargyris et al. (2018), the growth, antioxidant capacity, and essential oil yield of medicinal lavender (*Lavandula angustifolia* Mill.) plants exposed to salt stress were improved by K and Zn supplementation. These authors showed that K foliar spray changed the primary metabolite pathways in favor of biosynthesis of major volatile oil constituents under moderate salinity, revealing the potential of lavender plant for cultivation under prevalent semi-saline conditions. Exogenously applied Si, particularly at 1.5 mM concentration, resulted in an improvement in physiological parameters, thereby alleviating the negative effects of salt treatment on the anatomical attributes such as thickness of leaf blade and thickness of palisade parenchyma cells in borage (*Borago officinalis* L.) plants under saline condition (Torabi et al. 2015). The amelioration of salt toxicity effects by Si nutrition has been also attributed to enhanced chlorophyll and relative water content (Amiripour et al. 2021) and decreased oxidative damage (Farshidi et al. 2012). In salt-stressed *Glycyrrhiza uralensis* plants, Si treatment noticeably increased K^+/Na^+ ratio in plant tissues and SOD and POD activities and reduced MDA concentration, resulting in greater detoxification of reactive oxygen species and lower lipid peroxidation (Li et al. 2016). Some investigations have identified the Si-mediated selective ion uptake and regulating Na^+/K^+ ion channels (Liu et al. 2019).

Exogenous application of Ca can alleviate salt stresses by the regulation of antioxidant activities and plant defense mechanisms. Calcium is a second messenger that induces the growth and differentiation of cells and tissues in plants. Moreover, Ca is highly required by medicinal legumes for nitrogen fixation processes. Other targets of Ca effects on mitigating salt stress are related to intracellular processes. The *SOS* pathway can regulate plant ion homeostasis under salinity by Ca^{2+} signals. The *SOS3*, a Ca^{2+} sensor, interacts with *SOS2* protein kinase to transduce the signal downstream. The Na^+/H^+ antiporter of *SOS1* is activated by *SOS3/SOS2* complex, which reestablishes cellular ion homeostasis. The stimulation of the *SOS3/SOS2* pathway by calcium also induces vacuolar Na^+ accumulation by the vacuolar Na^+/H^+ antiporters (Naeem et al. 2013). In addition, the ameliorative effects of some other elements in the form of nanoparticles (NPs) have been investigated on various medicinal plants under salinity (Ghazi 2018; Abdoli et al. 2020). Application of 50 mg L^{-1} cerium oxide NPs (CeO_2 NPs) as the best concentration was resulted in boosting the growth of Moldavian balm (*Dracocephalum moldavica* L.) plants exposed to salinity, via improving agronomic traits, proline, photosynthetic pigments, and antioxidant enzymes. This higher antioxidant enzymatic activity caused a decline in MDA and H_2O_2 contents and electrolyte leakage (Hasan Zadeh Mohammadi et al. 2021). Using Ce-NPs has been proved to maintain cell structure due to Ce role in chlorophyll synthesis, ROS detoxifying, and maintaining chloroplast structure and cell wall (Jahani et al. 2019; Jurkow et al. 2020). Further information about the positive effects of nutrients on the mitigation of salt toxicity are provided in Table 6.1.

6.4.3 Bio-stimulants

Most plants can form symbiotic associations with the soil microorganisms that have the remarkable potential to improve the rhizospheric soil characteristics and availability of nutrients for plant growth (Navarro et al. 2013; Ahanger et al. 2014). Plants are colonized by various useful microorganisms, including endophytes, nitrogen-fixing bacteria, and mycorrhizal fungi, which closely collaborate with each other and can mediate important physiological processes, especially nutrient attainment and plant tolerance to abiotic stresses (Abd-Allah et al. 2015). Utilizing the potentially useful plant growth-promoting rhizobacteria (PGPRs) and mycorrhizal fungi (MF) is identified as an effective method for mitigating the toxic effects of salinity and improving the growth of plants (Bhat et al. 2020; Dastogeer et al. 2020; Kumar Arora et al. 2020; Moncada et al. 2020; Gupta et al. 2021). Moreover, various metabolic and genetic strategies used by these rhizosphere microorganisms can reduce the impact of salt stress on plants (Gopalakrishnan et al. 2015; Singh 2014). These microorganisms not only react to signal molecules secreted by plant roots but also release varied signaling molecules affecting plants, leading to increased stress resistance as well as root and shoot growth (Zhang et al. 2017). It has been also demonstrated that the tripartite plant-bacterial-fungal relationship could be a promising approach to alleviate the detrimental impacts of salt stress on the productivity of medicinal plants, through enhancing the activities of enzymatic and non-enzymatic antioxidants (Arora et al. 2020). In this section, the mechanisms of improving salt tolerance in PGPR- and MF-treated plants will be discussed.

PGPRs are involved in mitigating salt toxicity in plants by changes in the expression of defense-related genes, exopolysaccharide synthesis, osmolyte accumulation, reducing Na^+ absorption, and improving phytohormones and nutrient status in salt-stressed plants (Kumar Arora et al. 2020). Solubilizing inorganic phosphate, deaminizing ACC, and producing indole acetic acid and hydrocyanic acid under salt stress by plant growth-promoting bacteria have been well published.

Enhanced ammonia and α-ketobutyric acid contents as a result of PGPR inoculation are an indication of ACC reduction under salinity (Djebaili et al. 2021). Inoculation of coriander seeds with *Azospirillum brasilense* and *Azotobacter chroococcum* was resulted in improving chlorophyll a and b contents and CAT activity and reducing Na^+ uptake, leading to enhanced seed yield under salinity (Rabiei et al. 2020). The positive impacts of bio-stimulants in salt-stressed *Plectranthus amboinicus* are associated with the improved nutrient status of the plants (da Silva Mesquita et al. 2014). Using salt-tolerant PGPRs can be an effective strategy in elevating biomass and bacoside-A contents (a saponin) in Brahmi (*Bacopa monnieri*) plants under saline condition (Pankaj et al. 2020). According to Barnawal et al. (2016), ACC deaminase-producing *Brachybacterium paraconglomeratum* strain *SMR20* significantly protected *Chlorophytum borivilianum* from salt stress-induced biomass losses. This protection was attributed to hormonal and biochemical changes due to *B. paraconglomeratum*. The *SMR20* inoculation potentially reduced ethylene through deamination of ACC (precursor for ethylene synthesis). In addition, abscisic acid content and lipid peroxidation were decreased, and chlorophyll and indole-3-acetic acid were increased by *SMR20* under saline condition. Using *Pseudomonas stutzeri*, *Pseudomonas putida*, and *Stenotrophomonas maltophilia* enhanced internal IAA, cytokinin, and gibberellic acid in *Coleus* plants (Patel and Saraf 2017).

In a mycorrhizal association, the fungus either colonizes the root tissues of the host, intracellularly as in arbuscular mycorrhizal fungi, or forms extracellular exchange mechanisms outside of the root cells, as ectomycorrhizal fungi. Mycorrhizal fungi can noticeably enhance the rhizospheric soil characteristics, thereby altering plant growth (Navarro et al. 2013; Ahanger et al. 2014). Using arbuscular mycorrhizal fungi improves soil structure and promotes plant growth under optimal and stressful conditions (Rabie and Almadini 2005; Cho et al. 2006). The mycorrhizal fungi enhance plant growth and mitigate the adverse effects of environmental stresses by regulating morpho-physiological properties and nutrient status. In addition to influencing the plant physiological processes, MF can also promote root elongation, which increases water and nutrient absorptions (Aroca et al. 2013; Ahanger et al. 2014). Arbuscular mycorrhizal fungi play a critical role in alleviating salt toxicity by normalizing the uptake of essential nutrients and recovering the water relations (Carretero et al. 2008; Porcel et al. 2012). The advantageous effects of mycorrhizal fungi on morphological and physiological attributes have been well understood in various crops and medicinal plants (Asghari et al. 2005; Ahanger et al. 2014; Gheisari Zardak et al. 2018). Mycorrhizal fungi have been demonstrated to increase the growth of *Sesbania sesban* under salinity through enhancing antioxidant capacity and photosynthetic pigments and altering hormonal status. Increasing endogenous indole-3-acetic acid, indole butyric acid, and gibberellic acid and decreasing abscisic acid caused the amelioration of salt toxicity in plants (Abd-Allah et al. 2015). The nutritional homeostasis and K^+/Na^+ and Ca^{2+}/Na^+ ratios were improved in basil (*Osmium basilicum*) by mycorrhiza. The potential role of mycorrhiza inoculation as a bio-stimulant in enhancing salt tolerance of basil plants was also attributed to improving gas exchange, proline accumulation, water use efficiency, and photosynthetic pigments (Elhindi et al. 2017b). Inoculation with

Rhizophagus intraradices and *Funneliformis mosseae* induced an increase in growth, nutrient acquisition, and the ratios of K^+/Na^+, Ca^{2+}/Na^+, Mg^{2+}/Na^+, and total chlorophyll/carotenoids in salt-subjected *Valeriana officinalis* plants. The superiority of *F. mosseae* in the mitigation of salt toxicity was more than *R. intraradices* (Amanifar and Toghranegar 2020). Moreover, there are evidences of biochemical and molecular alterations caused by mycorrhizal fungi in medicinal plants. Various biochemical compounds may be accumulated in mycorrhizal-treated plants under salinity (Rivero et al. 2018). Differences in phenolic accumulation of untreated and MF-treated *Eclipta prostrata* plants under saline condition may be resulted from the differences in the biochemical and physiological status in the plants due to these bio-stimulants (Duc et al. 2021). Colonization of liquorice (*Glycyrrhiza glabra*) by arbuscular mycorrhizal fungus *Funneliformis mosseae* under salinity not only increased essential nutrients of K^+ and P and proline accumulation, which resulted in stimulating the expression of major genes participating in the glycyrrhizin biosynthesis including beta-amyrin synthase (*bAS*), squalene synthase1 (*SQS1*), and *P450* (Amanifar et al. 2019). Based on the notions presented in this section, the development of novel bio-stimulants for saline soils could be a sustainable approach for improving growth and productivity of salt-stressed medicinal plants.

6.5 Concluding Remarks and Future Prospects

Salt stress can change physiological and metabolic activities of medicinal plants. The main physiological processes influenced by this stress are ionic toxicity and nutrient imbalance. The high absorption of toxic sodium also results in oxidative stress, lipid peroxidation, and photosynthetic damages, which impair the growth and development of plants. Moreover, biochemical products and particularly secondary metabolites of medicinal plants can be strongly influenced by this environmental constraint. The essential oil synthesis and its major constitutes with considerable medicinal values might be enhanced or inhibited by salinity, depending on plant species and stress level. These variations in essence constitutes due to salt stress can be used in enhancing antioxidant potential of plants. Future research works may focus on physiological and molecular aspects of plant responses to salinity in varying temperatures due to climate change, in order to identify more superior methods for overcoming the harmful impacts of this stress on medicinal plants.

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Chapter 7
Horizontal Natural Product Transfer: A Phenomenon Which Is Responsible for the Widespread Alkaloidal Contaminations of Herbal Products

T. Hijazin, L. Lewerenz, M. Yahyazadeh, and D. Selmar

Abstract Induced by numerous alarming reports of the European Food Safety Authority on widespread contaminations of plant-derived commodities by poisonous alkaloids (nicotine, pyrrolizidine alkaloids), the origin of these alkaloidal contaminations had been investigated. These studies unveiled that alkaloids, which have been leached out from decomposing alkaloidal donor plants, are taken up by the roots of acceptor plants growing in the vicinity. These insights had been the basis for establishing the so-called horizontal natural product transfer. Meanwhile, it is verified that many other natural products, such as coumarins or stilbenes, are also taken up from the soil by plant roots and then are allocated into the leaves. Recent research revealed that alkaloids are also transferred from living and vital donor plants into plants growing in their vicinity. Moreover, it became evident that in a number of acceptor plants, the imported natural products are modified, whereas in others, they are just accumulated. These modifications comprise hydroxylation, methylation and glucosylation processes analogous to the modifications described for xenobiotics. In the past, it was presumed that these reactions are part of a deliberate detoxification mechanism, denoted as "green liver concept". But, since the mode and extent of these modifications strongly vary between different plant species, a general and universal mechanism such as the "green liver concept" can be excluded.

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Apart from the high relevance for preventing contaminations of plant-derived commodities, the novel insights in the “horizontal natural product transfer” will also impact our understanding of plant-plant interactions.

Keywords Horizontal transfer · Specialized metabolites, natural products · Pyrrolizidine alkaloids · Alkaloids · Coumarins · Allelopathy · Xenobiotics

7.1 Introduction

In the last decade, numerous alarming reports of the European Food Safety Authority unveiled widespread contaminations of plant-derived commodities by nicotine (EFSA 2011a), pyrrolizidine alkaloids (EFSA 2011b, 2016, 2017) and tropane alkaloids (EFSA 2018). The first time these problems arose was when nicotine was determined in many plant-derived products. It turned out that more than 70% of the tested herbal tea and spice samples contained nicotine significantly above the limit of quantification (EFSA 2011b). In the same manner, a comprehensive evaluation by Mulder et al. (2015) revealed that pyrrolizidine alkaloids (PAs) are present in more than 90% of all herbal tea samples tested. Due to their high toxicity for livestock, wildlife and humans (Fu et al. 2004; Wiedenfeld and Edgar 2011), PAs are of special interest when present in plant-derived commodities (Selmar et al. 2019a). Indeed, the genuine PAs are not poisonous, but in the liver of vertebrates, they are oxidized to yield the unstable highly toxic dehydropyrrolizidine alkaloids, also denoted as PA pyrroles (Mattocks 1986).

With respect to the widespread contaminations by PAs, it was rapidly argued that the contaminations are due to accidental co-harvest of alkaloid-containing weeds (Stegelmeier et al. 1999; Van Wyk et al. 2017; Selmar et al. 2019a). However, because of the rare and very restricted occurrence of nicotine-containing weeds, a corresponding path of contaminations for nicotine contaminations could be excluded. Thus, there must have been another cause for widespread alkaloidal contaminations of plant-derived commodities. Already more than half a century ago, it was demonstrated that alkaloids could be taken up from the soil (Winter et al. 1959c; Franz 1962). Accordingly, it seemed to be likely that the alkaloidal contaminations of the related staple plants could be due to an uptake from the soil. Meanwhile, it was verified that the alkaloids present in the corresponding staple plants are—at least in part—taken up from the soil, in which they had been leached out from rotting plant material or exuded from alkaloid-containing weeds grown in the vicinity (Selmar et al. 2019a; Table 7.1). In addition, it was demonstrated that a wide range of alkaloids is taken up by plant roots (Yahyazadeh et al. 2017; Lewerenz et al. 2020). These coherences had been the basis for the discovery and formulation of the so-called horizontal natural product transfer (Selmar et al. 2015a, 2019a, 2020; Nowak et al. 2017). In this treatise, the scientific background of this widespread phenomenon and its relevance for contaminations of plant-derived commodities are outlined.

Table 7.1 Documented transfer of natural products between plants of different species

Compounds	Donor plant	Acceptor plant	Authors
Arbutin[a]	*Arctostaphylos uva-ursi*	*Triticum aestivum*	Winter et al. (1960)
Aesculin/aesculetin[a]	*Aesculus hippocastanum*	*Triticum aestivum*	Winter et al. (1960)
Aristolochic acids	*Aristolochia clematitis*	*Zea mays*	Pavlović et al. (2013)
Aristolochic acids	*Aristolochia clematitis*	*Cucumis sativus*	Pavlović et al. (2013)
Atropine	Solanaceae	*Triticum aestivum*	Jandrić et al. (2013)
Benzoxazinoids	*Secale cereale*	*Vicia villosa*	Hazrati et al. (2020)
Coumarin	*Melilotus albus*	*Triticum aestivum*	Winter et al. (1960)
Nicotine	*Nicotiana tabacum*	*Mentha× piperita*	Selmar et al. (2015b)
Nicotine	*Nicotiana tabacum*	*Coriandrum sativum*	Selmar et al. 2018)
Nicotine	*Nicotiana tabacum*	*Mentha x piperita*	Selmar et al. (2018)
Nicotine	*Nicotiana tabacum*	*Ocimum basilicum*	Selmar et al. (2018)
Nicotine	*Nicotiana tabacum*	*Petroselinum crispum*	Selmar et al. (2018)
Pyrrolizidine alkaloids	*Senecio jacobaea*	*Matricaria chamomilla*	Nowak et al. (2016)
Pyrrolizidine alkaloids	*Senecio jacobaea*	*Melissa officinalis*	Nowak et al. (2016)
Pyrrolizidine alkaloids	*Senecio jacobaea*	*Mentha x piperita*	Nowak et al. (2016)
Pyrrolizidine alkaloids	*Senecio jacobaea*	*Petroselinum crispum*	Nowak et al. (2016)
Pyrrolizidine alkaloids	*Senecio jacobaea*	*Melissa officinalis*	Selmar et al. (2019b)
Pyrrolizidine alkaloids	*Senecio jacobaea*	*Petroselinum crispum*	Selmar et al. (2019b)
Pyrrolizidine alkaloids	*Chromolaena odorata*	*Zea mays*	Letsyo et al. (2021)

[a] In case of glucosides, it has to be considered that the substances leached out from the donor plants are hydrolysed in the soil (Winter and Brüsewitz 1960) into their corresponding aglycones, which—subsequently to their import into the acceptor plants—are re-glucosylated (Hijazin et al. 2019)

7.2 Plants Take Up Solutes from the Soil

In addition to various inorganic nutrients, such as cations and anions derived from minerals, plants take also up a wide variety of numerous organic substances from the soil. In contrast to the import of most ionic substances like nitrate, sulphate or metal ions, which requires specific transporters (e.g. Kobayashi and Nishizawa 2012), most of the organic compounds diffuse passively into the root cells. However, preconditions for such simple diffusion through the plasmalemma of the root cells are applicable physico-chemical properties, i.e. the substances have to be soluble in aqueous as well as in organic solvents. It is well established that the ability for such passive membrane transfer can roughly be estimated from the so-called K_{OW} value.

This parameter represents the distribution coefficient of a certain substance between octanol and water. In the related literature, generally its decadal logarithm, i.e. pK_{OW}, is displayed (Trapp 2004), which is frequently also denoted simply as log$P$ (Cronin and Livingstone 2004). Substances exhibiting log$P$ values between -1 and 3 (at times up to 4) are generally reported to diffuse easily through biomembranes (Trapp 2000; Limmer and Burken 2014). Indeed, most of these coherences had been elaborated for the uptake of xenobiotics, but they consistently also apply for all organic products. This was confirmed by Hurtado et al. (2016), who outlined that many so-called emerging organic contaminants (EOCs) are taken up by plants.

With regard to chemical ecology—up to recently—only the uptake of so-called allelochemicals had been in the center of focus. It is well established that these substances which affect germination or growth of putative competitors are frequently exuded from donor plants and exhibit their effects on plants growing in the vicinity (Wink 1983; Bertin et al. 2003; Kalinova et al. 2007). Yet, a requirement for a related growth inhibition is an uptake of the allelochemicals into the acceptor plants. A corresponding import is well documented in particular for xenobiotics, such as systemic herbicides or fungicides (Trapp and Legind 2011; Pullagurala et al. 2018). After their uptake by the roots, these compounds are generally translocated into the shoots (Trapp 2000; Collins et al. 2011; Eggen et al. 2013; Selmar et al. 2019a). Although an import of xenobiotics and allelochemicals was well established, a corresponding uptake of typical plant-derived natural products—nowadays denoted as specialized metabolites—was not taken into consideration, although it was well-known that such compounds are leached out from rotting plant materials (Rasmussen et al. 2003; Hoerger et al. 2011; Hama and Strobel 2019). However, the situation fundamentally changed when the origin of the widespread contaminations of plant-derived commodities was investigated (Selmar et al. 2019a).

7.3 Horizontal Natural Product Transfer

In order to identify the potential sources of the widespread alkaloidal contaminations, several research projects had been launched. In a first approach, in pot experiments dried tobacco leaf material was applied to the test plants. It turned out that high amounts of nicotine, which were leached out from the rotting tobacco material, were accumulated in the acceptor plants (Selmar et al. 2015b, 2019a). This transfer was strikingly confirmed by related field experiments, in which cigarette butts were discarded on the acreage (Selmar et al. 2018): only one cigarette butt per square meter was sufficient to cause nicotine concentrations in the crop plants that exceed the limit value set by the EU by the factor ten (Selmar et al. 2018). In analogy, pyrrolizidine alkaloids (PAs), which had been leached out from rotting PA-containing weeds (*Senecio jacobaea*), are also taken up and accumulated in the acceptor plants (Nowak et al. 2016; Selmar et al. 2019b; Letsyo et al. 2021). These results outlined that the uptake of alkaloids from the soil is—at least in part—

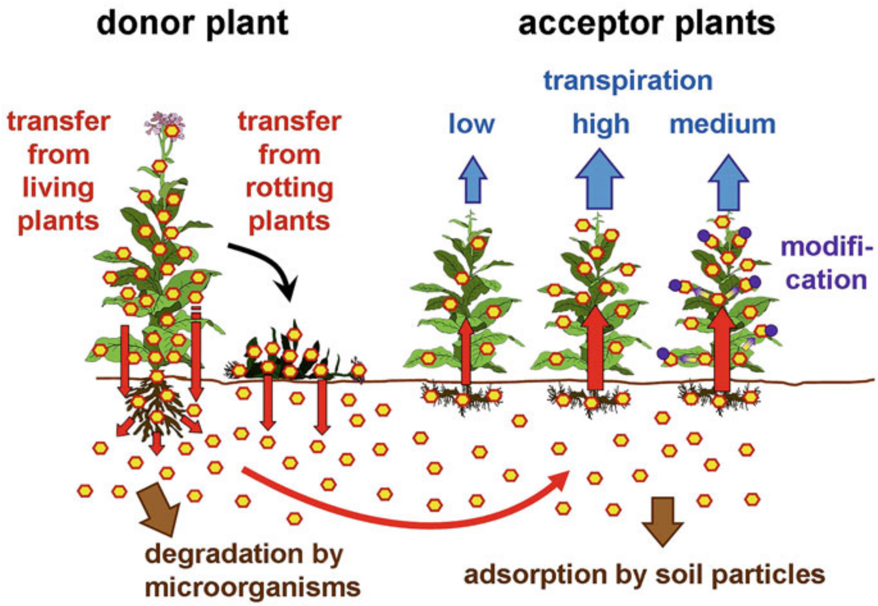

Fig. 7.1 Horizontal natural product transfer. The scheme displayed by Selmar et al. (2019a) is supplemented with the impacts of transpiration and rhizosphere

responsible for the numerous and widespread alkaloidal contaminations of plant-derived commodities (Selmar et al. 2019a). In the meantime, the uptake of many other alkaloids by plant roots has been manifested (Yahyazadeh et al. 2017; Lewerenz et al. 2020). In addition, various reports outline that apart from alkaloids, also other specialized metabolites, i.e. simple phenols (Winter and Schönbeck 1959; Winter et al. 1959a, b), coumarins (Hijazin et al. 2019), stilbenes (Abouzeid et al. 2019), aristolochic acids (Pavlović et al. 2013; Li et al. 2016) or betalains (Nowak et al. 2017), are imported by plant roots. These insights and coherences had been the basis to establish the concept of the “horizontal natural product transfer” (Selmar et al. 2015a, 2019a; Nowak et al. 2017): from decomposing plant parts—denoted as donor plants—natural products are leached out into the soil. From here, these compounds are taken up passively by the roots of other plants growing in the vicinity (Fig. 7.1).

As the underlying mechanisms are due to a quite general phenomenon, a corresponding import concerns all plants, which accordingly all act as acceptor plants. After their uptake by the roots, the substances are translocated via the xylem into the leaves (Trapp 2000; Limmer and Burken 2014). However, a precondition for such transfer is the ability of the substances to pass the plasmalemma of root cells. As outlined above, all substances exhibiting a log*P* value between minus one and roundabout three are able to diffuse across membranes (Trapp 2000; Limmer and Burken 2014). Accordingly, in contrast to the import of most ionic nutrients like nitrate, sulphate or metal ions (Kobayashi and Nishizawa 2012), no

Table 7.2 Log*P* values of various alkaloids imported by plant roots

Berberine	−1.0	Isoquinoline alkaloid	Winter et al. (1959c); Franz (1962)
Monocrotaline	−0.8	Pyrrolizidine alkaloid	Hijazin et al. (2020)
Theobromine	−0.7	Purine alkaloid	Winter et al. (1959c); Franz (1962); Yahyazadeh et al. (2017)
Caffeine	−0.1	Purine alkaloid	Winter et al. (1959c); Franz (1962); Yahyazadeh et al. (2017)
Theophylline	−0.04	Purine alkaloid	Yahyazadeh et al. (2017)
Jacobine	0.2	Pyrrolizidine alkaloid	Nowak et al. (2016)
Morphine	0.9	Isoquinoline alkaloid	Winter et al. (1959c)
Scopolamine	0.9	Tropane alkaloid	Winter et al. (1959c); Franz (1962)
Cytisine	1.0	Quinolizidine alkaloid	Franz (1962)
Seneciphylline	1.0	Pyrrolizidine alkaloid	Nowak et al. (2016)
Nicotine	1.1	Pyridine alkaloids	Winter et al. (1959c); Franz (1962); Weidner et al. (2005); Selmar et al. (2015b)
Anabasine	1.1	Pyridine alkaloids	Franz (1962)
Pilocarpine	1.1	Imidazole alkaloids	Franz (1962)
Codeine	1,3	Isoquinoline alkaloid	Winter et al. (1959c); Franz (1962)
Strychnine	1.5	Indole alkaloid	Franz (1962); Yahyazadeh et al. (2017)
Colchicine	1.5	Colchicine alkaloids	Franz (1962)
Atropine	1.6	Tropane alkaloid	Winter et al. (1959c); Franz (1962); Yahyazadeh et al. (2017)
Hyoscyamine	1.8	Tropane alkaloid	Winter et al. (1959c); Franz (1962)
Thebaine	1,9	Isoquinoline alkaloid	Franz (1962)
Cinchonine	2.2	Quinoline alkaloids	Franz (1962); Selmar et al. (2015b)
Narcotine	2,3	Isoquinoline alkaloid	Franz (1962)
Sparteine	2.5	Quinolizidine alkaloid	Franz (1962); Hijazin et al. (2020)
Noscapine	2.5	Isoquinoline alkaloid	Yahyazadeh et al. (2017)
Harmaline	2.7	Indole alkaloid	Hijazin et al. (2020)
Quinidine	2.7	Quinoline alkaloids	Winter et al. (1959c); Franz (1962)
Harmine	2.8	Indole alkaloid	Hijazin et al. (2020)
Papaverine	3.5	Isoquinoline alkaloid	Winter et al. (1959c); Franz (1962)

The data of log*P* were compiled and averaged from the databases “BioLoom”, “ChemSpider”, “chemicalize.org” and “ToxNet” as outlined in the mentioned literature

specific transporters are required. In consequence, the uptake of these natural products from the soil does not depend on the plant species, but only on the physico-chemical properties of the compound. And indeed, all substances reported to be taken up (Table 7.1; Yahyazadeh et al. 2017; Lewerenz et al. 2020) reveal appropriate log*P* values (Table 7.2). However, when dealing with alkaloids, in addition to the log*P* value, another factor, i.e. the pH, has to be considered (Hijazin et al. 2020). In case of their protonation, alkaloids exhibit a positive charge, and, consequently, they are not able any more to cross passively the biomembranes (Nowak and Selmar 2016). Moreover, the high percentage of protonation massively reduces the actual concentration of the unprotonated alkaloids and thereby is diminishing the concentration gradient between the rhizosphere and root cells, which determines the rate of influx into the root cells. Thus, apart from the log$P$, also the pKa values of the alkaloids have to be taken into account when evaluating the probability for their uptake (Hijazin et al. 2020).

In addition to the physico-chemical properties of the alkaloid and the pH of the soil, various other factors impact the actual concentration gradient between outside and inside, i.e. between the rhizosphere and the plasma of the roots cells, respectively. In this context, the properties of the soil have to be considered, since various soils exhibit quite different abilities to adsorb the alkaloids on the soil particles (Bolan et al. 1999). Furthermore, the concentration gradient massively could be influenced by microbial degradation of the alkaloids. It is well established that many microorganisms are able to efficiently degrade specialized metabolites including alkaloids and to use them as carbon sources. More than 60 years ago, Winter and Brüsewitz (1960) demonstrated that aesculin, which was applied to soils, is cleaved by microorganisms. Bartholomew et al. (1993) reported that *Pseudomonas* degrades atropine, and Mazzafera et al. (1996) outlined that *Serratia marcescens* is able to metabolize caffeine and related methylxanthines. Moreover, nicotine is degraded by various *Pseudomonas* species (Wang et al. 2013), and pyrrolizidine alkaloids are metabolized by a wide variety of different microorganisms (Joosten and van Veen 2011). Accordingly, when evaluating the uptake of natural products from the soil, microbial degradation has to be considered appropriately.

An additional feature that influences the concentration gradient between “inside and outside” is the fate of the imported alkaloid. In this context, the translocation of the alkaloids from the roots into the shoots is in particular of special concern. With respect to xenobiotics, it is well known that this transfer is accomplished via the xylem (Hsu et al. 1990; Trapp 2000), and also for alkaloids taken up from the soil, a xylem-based translocation is verified (Nowak and Selmar 2016). Accordingly, the extent of alkaloid uptake strongly depends also on the allocation velocity, which in turn is mainly determined by the rate of transpiration (Riedell and Schumacher 2009). As this property varies greatly among plant species, the extent of uptake indeed depends also on the plant species (Fig. 7.1), although the principle mechanism, i.e. the passive diffusion of alkaloids across the plasmalemma, is identical in all plants. Apart from the allocation, the actual concentration of imported substances can significantly be influenced by their biotransformation. Yet, the ability to modify or metabolize the imported compounds may vary between plant species and thereby could be responsible for differences in the velocity of uptake. The first report on such modifications of imported compounds was published by Winter et al. (1959b), who outlined that hydroquinone and phloroglucinol, which had been taken up by wheat plants, are subsequently glucosylated to yield arbutin and phlorin, respectively. Within the last decades, in particular the modification of imported xenobiotics had been in the center of focus. Accordingly numerous related information on the modification of xenobiotics in acceptor plants are available (Hatzios 1997; Burken 2003; Van Aken and Doty 2009). In analogy, also imported plant specialized metabolites are reported to be modified in the acceptor plants (Hijazin et al. 2019; Lewerenz et al. 2020). Further details of this interesting issue are outlined in the corresponding chapter below. However, one of the most astonishing results is the strong heterogeneity of the modifications, e.g. in barley seedlings, the imported umbelliferone is methoxylated to yield scopoletin, whereas it is converted to aesculin in garden cress. In contrast, in flax, pea or radish, no modification was observed at all (Hijazin et al. 2019). These data clearly show that—in addition to the differences in the translocation velocity via xylem—also the variations in the extent of modification strongly depend on the plant species. Consequently, the actual concentration of a substance in the root cells—and thus the extent and velocity of its uptake from the soil—could differ massively between plant species. This however means that—although the basic mechanism, i.e. the diffusion across the plasmalemma of the root cells, is identical for all the different plant species—various acceptor plants exhibit quite different behaviours in uptake and accumulation (Fig. 7.1). These coherences had been vividly confirmed by the variations in uptake and accumulation of pyrrolizidine alkaloids in different acceptor plants, i.e. parsley, melissa, chamomile, peppermint and nasturtium (Selmar et al. 2019b).

7.4 Transfer from Living Donor Plants

According to our insights in allelopathy, active compounds which inhibit germination or growth of potential competitors are released from various plants (Blum 2011; Seigler 2006). In principle, there are several options how these allelochemicals are released into the environment. The active compounds can be leached out from decomposing plant residues (Belz et al. 2007), or the substances are exuded from living plants, either by their roots (Bertin et al. 2003; Kalinova et al. 2007) or by their leaves (Tukey 1970; Nakano et al. 2003). When addressing the phenomenon of horizontal natural product transfer, it has to be taken into consideration that—in analogy to allelochemicals—also specialized metabolites might be released from living donor plants, too. This, in particular, seems to be consistent when considering the fact that numerous of these natural products are able to diffuse—at least to a certain extent—through biomembranes. In order to verify a putative transfer of specialized metabolites between neighbouring plants, co-culture experiments had been conducted: *Senecio jacobaea* plants, which contain high concentrations of pyrrolizidine alkaloids (PAs), were co-cultivated together with various acceptor plants in the same pot (Nowak et al. 2017). It turned out that after some weeks, the *Senecio* PAs were also present in the non-alkaloidal acceptor plants. The corresponding parsley leaves exhibited an average concentration of more than 200 μg PAs/kg d.w. (Selmar et al. 2019b). Since in pot experiments, due to an intimate contact between the roots of donor and acceptor plants, a direct transfer via root grafts (Basnet 1993) could not be excluded, appropriate co-culture experiments had been conducted under field conditions. For this, plants of various species had been cultivated in different spatial distances to the PA-containing *Senecio* plants (Selmar et al. 2019b). After several weeks of co-cultivation, the *Senecio* PAs could be detected in every plant, which genuinely did not contain these alkaloids.

However, the PA concentrations varied markedly between the different acceptor plants (Selmar et al. 2019b). These results vividly expound that the PAs, which originally had been synthesized and accumulated in the *Senecio* donor plants, were indeed transferred into the various acceptor plants.

As outlined for allelochemicals, in principle there are several options for the presence of the classical secondary metabolites in the soil, too. In the first instance, it could be assumed that the observed PA transfer between living plants is due to the shedding of *Senecio* leaves, from which the PAs might have been leached out. However, since it was reported that in the course of the related pot experiments hardly any abscission of leaves occurred (Selmar et al. 2019b), this possibility can highly be excluded in these experiments. Nonetheless this process certainly will be relevant when considering an entire vegetation period, especially when the senescence-induced shedding of leaves takes place. Alternatively, the PAs could have been bled out from minor leaf injuries, e.g. those caused by pathogens or herbivores. Yet, since the plants used in these experiments were described to be healthy and no herbivory had been observed, a corresponding bleeding of PAs from minor injuries of the leaves seems to be unlikely. This deduction is supported by the finding that the PA spectra of donor and acceptor plants were quite different. In case of an injury-induced release, all different PAs should have been leached out and subsequently been taken up by the acceptor plants to the same extent. Accordingly, it has to be assumed that the *Senecio* donor plants release PAs into the soil. Again, there are two options, i.e. a passive diffusion and an active exudation. Indeed, both possibilities might occur. In this context, it is relevant to mention that ptaquiloside, a carcinogenic phytotoxin produced by *Pteridium aquilinum*, indeed is passively leached out of the leaves of this fern (García-Jorgensen et al. 2020). Due to the analogous ability to diffuse through biomembranes, alkaloids (and other specialized metabolites exhibiting appropriate log*P* values) should also be either passively leached out from the leaves or released by the roots into the soil. However, efficient trapping mechanisms, i.e. the high acidity of the vacuole, prevent the diffusion across membranes: within the vacuole the alkaloids are protonated, and as consequence, they are unable to pass the tonoplast. But this “ion trap mechanism”, which was first described by Matile 1976), is only valid for alkaloids exhibiting high p*K*a values, which ensures ample protonation in the physiological pH range. In case of very low p*K*a values, e.g. of caffeine (p*K*a = 0.7), this trapping mechanism will not work. Alternatively, caffeine is trapped in the vacuole by complex formation with chlorogenic acids (Waldhauser and Baumann 1996).

When evaluating the literature describing the release of alkaloids, it turns out that the related statements are mainly based on organ or cell culture experiments. In this context, Bais et al. (2003) reported the exudation of harmine and harmaline from root cultures of *Oxalis tuberose*, and Ruiz-May et al. (2009) outlined that the indole alkaloid ajmalicine is secreted from hairy roots cultures of *Catharanthus roseus*. In the same manner, nicotine was found in the medium of root culture from *Nicotiana tabacum* (Zhao et al. 2013). Indeed, due to the coherences mentioned above, these data do not represent an unequivocal proof that the alkaloids are actively exuded into the medium. However, this option is supported by the findings of Toppel et al.

(1987) who showed that in *Senecio* root cultures, the spectrum of PAs present within the cells is quite different compared to that of the culture medium, i.e. in contrast to the wide variety of PAs within the cultured cells, in the medium nearly exclusively senkirkine could be detected. Accordingly, it can be assumed that the root cells indeed actively and specifically exude this PA into the culture medium. In this context it has to be mentioned that a related active transfer requires specific transporters, which enables the transfer of the protonated alkaloids out of the vacuole; and indeed numerous transporters for alkaloids are described (e.g. Shitan et al. 2003; Otani et al. 2005; Morita et al. 2009; for review see Yazaki et al. 2008). Unfortunately, in most of the reports dealing with membrane transfer of alkaloids, their ability to easily cross biomembranes is ignored. In contrast, the requirement for transporters, which allow the membrane transfer of the protonated alkaloids, is not considered appropriately.

Apart from the excretion of alkaloids from organ and cell cultures, only a few data are available which verify alkaloids are released from genuine plants into the soil. In this context, it has to be mentioned that the roots of *Agropyron repens* exude dihydroxybenzoxazinone into the soil (Schulz et al. 1994). In the same manner, the roots from *Oxalis tuberosa* are reported to release the carboline alkaloids harmine and harmaline (Bais et al. 2002), and caffeine is exuded by the roots of coffee seedlings (Baumann and Gabriel 1984). These findings are confirmed by the occurrence of quinolizidine alkaloids (lupine alkaloids) in soils, in which narrow-leaf and yellow lupines are growing (Hama and Strobel 2020). In the same manner, PAs, putatively derived from ragwort plants, could be detected in soils, in which the related *Senecio* plants were growing (Hama and Strobel 2021). Indeed, with respect to the exact origin of these alkaloids, the same questions as outlined above have to be quoted, i.e. did these alkaloids result from the leaching of shed or injured leaves, or are they released from vital tissues. Moreover, it has to be asked: is such release a result of an active exudation or of a passive diffusion? Nonetheless, although the actual path of the PA transfer from living donor plants into acceptor plants growing in the vicinity is still unknown, the co-culture experiments unequivocally verify that specialized metabolites, such as alkaloids, indeed are transferred from vital donor plants to acceptor plants growing in the vicinity. Accordingly, the concept of horizontal natural product transfer had to be broadened by including the transfer from vital plants (Selmar 2019b; Fig. 7.1). Yet, much more research is necessary to elucidate the exact path of release into the soil.

7.5 Modification of the Imported Substances

It is well established that xenobiotics, which are taken up from the soil, could be modified within the acceptor plants—in particular by oxidation, hydroxylation and conjugation (Burken 2003; Komives and Gullner 2005). These reactions are discussed to be part of the so-called green liver concept, which postulates a deliberate detoxification system for xenobiotics (Sandermann 1994). Accordingly, it seemed to be obvious that also the natural compounds, which had been taken up by the roots, could be modified within the acceptor plants, too. The first clue for corresponding processes is given by the finding that the concentration of alkaloids in acceptor plants strongly decreased over time (Selmar et al. 2015b; Nowak et al. 2016). Since in the related studies the quantification of PAs was based on the standard HPLC-determinations by summing up the contents of all genuine alkaloids, putative derivatives were not detected and quantified. Accordingly, in continuative studies (Selmar et al. 2019a), the quantification of PAs present in the acceptor plants was additionally performed by the so-called sum-parameter method (Cramer et al. 2013). Since this approach is based on a HPLC-ESI-MS/MS determination of the necine base, all PA-related structures, and thus also putative modification products of the genuine PAs, will be determined. As expected, in contrast to the previous investigations, the overall content of PAs and PA-related structures in the acceptor plants did not decrease over time. A modest comparison of this content with the PA amount calculated by summing up all genuine PAs (standard approach) revealed that 2 weeks after the mulching, more than two-thirds of the imported PAs had been modified to so far unknown derivatives (Selmar et al. 2019a). Unfortunately, up to now, any clues to the related modification products are lacking. Moreover, no information on the potential toxicity of these unknown derivatives is available.

Much more research is required to evaluate reliably the risk of the related PA contaminations. Nonetheless, these studies clearly outlined that—as known for xenobiotics—also PAs imported into the acceptor plants are modified to a large extent (Selmar et al. 2019b). Up to now, all attempts to elucidate the structure of the related PA derivatives failed, since it turned out that their identification in HPLC analyses is very difficult because of the weak UV absorbance and ambiguous fragmentation pattern in corresponding MS analyses. Indeed, an alternative strategy would be the employment of isotope-labelled compounds. But, such an approach is very time-consuming and cost-intensive. Accordingly, it seemed to be far more promising to study the putative modifications of imported natural compounds, when such substances are applied, whose derivatives are more feasible to detect. In this context, coumarins are quite auspicious, since the genuine substances as well as most of their derivatives could easily be detected due to their fluorescence (Jones and Rahman 1994). Hijazin et al. (2019) employed umbelliferone ($\log P = 1.5$) to exemplarily study the uptake of a typical specialized metabolite and its subsequent modification in various acceptor plants. As predicted, all the different acceptor plants employed took up umbelliferone by their roots and translocated it into the leaves. However, only in the seedlings of barley and garden cress that the imported coumarin was modified effectively. In garden cress, it was hydroxylated and glucosylated to yield aesculin (Fig. 7.2), whereas in barley seedlings, the imported umbelliferone was converted by methoxylation to scopoletin (Hijazin et al. 2019). As outlined above, corresponding reactions are known to be involved in the modification of imported xenobiotics (Burken 2003; Komives and Gullner 2005). Frequently, these reactions are catalyzed by cytochrome P450 enzymes (Yun et al. 2005; Siminszky 2006). To verify that the conversion of umbelliferone in barley and garden cress indeed is catalyzed by P450 enzymes, an additional approach was

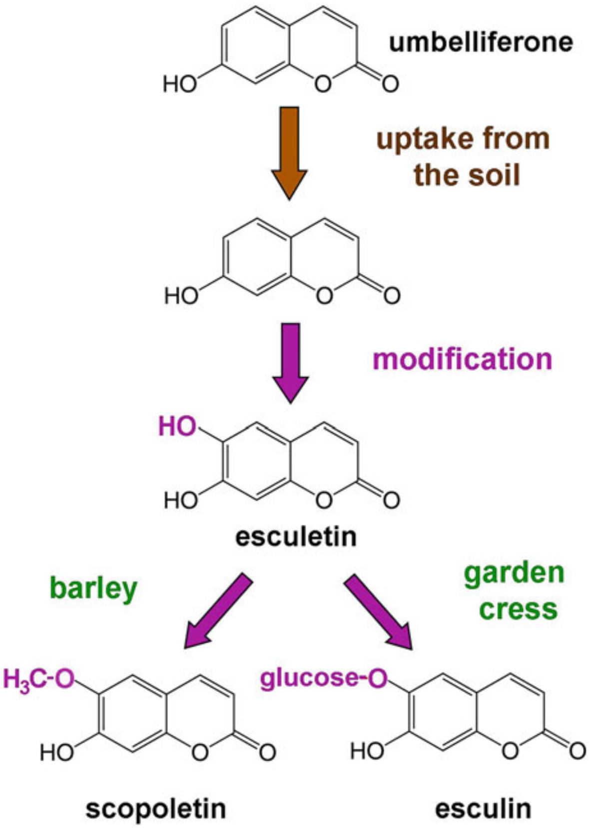

Fig. 7.2 Modification of imported umbelliferone in barley and garden cress seedlings according to Hijazin et al. (2019)

conducted: naproxen, a well-known inhibitor of P450 enzymes (Miners et al. 1996), was applied together with umbelliferone. This concomitant application of umbelliferone and inhibitor resulted in a strong reduction in the conversion of umbelliferone to both derivatives, to scopoletin in barley and to aesculin in garden cress (Hijazin et al. 2019). These findings demonstrate that imported natural products could also—analogue to the modification of xenobiotics—be modified in the acceptor plants by the means of cytochrome P450 enzymes. In consequence, the concept of horizontal natural product transfer had to be broadened by including the modifications of imported substances as outlined in Fig. 7.1 (Selmar et al. 2019a).

In summary, it has to be stated that the kind and extent of the modifications of the imported umbelliferone strongly depend on the plant species; it is either just accumulated in the acceptor plants (pea, flax, radish), or it is converted to aesculin or scopoletin in garden cress or barley, respectively. Corresponding species-related differences in such modifications are also described for typical allelochemicals. In this sense, biochanin A is converted differentially to various derivatives in different weed species (Shajib et al. 2012). In the same manner, the ability to hydroxylate and to glucosylate benzoxazolinone (BOA) is reported to vary markedly among various plant species (Schulz and Wieland 1999). The cognition that the fate of imported natural products is quite different in the various plant species indicates that the observed modifications are not part of a general and deliberate detoxification system as proposed by the "green liver concept" (Sandermann 1994; Burken 2003). By contrast, these modifications appear to be due to random "accidental" activities of enzymes, which generally are involved in the biosynthesis of specialized metabolites, which genuinely are present in the acceptor plants. In consequence, the issue of enzyme promiscuity (Kreis and Munkert 2019), which actually is getting more and more attraction, seems to be responsible for the differential modification of natural products as well as xenobiotics in the acceptor plants. There is a tremendous demand for further research to elucidate whether the modifications of imported substances are due to a particular "detoxification system" or to "side activities" of promiscuous enzymes involved in plant specialized metabolism (Selmar et al. 2019a).

7.6 Implications for Biochemical Ecology

The insights outlined above unveil that the "horizontal natural product transfer" represents a prevailing phenomenon in nature that is quite more spread than originally assumed (Selmar et al. 2019a). Accordingly, when studying plant-plant interactions, we always have to consider that specialized metabolites synthesized and accumulated in one plant species might also be present in the soil, from which they can be taken up randomly by other plants growing in the vicinity (Selmar et al. 2019a). Yet, as precondition for such exchange, the substances must exhibit appropriate physico-chemical properties, i.e. a $\log P$ value between -1 and about 3. Due to the unlimited number of permutations of donor and acceptor plants, the variation in the exchange of specialized metabolites is nearly infinite (Selmar et al. 2019a). Accordingly, for most of these cases, a certain or specific ecological effect of this phenomenon can be ruled out. This however, does not apply to the so-called typical allelochemicals. Due to their inhibiting impact on the acceptor plants, allelochemicals reveal a high relevance for the donor plant by suppressing potential competitors. It seems to be very likely that the evolution of allelochemicals indeed could have their inception in the random release of specialized metabolites. Accordingly, these novel insights will strongly influence our comprehension of chemical ecology, especially with respect to their evolution (Selmar et al. 2020).

A further cause for thought derived from our knowledge on the "horizontal natural product transfer" is related to the definition of xenobiotics. Up to now, xenobiotics are generally defined as non-natural substances, which are "foreign to life" (Godheja et al. 2016). Nonetheless, the authors already included in their definition "naturally occurring poisons". In the last century, the natural substances taken up by the acceptor plants had been denoted as "allochthonous substances"

(Winter et al. 1960). The knowledge that the random uptake of specialized metabolites and their subsequent modification in the acceptor plants directly correspond to the equal processes known for xenobiotics requires either a thorough re-evaluation of the classical definition of xenobiotics or a sound differentiation between "non-natural" and "naturally derived compounds" (Selmar et al. 2020). In the first case, specialized metabolites which are taken up by acceptor plants should be included in the denomination of xenobiotics. In other words, xenobiotic would comprise all metabolites, which are "foreign to the acceptor plants". In the second case, in contrast, the term xenobiotics would just comprise "non-natural compounds" and would be contraposed to all substances generated by organisms, i.e. allochthonous substances, which accordingly could be denoted as "allochtonics".

In addition to these reflections related to basic science, the novel insights also reveal relevance for applied plant biology and agriculture. The transfer of natural products from living donor plants might actually be the basis for increasing our understanding of various hitherto unexplained processes (Selmar et al. 2019a). In this context, the release of specialized metabolites by donor plants into the soil—either by active or passive exudation or by leaching from decomposing plant parts—and the subsequent uptake by plants growing in the vicinity should have a special relevance for new approaches to explain the beneficial effects of crop rotations or of co-cultivation of certain vegetables.

7.7 Conclusion

As outlined in the introduction, numerous alarming reports of the European Food Safety Authority unveiled that many plant-derived commodities are contaminated by various alkaloids. Meanwhile, as expounded in this treatise, it is well documented that these contaminations—at least in part—are due to the horizontal natural product transfer. Accordingly, in the future, a marked transfer of poisonous substances into crop plants has to be avoided. Indeed, when considering PA contaminations, the accidental co-harvest of PA-containing weeds is a major source of related contaminations. Thus, the farmers have to remove the PA-containing weeds efficiently from the fields. However, a simple chopping of the weeds is not appropriate. It is essential that the plants are removed from the field in order to prevent the leaching of PA from the rotting shoots. In addition, also the roots of the PA-containing weeds have to be extracted; otherwise, PAs will be further on released into the soil. Accordingly, the PA-containing weeds should not be chopped off, but the entire plants must be extracted completely and removed from the field.

In principle, these coherences apply for all other cases. Contaminations by specialized metabolites derived from collateral weeds could be prevented by removing the entire weed plants from the field.

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Chapter 8
Effect of Abiotic Stresses and Adaptation Strategies of Medicinal Plants

Sibgha Noreen, Muhammad Aasim, Umme Ummara, Muhammad Salim Akhter, Nawishta Saleem, Seema Mahmood, and Kausar Hussain Shah

Abstract The significance of medicinal plants for humans have been established since ancient times for curing diseases and ailments of themselves and domesticated animals based on their traditional knowledge. In recent years, the demand for medicinal plants has been increased and resulted in their enhanced cultivation. However, the production of medicinal plants is generally affected by biotic and abiotic stresses. Abiotic stresses like salinity, drought, cold, heavy metals etc. are the major constraints which affect the plant biomass production and subsequently their significant metabolite production. Under stress conditions, medicinal plants adapt and exhibit different physiological and molecular responses to cope with these stresses, and it is direly needed to understand these responses to overcome the issue. This study presents the information about some important medicinal plants and their uses and responses under variable stress conditions. Furthermore, different long-term and short-term strategies like plant breeding, genetic engineering and application of different chemicals and hormones have been summarized to overcome the issue of abiotic stress.

Keywords Abiotic stress · Medicinal plants · Secondary metabolites · Physiological responses · Molecular responses

8.1 Introduction

Medicinal plants play an important role in human life being a major source of valuable chemicals and direct source of food and medicines. Medicinal plants are used in the healthcare system since time immemorial. They play an important role in disease prevention and are rich resource of ingredients used in the synthesis of drugs

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(Sandberg and Corrigan 2001). A major portion of developed countries depends on traditional medicine (Abdalla and Laatsch 2012). It has been estimated that about 80% of global population depends on traditional medicines derived from medicinal plants. A list of some commonly used medicinal plants for curing common diseases is given in Table 8.1.

The use of traditional medicinal practices is common in India, China, Pakistan, Japan, Sri Lanka and Thailand. The plant extract and active compounds found in these plants are used in therapies against most diseases (Michel et al. 2020). The plants synthesize secondary metabolites using the primary metabolites, i.e. lipid, carbohydrate and amino acid (Hatami et al. 2016). Almost 100,000 secondary metabolites are known, having molecular weight < 150 KDa and constituting $<1\%$ of plant dry weight (Oksman-Caldentey et al. 2004). These metabolites besides being a unique source of food also produce plant-specific natural colours, taste, odour and toxins (Lajayer et al. 2017). Today there are at least 120 important drugs which are derived from medicinal plants are based on drugs are alkaloids (nitrogen-containing molecules), terpenoids (carbon- and hydrogen-containing units), flavonoids (phenol-containing compounds), glycosides (contain sugar molecules), tannins (polymeric astringent phenolic compounds), caffeine, aspirin, cocaine, morphine, digitalis etc. (Wink 2012; Salmerón-Manzano et al. 2020). There is a direct role of secondary metabolites in metabolic functions of plants like photosynthesis, pollination and fertilization. These metabolites also play an important role in plant's defence against system environmental adversities (Akula and Ravishankar 2011).

8.2 Influence of Abiotic Stresses on Medicinal Plants

Continuous changes in the environment make plants prone to abiotic stresses like drought, chilling, salinity, high temperature, heavy metal, high and low light intensity, UV-B, nutrient deficiency, ozone etc. (Mishra and Tanna 2017). The growth and the quality of aromatic compounds in these plants have been reported to be influenced by these environmental constraints (Table 8.2). The accumulation of salts in the rhizosphere causes specific ion toxicity, osmotic stress and ionic imbalances that lead to plant death or yield loss both in the medicinal plants and other crop plants. It directly affects the vegetative growth, ionic constituents, yield attributes and composition of essential oil of different medicinal plants. Furthermore salinity reduces biomass production of citronella (C*ymbopogon nardus*), lemongrass (*Cymbopogon flexuosus*) and vetiver (*Vetiveria zizanioides*). Water deficit conditions are life-threatening issue to plants. Drought stress adversely affects the medicinal value of many important medicinal plants including thyme (*Thymus vulgaris*), yarrow (*Achillea millefolium*), mint (*Mentha arvensis*), chicory (*Cichorium intybus*), balm (*Melissa officinalis*), calendula (*Calendula officinalis*) etc. It is also reported that drought stress drastically reduced the vegetative growth, water content and the percentage of essential oil content of lemongrasses (*Cymbopogon nardus*). These

Table 8.1 Commonly used medicinal plants and their usage under specific diseases

Plant name	Used in disease
Abutilon indicum	Leaves are used in boils and ulcer Seeds are laxative and used in piles
Acacia catechu	Bark is cure of chronic diarrhoea
Acacia nilotica	Twigs are used as toothbrush Bark extract is used as tonic.
Adhatoda vasica	Leaf liquor cures asthma and bronchial disorders
Aloe vera	Leaf peelings used in skin burn Gel cures ulcers Fleshy part of leaf is used in face mask
Andrographis paniculata	Used in malarial fever and tonic to liver
Anisomeles indica	Leaves cure cough and cold
Anogeissus latifolia	Gum is used as tonic, while leaves are used in diarrhoea
Ammi majus	Leaf extract used for many skin diseases
Argemone mexicana	Extract cures skin diseases Latex is applied to the eyes in conjunctivitis
Azadirachta indica	Bark cures malarial fever; twigs are used as toothbrush; and oil of seeds cures skin diseases and lice
Boerhavia diffusa	Plant material used in urinary troubles, jaundice and skin diseases
Cassia angustifolia	Plants materials are used as a laxative
Catharanthus roseus	Flowers and leaves reduce sugar level
Chlorophytum spp.	Roots are used in general weakness as tonic
Curculigo orchioides	Roots are used as tonic and aphrodisiac; in leucorrhoea and menstrual irregularities
Curcuma caesia	Rhizomes cure sprains, internal injuries and bruises
Cyperus scariosus	Tubers are used in heart and urinary diseases
Datura metal	Smoke from seeds cures bronchial troubles
Gymnema sylvestre	Leaves are used in diabetics
Jatropha curcas	The fruit is used for treating dysentery and diarrhoea, latex contains an alkaloid known as “jatrophine” having anticancer properties
Momordica charantia	The fruit has hypoglycaemic effect and hence can be used for diabetes patients
Ocimum sanctum	Leaves are used in cough and cold and also in boils and ulcers
Phyllanthus amarus	Commonly used to treat jaundice
Salvia officinalis	Plant is commonly used to treat upper respiratory and gastrointestinal tract infections
Solanum nigrum	Leaves cure jaundice and skin diseases
Syzygium cumini	Seed powder helps to cure dysentery, diarrhoea and diabetes
Trachyspermum ammi	Pods and leaves are edible and it is used as an antiflatulent
Tylophora indica	Leaves are used in asthma
Urginea indica	Bulb juice is given in respiratory disorders

(continued)

Table 8.1 (continued)

Plant name	Used in disease
Vitex negundo	Leaf extract is used in body pain and in skin diseases
Withania somnifera	Plant parts are used to treat asthma, diabetes, hypertension, stress, arthritic diseases and cancer

abiotic stresses in plants showed significant increase in production of reactive oxygen species (ROS), superoxide, hydrogen peroxide and hydroxyl radicals (Fig. 8.1). Detoxification system (enzymatic and non-enzymatic antioxidant enzymatic system) of plants is activated in response to ROS. These systems include increased production of the antioxidant enzymes SOD, CAT, APX and GPx (Hasanuzzaman et al. 2013). Reactive oxygen species act as signaling molecules in processes involved in combating stress. Increase in the production of ROS in response to abiotic stresses can lead to significant oxidative damage and also death of the plant (Sharma et al. 2012).

8.2.1 Salinity Stress

Salinity stress is shown to have a directly damaging effect on the medicinal properties of plants. Plant responses to different types of stresses vary, e.g. manifold induced water stress in plants is shown to reduce growth, causing high root-to-shoot ratio. When NaCl is applied to water-stressed plants, it improves the response to mannitol-induced water stress. Relative water content and growth of seedlings (reduced by water stress) are enhanced by NaCl application. Salt stress is observed to cause significant reduction in gas exchange, plant growth, photochemical quenching and shoot and root potassium ion content. Salinity is also associated with increase in leaf glycine betaine, free proline and sodium ion content (Shahbaz et al. 2013; Noreen et al. 2019a). In *Bacopa monnieri* mild and moderate salt stresses result in a 36% and 76% increase in secondary metabolite content, respectively. Bacoside A (a saponin) is an important secondary metabolite of *Bacopa monnieri*, having many medicinal properties. It can be concluded that treatment with salt results in enhancement of biomass yield and saponin content in *B. monnieri* (Bharti et al. 2013). Subjection of plants to environmental constraints results in overexpression of endogenous melatonin. Melatonin is observed to have a key role in plant to tolerate stress (Zhang et al. 2015).

8.2.2 Drought Stress

Severe drought conditions in medicinal plants are associated with high phenolic contents and elevated antioxidant enzymes (Saeidnejad et al. 2013). The plants of

Table 8.2 Effect of abiotic stresses in medicinal plants

Medicinal Plant	stress	Effect	Reference
Ocimum tenuiflorum L.	Cold stress	Reduction in eugenol and methyleugenol concentration. No subsequent alteration caryophyllene concentration	(Rastogi et al. 2019)
Ocimum basilicum L.	Cold stress	Reduction linalool, α-pinene and camphene production. Enhanced α-bergamotene, cineole, γ-cadinene, geraniol and germacrene D production	(Senji and Mandoulakani 2018)
Fagopyrum tataricum Gaertn.	Cold stress	Enhanced flavonoid production and antioxidant activity	(Jeon et al. 2018)
Artemisia annua L.	Cold stress	Enhanced artemisinin production associated secondary metabolites which include artemisinic acid, artemisinin B and dihydroartemisinic	(Liu et al. 2017)
Ajuga bracteosa Wall. ex. Benth.	Cold stress	Flavonoids, quercitol, caffeic acid, cinnamic acid, total phenolic production enhanced	(Rani et al. 2017)
Withania somnifera (L.) *Dunal*	Cold stress	Enhanced withanolide contents	(Mir et al. 2015)
Vitis vinifera L.	Cold stress	Mitigation in ferulic acid, caffeic acid and ρ-coumaric acid and reduced scavenging of radicals	(Król et al. 2015)
Cucumis sativus L.	Cold stress	Enhanced flavonoids, cinnamic acid, ρ-coumaric acid, caffeic acid, ferulic acid, lignin, phenol production	(Chen et al. 2013)
Camellia sinensis L.	Cold stress	Phenolic compound production increased	(Upadhyaya 2012)
Eleutherococcus senticosus	Cold stress	Reduction in eleutheroside, eleutheroside E, flavonoids and total phenolic production	(Shohael et al. 2006)
Hypericum perforatum L.	Cold stress	Reduction in hypericin content, hyperforin content, pseudohypersin content	(Zobayed et al. 2005)
Hypericum brasiliense Choisy	Cold stress	Reduction in accumulation of total phenolic compounds and betulinic	(de Abreu and Mazzafera 2005)
Crataegus laevigata and *C. monogyna*	Cold stress	Upregulation of acetylvitexin-2″-O-rhamnoside, hyperoside, quercetin, vitexin-2″-O-rhamnoside	(Kirakosyan et al. 2004)
Artemisia annua L.	Cold stress	Boast upregulation of artemisinin	(Wallaart et al. 2000)
Catharanthus roseus	Salinity	Production of secondary products (vinblastine)	(Jaleel et al. 2008c)
Orthosiphon stamineus	Salinity	Production of specialized metabolite (polyphenols)	(Ting et al. 2009)
Jatropha curcas	Salinity	Enhanced curcin contents	(Gao et al. 2008)
Thymus maroccanus Ball.	Salinity	Enhanced thymol contents	(Belaqziz et al. 2009)

(continued)

Table 8.2 (continued)

Medicinal Plant	stress	Effect	Reference
Olea europaea L.	Salinity	Enhanced oleoside contents	(Rejšková et al. 2007)
Senecio jacobaea	Drought	Enhanced pyrrolizidine alkaloid production	(Kirk et al. 2010)
Phellodendron amurense	Drought	Increased benzylisoquinoline production	(Xia et al. 2007)
Catharanthus roseus	Drought	Enhanced indole alkaloid production	(Jaleel et al. 2007a)
Ocimum sp.	Drought	High eugenol contents	(Khalid 2006)

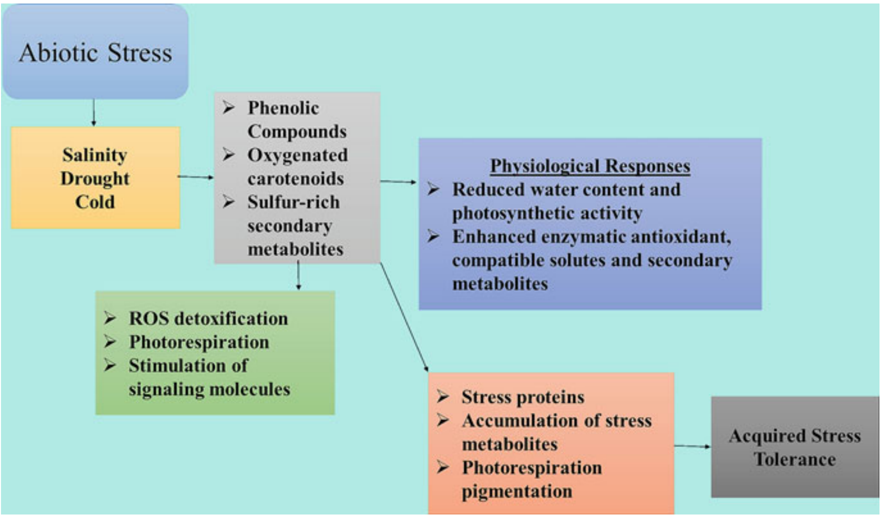

Fig. 8.1 Various responses of medicinal plants against abiotic stress condition

Hypericum brasiliense grown under drought stress produced 10% high total phenolic contents were produced in stresses plants as compared to control ones (de Abreu and Mazzafera 2005). The flavonoids were nearly the same, while the content of furoquinones was slightly low, in drought and control red sage plants (*Salvia miltiorrhiza*) (Liu et al. 2011).

8.2.3 Heavy Metal Stress

In medicinal plants the toxicity due to heavy metals results in decreased plant growth and chlorophyll concentration in leaves. Higher concentrations of cadmium are associated with total damage of leaf structure and fusion of cell membrane and cell wall (Fu and Cui 2013). It was observed in *Mentha pulegium* that heavy metal stress induces the synthesis of secondary products stimulating plant body defence system to combat stressful conditions. A significant increase in secondary metabolites has been observed under heavy metal stress in some medicinal plants (Hussein and El-Anssary 2019; Li et al. 2020).

8.3 Abiotic Stresses and Medicinal Plants

8.3.1 Ammi Majus

Ammi majus is a traditional medicinal plant commonly called bishop's flower or bishop's weed. This plant belongs to the Apiaceae family. 46-day-old *A. majus* plants exposed to NaCl stress showed a significant reduction in biological yield of both root and shoot organs and a 50% reduction in seed yield at 104 mM salinity treatment. *A. majus* is a glycophyte, and experiments have shown that as the concentration of NaCl ions increases in shoots, the concentration of potassium and calcium ions decreases. These plants can therefore be seen as moderately salt tolerant (Ashraf et al. 2004).

8.3.2 Bupleurum Chinense

Bupleurum chinense is a part of the Apiaceae family of medicinal plants. It is commonly known as Chinese thorowax roots, and the root is used for its medicinal properties. Under drought stress, *B. chinense* roots are observed to be highly resistant. Increase in drought treatment results in a significant increase in saikosaponin a (SSa) and saikosaponin d (SSd). Moderate drought stress results in increased SSa concentration up to 83% and SSd concentration up to 22%. However, increased levels of SSa and SSd are associated with enhanced oxygen ion content and activity of SOD, CAT and APX. *B. chinense* roots are observed to possess effective mechanisms that protect them from antioxidants which confer resistance to drought stress (Zhu et al. 2009).

8.3.3 Cassia Angustifolia

Cassia angustifolia is also known as Indian senna and belongs to the Fabaceae family. In one experiment, 45-day-old plants were subjected to lead stress. Significant changes in the levels of ascorbate, glutathione, proline and sennosides were observed. As compared to control plants, experimental plants showed an increase in the content of thiobarbituric acid-reactive substances and dehydroascorbate. Proline content is also observed to increase significantly at 60 days after sowing, and after that it decreases (Qureshi et al. 2007).

Salt stress applied to *C. angustifolia* plants showed that NaCl stress alters physiological mechanisms, which changes the rate of germination, seedling growth and biomass production (Shitole and Dhumal 2012). Drought-stressed *C. angustifolia* plants demonstrate increased proline levels and glutathione reductase activity but decreased seed yield. Superoxide dismutase, catalase and glutathione peroxidase activities are also observed to change drastically in these plants. However, the application of nitrogen fertilizer to *C. angustifolia* plants results in enhanced proline content, antioxidant enzyme activity and yield (Khammari et al. 2012).

8.3.4 Catharanthus Roseus

Catharanthus roseus is commonly known as Madagascar periwinkle or simply periwinkle. It belongs to the Apocynaceae family. The plant is effective against diabetes, malaria, leukaemia and Hodgkin's lymphoma (Marcone et al. 1997). *C. roseus* plants subjected to water deficit stress showed a change in abscisic acid level, DNA content, RNA content and activities of ATPase and protease. Exposure to drought stress doubles the abscisic acid concentrations in all parts of the plant. It also causes a decrease in DNA and RNA content in experimental plants in comparison with control plants. The activities of ATPases and proteases are also enhanced with increase in water deficit stress (Jaleel et al. 2008a).

It is observed that *C. roseus* plants subjected to NaCl stress show a reduction in overall growth, chlorophyll content, proteins and antioxidant enzymes. Secretion of the medicinally important alkaloid ajmalicine increases as a response to NaCl stress. It increases biological yield, green pigments, soluble proteins and the activities of antioxidant enzymes. Increase in the rate of ajmalicine production after triadimefon treatment on salt-stressed plants is observed (Jaleel et al. 2008b).

8.3.5 Jatropha Curcas

Jatropha curcas is a flowering plant belonging to the Euphorbiaceae family. It is known to be hepatoprotective. *J. curcas* is a stress-resistant plant which grows perennially on marginal soils. *Jatropha curcas* plants subjected to water deficit stress show that as the stress increases, carbon dioxide assimilation, transpiration and stomatal conductance decrease. Stressed plants and control plants do not differ in relative water content and succulence. Plants with more severe water deficit are observed to possess the highest levels of total soluble sugars. Amino acids and glycine betaines are involved in the management of osmotic potential (Silva et al.

2010). With chromium stress, increasing chromium concentration results in increased response of catalase, ascorbate peroxidase and glutathione-S transferase, illustrate that antioxidant enzymes have a key role in protecting plants from chromium toxicity. *J. curcas* plants are somehow tolerant to chromium and can grow in chromium-affected environments (Yadav et al. 2010).

Effects of salt stress are more deleterious compared to heat stress, but the effects of both stresses increase when they are combined. Heat favourably stimulates the accumulation of amino acids, i.e. glycine betaine and chlorophyll molecules, in salt-stressed leaves. Antioxidant enzymatic defence is initiated as a result of combined heat and salt stress (Silva et al. 2013). Exposure to chill hardening stress also results in increased activation of antioxidant enzymes which leads to enhancement of chilling tolerance (Ao et al. 2013).

8.3.6 Momordica Charantia

Momordica charantia is commonly known as bitter melon or bitter gourd. It is a common garden vegetable and has many medicinal uses. The plant belongs to one of the leading medicinal plant families, the Cucurbitaceae. *M. charantia* showed an increase in activity of SOD, CAT, PPO, GR and APX on treatment with salt stress. Protein concentration of plants under salt stress decreases in all stages except pre-flowering (Agarwal and Shaheen 2007). Bitter gourd plants treated with NaCl show a significant decrease in germination rate and vitality index. These symptoms were significantly enhanced when the salt-stressed plants were treated with silicon. Malondialdehyde concentrations in leaves notably decrease as silicon treatment is initiated. Activities of superoxide dismutase, peroxidase and catalase are drastically increased on application of silicon treatment to salt-stressed plants (Wang et al. 2010).

8.3.7 Phyllanthus Amarus

Phyllanthus amarus is a small herb belonging to the Euphorbiaceae family. It is known for its medicinal properties. Effects of different cadmium concentrations were observed on *P. amarus* plants. Cadmium causes a significant decrease in fresh and dry biomass, length of root and shoot organs, protein, chlorophyll, carotenoids and various sugars (Rai et al. 2005). After 90 days of salt stress, the plants show an increase in lipid peroxidation, hydrogen peroxide, glycine betaine and proline content. An increase in NaCl concentration decreases the proline oxidase activity in experimental plants (Jaleel et al. 2007a).

8.3.8 Salvia Officinalis

Common or garden sage is also known as *Salvia officinalis* and is a perennial shrub belonging to the Lamiaceae family. *Salvia officinalis* plants subjected to salt stress show a 61% decrease in growth. Application of 100 mM NaCl stress reduces total fatty acid content by up to 32%. Polyunsaturated fatty acids decrease with increase in NaCl (NaCl) content, whereas content of monounsaturated fatty acids increases with progression in salt stress (Taarit et al. 2009). Sage plants grown under drought stress have significantly high concentration of total monoterpenes compared to well-watered plants (Kleinwächter and Selmar 2014).

Sage exposed to water deficit shows leaf senescence at a high rate. Initiation of leaf senescence increases salicylic acid accumulation and decreases jasmonic acid levels. This is further associated with degradation of chlorophyll contents and increase in deepoxidation (Abreu and Munné-Bosch 2008). Similarly, applying water deficit stress on sage results in a 73% decrease in dry weight, reduces essential oil production by 69% and increases nitrogen content by 15%. Decrease in water supply results in 21% decrease in phosphorus levels, 25% decrease in potassium levels and 10% decrease in magnesium levels (Corell et al. 2012).

8.3.9 Withania Somnifera

Withania somnifera, belonging to family *Solanaceae*, is a medicinally important Indian plant. Exposure of *W. somnifera* to drought stress leads to decrease in leaf area, photosynthetic pigment, root length, shoot length and photosynthetic activity. Withaferin A contents were increased by 5% under drought stress (Kannan and Kul 2011). The salt-stressed *W. somnifera* showed decreased germination percentage, seedling vigour and chlorophyll content. It also affects antioxidants like AsA and results in decreased glutathione and α-tocopherol contents. However, the activities of CAT, SOD, POD and PPO were also significantly affected as a result of salt stress (Jaleel et al. 2007b).

8.3.10 Trachyspermum Ammi

Ajwain is a common name for the traditional medicinal plant scientifically known as *Trachyspermum ammi*, which belongs to the Apiaceae family. In one experiment, 67-day-old ajwain plants were potted in saline soil. Increase in salt levels leads to the reduction in biological yield of leaves and roots and reduces seed yield. A 50% reduction in seed yield is observed at 120 mmol/L of NaCl (Ashraf and Orooj 2006). Seed germination and seedling growth are significantly retarded in the presence of

NaCl stress. Reduced sugars, proline, glycine betaine and total sugars are observed to accumulate in plants with increasing salinity (Yogita et al. 2014).

Drought stress significantly alters plant mechanisms. Transpiration rate and stomatal conductance decrease drastically with progressive increase in stress levels, and carbon dioxide concentration is also increased. Plant height and fresh and dry weights are reduced due to drought stress. The total phenolic and chlorophyll content is increased with increased exposure to drought stress. Secondary metabolite production is also enhanced significantly under stress (Azhar et al. 2011).

8.3.11 Carthamus Tinctorius

Carthamus tinctorius (safflower) is an aromatic oilseed crop (Kumar and Kumari 2005). The flowers of safflower are used to cure several chronic diseases and are extensively used in Chinese herbal medicines (Li and Mündel 1996; Hussain et al. 2016). Different environmental stresses affect safflower growth and productivity. It has been reported that due to salt stress, safflower loses fresh weights and relative water contents, thus hampering its growth even in moderately salt-tolerant American safflower (Hussain et al. 2016). Under drought stress the contents of flavonoids are significantly increased, and the synthesis of carbohydrates is diverted to secondary metabolites as reported by Salem et al. (2014) in safflower.

8.3.12 Coleus

Coleus has important medicinal values having several therapeutic properties. Kotagiri and Kolluru (2017) reported that salinity stress has considerably decreased growth attributes including leaf water potential and relative water content in *Coleus* species.

8.4 Adaptation Strategies to Tolerate Abiotic Stresses in Medicinal Plants

Medicinal plants consist of a large number of plant species with diversified biological particularities and attributes that are cultivated on a small area as compared to other cultivated plant groups (Pank 2006). The anthropogenic effects on the biosphere, the obstacle of the biosafety rules for the commercial medicinal plants and enhancing climate change challenges are severe intimidations for biorepository (Kurnaz and Kurnaz 2021).

Therefore, to increase the cultivation of medicinal plants, many long-term (screening and selection, plant breeding and genetic engineering) and short-term strategies (exogenous application of potential osmoprotectants, hormones, amino acids and minerals) are adapted under various biotic and abiotic stresses for improving their growth as well as cultivation techniques (Fan et al. 2019; Ghassemi et al. 2020; Rana et al. 2020).

8.4.1 Long-Term Strategy

In order to cultivate medicinal plants to achieve sustainable production on a large scale, it is imperative to use modern breeding techniques. More sophisticated selection criteria through appropriate breeding strategy can help to achieve realistic targets (Wang et al. 2020).

8.4.1.1 Plant Breeding

Plant breeding portrays the importance of specific medicinal plant species to a required demand with high quality which leads to a sustainable plant production. While genetic engineering techniques are very expensive and precise procedures, classical breeding methods are more preferable for cultivation of medicinal plants (Pank 2006; Xiao et al. 2016). However, successful breeding techniques lead to the availability of particular characteristics of the desired new varieties; according to these approaches, breeders initiate breeding programs by screening the accessible germplasm of different plant populations. It is also a fact that it relies on the availability of a variety of germplasm with genes of desired characteristics; the larger the variety of plant species, the larger the chance to recognize an appropriate donor accession (Pank 2007; Ozaki and Shibano 2014; Xiao et al. 2016; Wang et al. 2020).

The most essential requirement of successful breeding program is genetic variation of initial population. Prior, the breeder deals with available natural population, this leads to the development of a new variables (Wang et al. 2020). Once a population with a suitable variable is accessible, the breeder starts to choose genotypes with desired gene expression. Successful breeding strategies led to the development of precise breeding procedures. The classical breeding methods are less expensive as compared to advanced biotechnological techniques (Pank 2007). These methods include

- Exploitation of available natural population
- Generation of new genetic variable
- Utilization of crossing procedures for new combination
- Using hybrid technique for breeding
- Synthetic cultivars

- Induced mutation
- Somaclonal variation
- Fusion of protoplasm (somatic hybridization)
- Molecular gene transfer by using somatic hybridization
- Selection

The are several limitations of breeding techniques: less results of breeding programs are available, medicinal plants consist of greater population, expensive analysis of important constituent, limited dimension for breeding research and breeding methods are used for minor cultivars and very low seed turnover because of limited available cultivation area (Pank 2007; Wang et al. 2020). So, preferably advanced biological techniques such as genetic engineering approaches are used to meet the need of medicinal plant production.

8.4.1.2 Genetic Engineering

Genetic engineering techniques in advanced crop production methods exhibit exceptional brilliant performance to meet food scarcity. Conventional medicinal plant production also deals with several challenges which include resource shortage, environmental destruction, decline in germplasm availability and number of complications (Teng and Shen 2015). It is regarded as a tool to enhance medicinal plants' yield and resistance against diseases, insects and herbicides and enhance the level of active ingredients in targeted medicinal plant species (Wang et al. 2008). Thus, potential approaches of genetic engineering are a vital player in the protection and huge area cultivation of medicinal plants. However, in the process of developing transgenic medicinal plant, the safety protocol regarding medicinal plant is of great significance. And it depends on the targeted transgenic medicinal plant (Teng and Shen 2015).

Secondary metabolites are extensively found in medicinal plants and are of great importance exhibiting different biological activities used in various applications (Kliebenstein and Osbourn 2012). Genetic engineering facilitates to mitigate the difficulties in getting secondary metabolites through medicinal plant cultivation. For the production of target compounds from valuable medicinal and aromatic plants (*Artemisia annua* L., *Atropa belladonna* L., *Papaver somniferum* L., *Dioscorea* sp. and *Panax ginseng* L.), various genetic transformation procedures have been standardized (Bindu et al. 2018). The various strategies such as decreasing catabolism, reducing flux through competitive pathways, overwhelming rate-limiting stages and regulatory gene overexpression are used to enhance the production of secondary metabolites (Li et al. 2020). The advanced biotechnological tools including metabolic engineering and RNAi technology open an avenue for targeted metabolite production for commercial applications as given in Fig. 8.2.

Plant	Targeted product	Enzyme used	References
Artemisia annua	Artemisinin and salicylic acid	Cinnamate-4-hydroxylase	Kumar *et al.*, 2016
Withania somnifera	Withanalides	Cycloartenol synthase	Mishra *et al.*, 2016
Salvia miltiorrhiza	Rosmarinic acid	Phenylalanine ammonia-lyase	Song & Wang, 2011
Artemisia annua	Artemisinin	Squalene synthase	Zhang *et al.*, 2009a; Zhang *et al.*, 2009b
Catharanthus roseus	Tryptamine	Tryptophan decarboxylase	Runguphan *et al.*, 2009)
Papaver somniferum	Morphinan alkaloids	Salutaridinol7-O-acetyltransferase	Allen *et al.*, 2008; Kempe *et al.*, 2009
Panax ginseng	Ginsenoside	Dammarenediol synthase gene	Han *et al.*, 2006
Papaver somniferum	Codeine and morphine	Codeinone reductase (COR)	Allen *et al.*, 2004
Papaver somniferum	Morphine, codeine, sanguinarine	Berberine bridge enzyme (BBE) and N-methyl coclaurine 3′-hydroxylase (CYP80B1	Frick *et al.*, 2004
Mentha×piperita	Limonene	Limonene-3-hydroxylase gene	Mahmoud *et al.*, 2004
Mentha×piperita	Menthofuran	Cytochrome P450 (+) menthofuran synthase	Mahmoud & Croteau, 2001

Fig. 8.2 RNAi-mediated gene silencing in medicinal plants

8.4.2 Short-Term Strategy

8.4.2.1 Application for Osmoprotectants

Osmoprotectants assist the plant to conquer variable stressful environment. Osmolyte accumulation having low molecular weight and water-soluble character in the plant cells is common under stress conditions (Janmohammadi 2012; Noreen et al. 2019a). However, plant's physiological, biochemical and molecular functions differ widely in response to various biotic and abiotic stresses (Ghassemi et al. 2020). These stresses respond via changes in morphological, growth and gene expression modulation (Arbona et al. 2017). In this environment plants adapt embolic changes for the production of various osmolytes such as proline and glycine betaine. They play an important part in the structure of cell and stabilization of proteins, in addition to mediating osmotic adjustment and redox metabolic activities to manage turgid pressure (Janská et al. 2010). While in cellular respiration and signaling processes, total soluble carbohydrates (osmolyte) act as substrates (Janmohammadi 2012; Noreen et al. 2020).

The cell membrane proves to be a more sensitive plant organ in chilling stress (Levitt 1980). The saturated and unsaturated fatty acids are the components of membranes, and ratios of fatty acid may alter when exposed to cold environment. This variation is an index of plant tolerance to cold stress (Karabudak et al. 2014). Therefore, osmolytes can enhance resistance to cold stress by representing "over-all" mechanism of resistance. Primary metabolites produced in plants are involved in mineral nutrition and necessary biochemical function of the cell, whereas secondary metabolites mediate defensive mechanism, such as biotic and abiotic environmental stress conditions (Croteau et al. 2000; Berli et al. 2011; Gil et al. 2012; Escoriaza et al. 2013).

8.4.2.2 Salicylic Acid

Most groups of plant phenolics include salicylic acid (SA) that regulates the germination of seeds, opening and closing activity of stomata, accumulation of photosynthetic pigments and function, activities of enzymes, production of heat, biosynthesis of ethylene, uptake of minerals, sprouting of flower, functions of membranes and developmental process of medicinal plants (Ali 2020) as well as in crop plants like wheat (Noreen et al. 2019b), sunflower (Noreen et al. 2009, 2012), canola (Rehman and Khalil 2018) and maize (Fahad and Bano 2012) . The hormonal activity exhibited by SA has been applied to various medicinal plant species in vivo and vitro conditions to highlight its significant role in the synthesis and accumulation of secondary metabolites (Ali 2020). The environmental stress conditions like cold stress enhanced the effective level of SA in medicinal plants which includes *Capsicum annuum* L. (Fung et al. 2004), *Phaseolus vulgaris* L. (Gharib and Hegazi 2010), *Musa acuminate* L. (Mirdehghan and Ghotbi 2014), *Citrus limon* L. (Soufi et al. 2015) and *Stevia rebaudiana* L. (Rajashekar et al. 1999). Exogenous application of SA enhanced subsequently the primary and volatile metabolites in medicinal plant, i.e. *Egletes viscosa* L., under saline stress condition (Batista et al. 2019).

8.4.2.3 Ascorbic Acid

Ascorbic acid (AsA) plays an important role in the mediation of defensive mechanisms of medicinal plants under stressful environmental (Ghassemi et al. 2020; Noreen et al. 2021). AsA was used to increase the solubility of alginate-chitosan in medicinal herbs such as *Urtica dioica*, *Crataegus laevigata*, *Rubus idaeus*, *Olea europaea*, *Achillea millefolium* and *Glechoma hederacea*. AsA enhanced the antioxidant activity of encapsulated herbal-phenolic extracts (Belščak-Cvitanović et al. 2011). AsA takes part in the acclimation of medicinal plants (Chen and Paul 2002). The decreased level of AsA was observed in pear fruit due to the development of brown core (Veltman et al. 1999). The content of ascorbic acid enhanced during the development of fruit in tomato and pepper (Yahia et al. 2001) and also in muskmelon (Al-Madhoun et al. 2003) occurs in the apoplast of the cell. The content of AsA was regulated by the synthesis, transportation and breakdown of apoplastic ascorbate oxidase (Pignocchi and Foyer 2003).

8.4.2.4 Jasmonic Acid

Jasmonic acid (JA) is a phytohormone which regulates important functions in medicinal plants which includes retardation of plant root and inhibition of male fertility process and biotic and abiotic stresses (Ghassemi et al. 2020). JA level in medicinal plant enhanced under cold stress modulated by LOX genes (Kosová et al. 2012) and the biosynthetic pathway of JA regulated by four genes (AOC, JAR1, LOX1, LOX2) in response to cold stress (Liu et al. 2017). The regulation of JA through the cascade series of pathways is controlled by various ICE-CDF-independent and ICE-CBF-dependent transcriptional factor. The ICE binding to cis-element enhances COR gene expression resulting in enhanced resistance to stress condition in medicinal plants (Zhao et al. 2016). The environmental stress conditions enhanced the effective level of JA in medicinal plants which includes *Caragana jubata* (Bhardwaj et al. 2011), *Eriobotrya japonica* (Cao et al. 2011), *Musa acuminate* (Zhao et al. 2013), *Punica granatum* (Mirdehghan and Ghotbi 2014) and *Solanum lycopersicum* (Wang et al. 2016).

8.4.2.5 Abscisic Acid

Abscisic acid (ABA) is a phytohormone which mediates growth and developmental processes and enhances antioxidant activity in medicinal plant under stress condition (Ghassemi et al. 2020). The exogenous application of ABA is a technique used for the phenolic compound production in different medicinal herbs by adapting spraying strategy on the aerial plant parts (Marcińska et al. 2013). The ABA production inside the plant is involved in photosynthetic and gas exchange attributes, closing of stomata, seed dormancy, overcoming different environmental stress; exogenously applied ABA is involved in enzyme synthesis that changes subsequently the production of primary and secondary metabolites and may directly enhance phenolic compound synthesis (Murcia et al. 2017; Shamsi et al. 2019). The level of ABA is enhanced in *Fragaria x ananassa* to alleviate the harsh effect under cold stress condition (Rajashekar et al. 1999). *Physalis angulata* is a medicinal herb that cures various diseases because of its production of secondary metabolites (phenolic compounds). Exogenous application of ABA enhanced the production of phenolic compounds in *Physalis angulate* (*Moreira et al.* 2020). The exogenous application of ABA was used for the production of secondary metabolites in grapevine (Fanzone et al. 2012; Gonzalez et al. 2015; Murcia et al. 2017).

8.4.2.6 Gibberellic Acid

Gibberellic acid (GA) is a phytohormone which controls many important processes in medicinal plants and is also known as growth regulators. The downregulation of GA genes was observed in medicinal herbs under environmental stress conditions (Ghassemi et al. 2020). GA played a very important role in plant developmental stages; due to that reason, it is very significant to highlight these respective genes to enhance abiotic plant tolerance in stressful environment. GA2 gene is involved in the biosynthetic pathway of GA (Murcia et al. 2017). GA used for the priming of seed cloves (*Allium sativa*) enhanced the growth and developmental processes of plant, especially regulating axillary bud outgrowth; however, GA endogenously enhanced the content of sugar (fructose and sucrose) in the stem and leaf of *Allium sativum* to govern growth processes (Liu et al. 2020).

8.4.2.7 Plant Growth-Promoting Rhizobacteria (PGPRs)

The close vicinity of the plant root in which complicated association between plant root, soil microbes and soil defines as "rhizosphere". The diversity of microbial population in the plant rhizosphere than in the bulk soil because physiochemical and biological characteristics of plant-microbes interactions (Glick 2012, 2015; Compant et al. 2019). Rhizosphere microbes are beneficial for the plant growth and establish sustainable agriculture practices (Mukasheva et al. 2016; Afzal et al. 2019; Ummara et al. 2020). The PGPRs found in the root surrounding of medicinal plants like *Aloe vera*, *Catharanthus roseus*, *Coleus forskohlii* and *Ocimum sanctum* are isolated from bioinoculants and play an important role in enhancing the growth and yield of medicinal plant (Attia and Saad 2001; Karthikeyan et al. 2008). The microbial inoculants include *Azotobacter chroococcum*, *Azospirillum lipoferum*, *Bacillus megaterium* and *Pseudomonas fluorescens* used for the better growth of medicinal plants and subsequently increased rate of germination, seed vigour index and photosynthetic pigment of medicinal herb *Catharanthus roseus* (Lenin and Jayanthi 2012).

The isolated bioinoculants exhibit a potential to establish in the microenvironment of the root and soil of medicinal plants even under abiotic stress by optimizing stressed condition of plants and mitigating drastic effects of plant disease. The role of PGPR in abiotic stress reduction (Dimkpa et al. 2009) includes alleviation of drought stress (Alvarez et al. 1996), lessening oxidative stress (Štajner et al. 1997) and mitigation of saline stress (Weyens et al. 2009; Yang et al. 2009). The yield of the *Bacopa monnieri* is improved by applied salt-tolerant inoculant (*Exiguobacterium oxidotolerans*) (Bharti et al. 2013). The microbial inoculum improved the growth of pepper and tomato under drought stress (Aroca and Ruiz-Lozano 2009). “Induced systemic tolerance” of plant is defined as tolerance against abiotic stress because of physiochemical alteration caused by PGPB (Sandhya et al. 2010). Endemic microbial populations especially rhizosphere bacteria under water-deficient condition such as in arid areas are most effective in increasing tolerance against drought stress (Marulanda-Aguirre et al. 2008; Ilyas and Bano 2010).

8.5 Conclusion and Future Prospects

Pharmaceutical importance and a variety of chemical and biological diverse functions are characteristics of plant secondary metabolites that constitute a significant and interesting research (Ghassemi et al. 2020). Biotechnological strategies and metabolic engineering facilitate the production of secondary metabolites on commercial level. These techniques are of great importance and have not been fully understood in medicinal plants as in other crops. RNAi opens an avenue for genomic understanding. The greater emphasis on time-specified and inducible promoters functional in the target tissues and the development of additional RNAi procedures for genomics screening may assist in successful plant secondary metabolite production (Bindu et al. 2018). However limitations include gene silencing, erratic results due to complicated gene networking, constant and desirable amount of secondary metabolite production and biosafety rule regarding transgenic medicinal plants required to be enforced in the future. There is a need of more work to recognize targeted genes and enzymes that regulate the synthesis of secondary metabolites using genomics, proteomics and metabolomics strategies (Kumar et al. 2016).

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Chapter 9

Impact of Various Environmental Factors on the Biosynthesis of Alkaloids in Medicinal Plants

Arian Amirifar, Arash Hemati, Behnam Asgari Lajayer, Janhvi Pandey, and Tess Astatkie

Abstract Alkaloids are a group of nitrogenous heterocyclic compounds derived from amino acids, and plants can synthesize them as secondary metabolites. Such compounds play a key role in the life of organisms. Alkaloids can be easily available in our daily food items and act as a consequential element in various pharmaceutical products. Moreover, they have a significant role in the defense mechanism of plants during stress conditions. In this review, we discuss the impact of various stress factors like abiotic stress; potentially toxic element (PTE)-induced stress (cadmium, lead, zinc, chromium); stress caused by salinity, heat, drought, reactive oxygen species, and free radicals; and nutrient scarcity on the synthesis and production of valuable alkaloids in several medicinal plants.

Keywords Secondary metabolites · PTE stress · Drought stress · Salinity stress · Oxidative stress

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9.1 Introduction

Many secondary metabolites can be derived from plants. Several types of secondary metabolites exist in plants to protect and regulate functions under various stress conditions. Based on biosynthetic pathways and chemical nature, plant secondary metabolites are categorized into three main groups: (1) phenolic (phenolic acids, lignin, coumarins, stilbenes, lignans, flavonoids, and tannins), (2) terpenes (carotenoids, plant volatiles, sterols, and glycosides), and (3) nitrogen-containing compounds (glucosinolates and alkaloids) (Fig. 9.1) (Asgari Lajayer et al. 2017).

The most important group of secondary metabolites is alkaloids, due to their numerous physiological and pharmacological impacts and utilizations. Since there is no clear boundary between naturally occurring alkaloids and complex amines, the exact definition of the word "alkaloid" is somewhat difficult to elaborate. From chemists' point of view, these are a group of heterocyclic compounds comprising nitrogen in their structure, with strong physiological activity, which are often toxic and have alkaline chemical properties. According to biologists, alkaloids are nitrogenous heterocyclic chemical compounds, which have ecological and pharmacological applications. Plants produce alkaloids and other secondary metabolites for specific purposes, which are often important for the survival of organisms. One of the functions of alkaloids in plants is chemical defense against herbivory attributing to their toxicity and bitter taste. For example, it has been reported that glycoalkaloids emanated from potato leaves exhibit negative effects on the contractile activity of

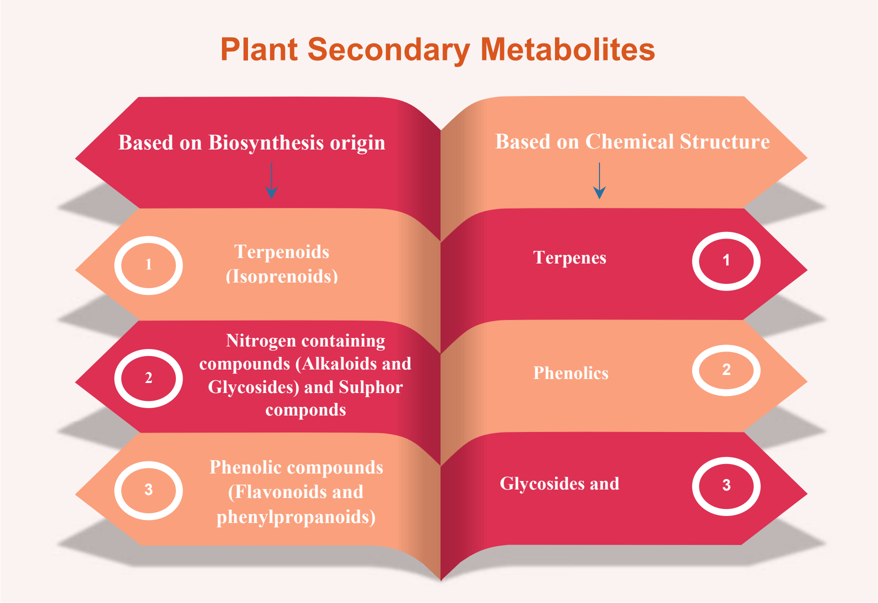

Fig. 9.1 Schematic representation of a detailed classification of plant's secondary metabolites

three species of beetles *Zophobas atratus*, *Tenebrio molitor*, and *Leptinotarsa decemlineata* (Marciniak et al. 2010). Antifungal effects of alkaloids have also been suggested for several plant-related fungi via bioassay experiments (Wippich and Wink 1985). Studies conducted by the World Health Organization revealed that the application of herbal medicines has recently increased dramatically worldwide. With the enhancement in the popularity of medicinal plants, health, safety, and quality of raw materials of medicinal herbs and their processed outcomes have become a major concern of global organizations. Environmental pollution extremely influences the quality of medicinal herbs and their processed products. In this review, we will examine the effect of biological and non-biological stresses on the production and quality of alkaloids in medicinal plants.

9.2 Impact of Potentially Toxic Elements on the Biosynthesis of Alkaloids in Medicinal Plants

Several studies have manifested that the accumulation of potentially toxic elements (PTEs) in medicinal plants rely on their cultivation environment, type of plant species, drying conditions, storing, transportation, and processing. Contamination of the growing environment of medicinal plants with PTEs can cause substantial alterations in the quality and amount of these metabolites by affecting the biological pathway of their production. At present, one of the alarming environmental issues is soil contamination with PTEs. PTEs can be described as elements with atomic number greater than 20 and densities >5 grams per cubic meter (Alloway 2010). Some of these elements are crucial for oxidation and reduction reactions, normal growth, electron transfer, and many metabolic activities, but their excess concentration in the soil gives rise to metabolic disorders and growth retardation in various plants. Others, such as cadmium, lead, mercury, and chromium, are rather not required and are toxic to plants even in low concentrations (Asgari Lajayer et al. 2017). Soil contamination by PTEs occurs either by human or some natural activities such as weathering of rocks in the soil (Moattar et al. 2010). In fact, human activities such as metal smelting, industrial expansion, mining, and the use of chemical fertilizers that have PTEs in them may lead to their further accumulation in the soil (Megateli et al. 2009). Ions of PTEs can get absorbed by plant roots and then transported to the aerial parts, disrupting plant metabolism leading to reduced growth (Li et al. 2010). Several cases of PTE contamination in the medicinal herbs and their high translocation potential to usable plant parts have been reported by various researchers (Zheljazkov et al. 2006; Baye and Hymete 2010), although contamination is most likely to happen in medicinal plants and their end products during the process of cultivation, processing, etc. (Denholm 2010).

In recent years, a significant positive trend in the application of traditional medicine, especially herbal medicine, worldwide has been noted, which might be attributed to advertising, organic nature, and their low side effects. Unfortunately, along with the popularity of traditional and herbal medicines, reports on the negative impacts of excessive usage of herbal medicine without proper consultation have also increased, which has paved the way for the entry of low quality of herbal raw materials in the industry. Until now, little attention was given to the quality standards of medicinal plants' raw material, but recent enhancement in the popularity and trading, the quality of medicinal plants, and especially their environmental quality has become a major concern of global organizations (WHO 1998; Dietrich's et al. 2006). However, due to the lack of global standards and laws, there is a huge difference between countries in terms of obligation to quality and safety of these kinds of products (Dietrich's et al. 2006). Until 1998, there were only 14 members of the World Health Organization that had ordained some regulations over the utilization of medicinal plants, but till 2003 the number of members increased to 53, among which 49% of the countries reported that they are looking forward to approving these laws in the benefit of mankind. Countries such as Canada, China, Malaysia, Singapore, and Thailand have developed their own guidelines to guarantee the minimum possible concentration of PTEs in medicinal plant precursors and their products (WHO 1998; Kosalec et al. 2009). In 1998 and 2005, the WHO declared the maximum permissible levels of cadmium, arsenic, and lead for medicinal plants at 0.3, 1, and 10 mg/kg, respectively. Provisional tolerable intake (PTI) is another index to determine and explain the maximum permissible amount of PTEs that can be present in the medicinal plants, values of which are determined by the World Health Organization or the FAO. The two organizations have suggested the permissible concentration of PTEs that is rather safe for provisional tolerable weekly intake (PTWI). According to these organizations, the word "weekly" is used to emphasize the importance of limiting the consumption period for such substances.

PTWI for mercury, arsenic, lead, and cadmium is 5, 15, 25, and 7 micrograms per kilogram of body weight, respectively. Also, the minimum and maximum concentration required in the daily diet is 0.05–0.5 for copper and 0.3–1 mg/kg for zinc body weight (Kosalec et al. 2009). Studies exhibited that under stress conditions generated by some PTEs, certain secondary metabolites are significantly enhanced in the plant parts (Rai et al. 2005). It has been reported that PTE contamination in the air and soil at 400 meters distance from the source of the pollution caused the essential oil yield of peppermint (*Mentha piperita* L.) and wild mint (*Mentha arvensis* L.) to be reduced by more than 14%, but on the other hand, no functional reduction in the amount of essential oil and quality in lavender plants was recorded at the same distance from the source of contamination (Zheljazkov and Nielsen 1996). Research findings on the impact of PTEs on various plant alkaloids are summarized in Table 9.1.

Table 9.1 Findings of studies on the impact of PTEs on various plant alkaloids

PTEs	Main findings	Reference
Cd	*Catharanthus roseus* plants treated by $CdCl_2$ exhibited a significant reduction in alkaloid levels	Pandey et al. (2007)
Cd	$CdCl_2$ treatment displayed the same impact on the concentration of alkaloids in the roots of *Catharanthus roseus*. Due to stimulating effects of Cd on transcription of tryptophan decarboxylase (TDC), it caused an increase in ajmalicine concentration in the culture medium by increasing cell tryptamine and ajmalicine excretion	Zheng and Wu (2004)
Pb	$PbCl_2$-treated *Catharanthus roseus* exhibited a drastic reduction in the yield of alkaloids	Pandey et al. (2007)
Pb	Although Pb treatment enhances alkaloid concentration in the roots, it has a completely opposite effect on leaves leading to the reduction of vindoline concentration. Moreover, Pb treatment does not seem to have any noticeable effect on ajmalicine content in roots, but the total content was increased	Srivastava and Srivastava (2010)
Zn	Usually, stimulants increase secondary metabolite content via promoting the transcription and translation of genes that are involved in the synthesis of secondary metabolites. ZnO is one of the stimulants that increases hyoscyamine and scopolamine concentration by overexpressing their relative genes called h6h genes. Studies have shown a greater association between scopolamine content and ZnO, which enhanced the transcription of h6h genes. The accumulation of both targeted secondary metabolites, hyoscyamine and scopolamine, exhibited a 5% increase	Asl et al. (2019)
Cr	In both varieties of *C. roseus* (Rosea and Alba) treated with chromium, the accumulation of vincristine content increased to 100 M Cr and 50 M Cr in the roots and shoots, respectively. In the presence of chromium, vinblastine content increased by 2.29 times	Rai et al. (2014)

9.3 Salinity Stress Impacts on Alkaloid Content in Medicinal Plants

Salinity plays a major role in the survival of plants and viability of the soil (Hilal et al. 1998). Salinity stress is likely to come along with osmotic stress and ion toxification that deteriorate plant growth by reducing or misbalancing plant's available water and nutrition. Also, high levels of salinity reduce biomass of root and leaves as well as plant's photosynthesis rate in addition to root's length (Seiler and Raul 2005). Putrescine (diamine), spermidine (triamine), and spermine (tetraamine) are important polyamines that exist in all living organisms and are part of a larger group called polycations compounds that are usually found comprising two or more amino groups. The most important aspect of these compounds is their polycationic feature at physiological pH. Polyamines are essential for plant development; they stimulate cell division, synthesize DNA and proteins, control rooting and germination, and respond to environmental stresses (Tang and Newton 2005). They also participate in plant's stress responses depending on the type of plant species and stress (Abdel Rahman et al. 2013). Reports suggest that the utilization of exogenous polyamines reduces stress influence on plant growth. For example, during salinity stress these compounds can prevent the accumulation of Cl^- and Na^+ in plant tissues (Del Duca et al. 1994). Due to these impacts, a positive interrelationship might be considered between polyamines and plant secondary metabolites. Among all the mentioned polyamines, putrescine seems to be a pioneer in alkaloid production (Foster and Walters 1991). Hyoscyamine is one of the alkaloids found in *Datura stramonium* plants belonging to family Solanaceae and has several medicinal applications. Although it is used externally, this plant has analgesic properties that make it applicable during surgeries or childbirth. Moreover, it can be used as an analgesic, disinfectant, narcotic, anti-asthma, and anti-seizure, when used internally. But on the other hand, its overapplication has some side effects and might cause cancerous wounds, burns, rheumatoid arthritis, eye or scalp pain, superficial nerve pain (e.g., facial pain), etc. Also, it can help to reduce liver pain and menstrual cramps and relieve gout and cough. Studies have shown that the amount of soluble carbohydrates and alkaloids in *Datura stramonium* under potting conditions scales with the level of salinity.

It has been reported that the amount of wet and dry weight of soluble and insoluble sugars and alkaloid compounds enhances in *Datura stramonium* fruits as soil salinity increases (Ali 2000). Salinity treatment has also been utilized to enhance the concentration of scopolamine and atropine in callus of two cultivars of *Datura stramonium* (Ahmed and Leete 1970). Research indicates that compounds and active ingredients of medicinal plants, including alkaloids and secondary metabolites, increase in reaction to environmental stresses. And it is important to mention that in the biosynthetic pathway of tropane alkaloids, a polyamine called putrescine acts as precursor in the process (Flores and Galston 2011). A study indicated the presence of an association between the salinity and the alkaloid content in *Hyoscyamus muticus* and *Datura stramonium*, as both species responded positively to increasing levels of salinity by enhancing their alkaloid content (Ali 1991). It was reported that the concentration of alkaloids in *Datura stramonium* plants increased by up to 3000 mg/L with increasing salinity, but further enhancement reduced the content. Ornithine, proline, and glutamic acid are the three common precursors for alkaloids. For example, ornithine and proline are precursor for tropane alkaloids. Salinity can cause acid buildup by suppressing transaminase reactions. Glutamic acid, with the help of some other substances, can make some nitrogenous compounds such as ornithine, and then ornithine can be turned into tropane alkaloids (Ahmed et al. 1988). Research has shown that treating *Datura stramonium* plant that has been under medium salinity stress (40 mM salt), along with putrescine at a concentration of 0.05 mM, drastically increases the concentration of alkaloids in roots and shoots. Polyamines are essential for tropane production in *Datura* plant (Abdel Rahman et al. 2013). But in *Atropa belladonna* seeds, the presence of putrescine (1.00 mM) reduces alkaloid content and prevents growth and germination (Alet et al. 2011). In *Catharanthus* plants, increased sodium-to-potassium uptake ratio and being under seawater stress have exhibited an accumulating effect on vinblastine, vincristine, and catharanthine alkaloids (Jing-Yan and Zhao-Pu 2010).

Trigonelline is one of the alkaloids, which is vastly present in many plant species including fenugreek (*Trigonella foenum-graecum*), sea urchins, and starfish and in mammalian's urine after using nicotinic acid (Yuyama and Suzuki 1991). Constituents of trigonelline include nicotine (derived from pyridine nucleotide) during pyridine nucleotide biosynthesis cycle; and it is also produced by niacin because 5% of consumed niacin is converted into trigonelline (Yuyama and Suzuki 1991). In response to salinity stress, many plants utilize osmotic regulators such as glycine betaine, proline, and trigonelline to minimize wastage of water (Tramontano and Jouve 1997). Research conducted by Tramontano and Jouve (1997) suggested that alfalfa plants that were put under salinity stress had a two- to five-fold increase in the concentration of proline and trigonelline. Also, an enhancement in the amount of specific nuclear molecules in the G2 phase was reported by treating chickpea root meristems with concentrations of 10^{-7}–10^{-4} mol/L trigonelline. In comparison, proline was ineffective, and glycine betaine had a negligible effect on improving the accumulation of these substances in G2 phase. All these results emphasize the importance of trigonelline for cell cycle and osmotic regulatory role in plants under salinity stress.

In most soybean plants that have been under salinity or dehydration stress and even those that have acclimatized to these conditions, the content of trigonelline noticeably increased in the younger leaves, but with the growth of pods and seed ripening in reproductive stages, the content of trigonelline diminished (Minorsky 2002). Under moisture stress conditions, growth in plants is crucial for node production efficiency and nitrogen stabilization. Node production and nitrogen stabilization are far more influenced by drought stress during growth stage in comparison with the reproductive stage. There are few plants that are not capable of providing their own nitrogen and are dependent on the soil nitrogen. Developing adaptation for dry conditions is crucial for their future growth. Trigonelline is one of the dry stress-associated alkaloids that function as a defense system for plants that leads to decrement in the number of nodes. Application of *Rhizobium* can help the plants to prevent this phenomenon leading to yield enhancement, especially under adequate irrigation. It has been reported that in *Arachis hypogea* plants under low irrigation, the trigonelline content increases in comparison with complete irrigation (Cho et al. 2011). In *Rauvolfia tetraphylla*, reserpine alkaloid content was reported to increase under salinity stress (Anitha and Kumari 2006). According to a research conducted by Jaleel et al. (2007a), accumulation of indole alkaloids was observed after the application of 80 mM NaCl in *C. roseus* plants. Another research exhibited a significant accumulation in the amount of vincristine by treating shoots of *C. roseus* with 150 mM NaCl for 2 months (Osman et al. 2007). During salinity stress, the content of some alkaloids such as 6-dihydronicotine, portulacaxanthin II, papaveroxin, and secoberbin in *S. brachiata* leaves was reported to enhance, while the amount of some alkaloids like harmol and ricinine diminished. Application of 200 mM NaCl on *Sesuvium portulacastrum* leaves led to the enhancement of some alkaloids such as cyclo-dopa, N-formyldemecolcin, 5-O-glucoside, and colchicine along with reduction in the content of some alkaloids such as cyclo-acetoacetyl-L-tryptophan, castanospermine, and chelirubin (Benjamin et al. 2019). The content of chelirubin, 2-descarboxy-betanidine, deoxypumiloside, and noscapine was reported to diminish in *S. maritime* plants that have been under influence of salinity stress.

Research indicated that treating plants with salicylic acid (SA) can reverse the effects of salt stress, leading to alkaloid buildup in stressed *Catharanthus roseus* plants. Salicylic acid has the property that increases alkaloid biosynthesis in unstressed *Catharanthus roseus* plants (Idrees et al. 2011) and has promotional effects on genes that are decisive in the production of some groups of secondary metabolites that have antioxidant properties in stressed plants (Malarz et al. 2007). Even the content of some compounds that are in different structural groups such as phenolics, terpenoids, alkaloids, and others can be enhanced by SA application. In addition to that, it can increase the amount of hyoscyamine 6b-hydroxylase and putrescine N-methyltransferase in the roots of *Scopolia parviflora*. These compounds act as enzymes in the bioproduction of tropane alkaloids (Kang et al. 2004). Compounds such as vincristine and paclitaxel are two examples of anticancer drugs that, like many other active compounds, are extracted from plants due to their medicinal properties (Verpoorte et al. 2002). Many studies have been conducted to investigate the influence of environmental factors on the biosynthesis of these compounds in medicinal and aromatic plants to screen out high-efficiency genotypes; and salinity stress is one of the factors that stands out (Said-Al Ahl and Omer 2011). Salinity stress can influence plants in many stages, especially during seed germination phase that can be drastically restricted by salinity stress (Sosa et al. 2005). Salinity and drought stress can both cause further accumulation of secondary metabolites in medicinal plants compared to control groups of test plants (Selmar 2008). The increased accumulation of tropane alkaloids in *Datura inoxia* plants under salt stress is one example.

9.4 Heat Stress Impacts on Alkaloid Biosynthesis in Medicinal Plants

With the rapid increase in the population of the world, food demand is rising steadily due to which there are rising concerns about the sustainability of the global environment. Agricultural productivity is highly affected by several factors including water availability, air pollution, and soil fertility. Although medicinal plants can acclimatize to a wide range of conditions, there is still a concern that their production efficiency is influenced by extreme environmental stresses. There is a variety of abiotic stresses that can impact plant's growth and production such as temperature, salinity, drought, and anaerobic and flood conditions (Lawlor and Cornic 2002). These abiotic stresses are some of the most important impacts caused by global climate change (Wani et al. 2008). Due to greenhouse effect and increased total temperature of Earth, heat stress has become one of the major abiotic stresses particularly in dry and semi-dry areas. Extreme temperatures can profoundly affect plant's biological and chemical processes and result in deteriorated production (Wahid et al. 2007; Ghasemi et al. 2016).

Temperature is a detrimental factor for the development and growth of plants, and each species has its own optimal growth temperature. Recurrent enormous heat waves have been predicted (Bhatla and Tripathi 2014); and a vast majority of biological processes and metabolites are negatively influenced by such extreme conditions. Some examples are signal transduction processes, physiological adjustments, and defensive reactions. Even some primary metabolites such as amino acids and sugars are affected by these factors. Also, because heat stress can induce the biosynthesis of some secondary metabolites that act as cross-protective mechanism against biotic threats, it can be a link between biotic and abiotic stresses (Arbona et al. 2013). Among all types of adaptation to climatic changes, genotypic changes are the best and the most effective (Springate and Kover 2014). Different temperatures are highly effective over metabolic processes and ontogeny. For example, high temperature can accelerate leaves' senescence along with its effect on secondary metabolites (Morison and Lawlor 1999). Effects of temperatures and phenological phases on secondary metabolite accumulation in *Rhodiola rosea* clones are other examples of temperature's impact on plants (Thomsen et al. 2012). In *Helicteres isora* callus cultures, secondary metabolite content exhibited a positive correlation with light and temperature (Joshi 2015). Accumulation of polyamines followed by production of phenylamides was observed in bean and tobacco plants under water stress and temperature shocks alongside with phenylamide ascribed ROS-limiting activity through the stress (Edreva et al. 1995, 1998, 2008).

Heat stress can be used as a way of extraction in *P. brachyceras* plants. In two experiments that investigated the impact of temperature on leaf disks of *P. brachyceras*, the content of brachycerine almost doubled. In both experiments the temperature increased from 25 °C to 40 °C, but in the first one temperature increased suddenly and stayed at 40 °C for 3 days, while in the other one, temperature increase was slow and steady, and it increased by 5 °C per day during 1 week. The expression of tryptophan decarboxylase was reduced in leaf disks that were under severe temperature changes (at 12 h and 24 h), which suggests that adjustment of brachycerine accumulation by heat is mainly posttranscriptional. This idea was supported by a study where heat-treated leaf disks showed a higher amount of tryptamine and TDC activity (Magedans et al. 2017). Studies on *Datura stramonium* plants under heat stress indicated that alkaloid accumulation occurred both under warm and cold conditions (Shriya et al. 2019).

9.5 Impacts of Drought Stress on Alkaloid Biosynthesis in Medicinal Plants

Drought crisis in agriculture is associated with the reduction of water sources and day by day increasing need for food due to fast population growth (O'Connell 2017). Because of uneven and unreliable rainfall distribution, evapotranspiration, and water-holding capacity around the rhizosphere, it is hard to predict drought (Passioura and Angus 2010). Other than this, in some circumstances, plants may experience lack of water even if there is enough water available (Daryanto et al. 2020). Lack of water can significantly reduce plant's production and quality of crops (Battaglia et al. 2018). Growth stages, age, plant species, and drought severity and duration are the most important factors that can affect plants' growth and development (Gray and Brady 2016). Depending on the plant species, there are a variety of different mechanisms that plants utilize to overcome drought stress. They can reduce and adjust their metabolism, resource usage, and growth in order to resist inadequate situations such as drought (Osakabe et al. 2014; Bielach et al. 2017). To improve their response, plants utilize many other mechanisms such as molecular-level networks that participate in the signal transduction (Zandalinas et al. 2020; Kaur and Asthir 2017).

Jasmonic acid is a compound that has growth regulatory role and participates in developing responses to environmental stresses (Avanci et al. 2010). In fact, there are some other hormones like auxin and cytokinin that regulate plant growth, but jasmonic acid is unique due to its regulatory role on both growth and defense against various stresses. It is important to mention that jasmonates work as messengers in the process of accumulation of plant secondary metabolites (Yu et al. 2006). They accelerate the activity of a group of enzymes that take part in processes that result in the formation of secondary metabolites (Montiel et al. 2011). Periwinkles are a group of plants that are known to be one of the most resistant ones towards stresses like heat, salinity, and drought. They are capable of thriving under some extreme conditions where not many plants survive. To maintain this ability, they tend to reduce their need of water and nutrition (Pandey 2017). Research has revealed that under drought stress, butterfly plants are able to produce and accumulate antioxidants in all their parts. This feature makes them a putative candidate to be cultivated in places under frequent drought stress (Jaleel et al. 2007b). Findings of a study on the impacts of growth regulatory compounds on the biosynthesis of indole terpenoid alkaloids in the short term during the flowering period suggest the utilization of a combination of treatments in order to increase the content of alkaloids rather than using one plant growth regulatory treatment alone (El-Sayed and Verpoorte 2004). In conclusion, it is possible to take advantage of water shortage in some areas by using this feature in plants like butterfly plant, to effectively increase the alkaloid production (Jaleel et al. 2008).

Another study implies that the impact of drought on secondary metabolites production is highly dependent on two aspects: the species itself and the intensity of water stress (Azhar et al. 2011). Lack of water up to some extent has some positive implications in plants such as biosynthesis of secondary metabolites, enzymatic activities, and soluble substances accumulation. Drought stress can profoundly change the amount and structure of aromatic compounds and secondary metabolites in aromatic and medicinal plants as well as their yield (Bettaieb et al. 2009). Secondary metabolites are meant to function as a defense mechanism to overcome environmental stresses; hence, it is clear that these stresses can cause the accumulation of secondary metabolites (Ezz et al. 2009). Increased concentration of vinblastine and vincristine was observed in the aerial sections of periwinkle under drought stress. This indicates that the alkaloids are produced and can be increased in response to drought stress (Amirjani 2013). Oxidative stress caused by water shortage can noticeably increase the amount of alkaloids in the roots and aerial organs in some plants (Karadge and Gaikwad 2003). During drought stress, plants tend to assign more carbon on the production of secondary metabolites than on their growth, which implies that there is a balance between growth and defense (Bettaieb et al. 2009). In the presence of salicylic acid and under the effect of different drought stress levels, it has been reported that the medicinal and economic value of periwinkle is refined due to the enhancement in plant biomass and alkaloid yield. Salicylic acid is also effective in improving the content of bioactive compounds via stimulating the expression of genes that code some enzymes that participate in secondary metabolic pathways (Ali et al. 2006). One of the most important sources of analgesics is the opium poppy (*Papaver somniferum* L), which provides codeine, morphine, and semi-synthetic analogues like hydrocodone, oxycodone, buprenorphine, and naltrexone (Ali and Abbas 2003). Based on the experiments conducted to investigate the effects of drought stress on alkaloid content in *P. somnife*rum, drought stress drastically increases the amount of morphine, codeine, and narcotine in opium poppy (Szabo et al. 2003).

9.6 Impact of Free Radicals and ROS on Alkaloids in Medicinal Plants

Free radical is referred to as any molecular species that possess an unpaired electron in an atomic orbital and can independently exist. The common features shared by all free radicals are due to these unpaired electrons. Most of the free radicals are unstable and highly reactive. Free radicals are capable of either donating an electron or accepting one from other compounds; because of this feature, they can act as oxidants or reductants (Cheeseman and Slater 1993). The most important free radicals that contain oxygen are hydroxyl radicals, superoxide anion radicals, hydrogen peroxides, oxygen singlets, hypochlorites, nitric oxide radicals, and peroxynitrite. These highly reactive species have the potential to cause damage to some biologically important molecules such as DNA, proteins, carbohydrates, and lipids in nucleus and membranes (Young and Woodside 2001). Reactive oxygen species also known as ROS are a group of oxygen species that are naturally non-reactive but can produce free radicals. These free radicals can come to existence in different ways; for example, they may emerge from the environment, physiological processes, or endogenous sources (Sultan 2014). Many physiological processes can lead to the generation of free radicals. These free radicals tend to react with other molecules to become stable.

1. Superoxides are basically oxygen molecules with an extra electron. There are many biological reactions that can lead to the creation of these types of free radicals (Halliwell and Gutteridge 1992).
2. Another type of free radical is hydrogen peroxide. These molecules are generated in the biological systems by spontaneous dismutation reaction of superoxides. In addition to that, there are several enzymatic reactions that lead to the creation of these molecules such as reactions catalyzed by D-amino acid and glycolate oxidases (Chance et al. 1979). Hydrogen peroxide is not a free radical itself. It is a ROS and can turn into other free radicals like hydroxyl. Most of the negative effects that are assigned to H_2O_2 are actually because of this hydroxyl form (Halliwell and Gutteridge 1995).
3. Hydroxyl radical is the most important and most reactive free radical among others as it is capable of reacting with many types of biomolecules such as amino acids, sugars, lipids, and nucleotides. It is also the common product of ROS reactions and is the reason for most of the free radical tissue damages (Lloyd et al. 1997).

Continuous occurrence of ROS reactions and accumulation of free radicals from inner and outer sources can cause oxidative damage to cellular components and is capable of impairing many cellular functions (Comporti 1989). The production pathway of some secondary metabolites can be enhanced by oxidative stresses. Such impacts of oxidative stresses have been proven in both field conditions and controlled bioreactor environments. The introduction of air and stimulation in bioreactors in plant cell and organ cultures can eventually increase oxidative stress and influence both growth and secondary metabolism via providing proper accessibility and distribution to nutrients, metabolites, and other biologically important molecules as well as homogeneity of mass and heat transfer (Georgiev et al. 2009). It was found that oxygen deficiency in the cell cultures can also increase oxidative stress (Nisi et al. 2010). The level of phytoalexin-like compounds has been reported to increase under some specific stress conditions, but phytoanticipin-like compounds are continuously present regardless of the circumstances. A majority of metabolites seem to exhibit an accumulation profile somewhere in between these extremes. These compounds contribute to overall plant resilience. Their contribution may be direct like bitterness, toxicity, repellent properties, and proteinase inhibitors or indirect such as intra- and inter-specific signaling and internal signaling, antioxidant compounds. Antioxidants are important for alleviating or spatially restricting oxidative reactions. This way they participate in helping the plants to overcome harsh situations (Matsuura and Fett-Neto 2013).

A noticeable number of plant antioxidants are part of secondary metabolites. These antioxidants greatly participate in developing adaptability towards biotic and abiotic stresses and creating mechanisms that defend plants against reactive oxygen species that are capable of damaging membranes, organelles, and macromolecules. The role of natural products in developing adaptation to the environment and the initiation of plant responses depending on the situation is the reason of the great variety and complexity of these compounds. They are also the source for the formation of new drugs (Matsuura et al. 2013). Alkaloids can be called as the most important secondary metabolites. Redox imbalances can stimulate few signal transmission pathways that ultimately leads to alkaloid accumulation. Some studies showed a connection between oxidative stress and monoterpene indole alkaloid production. Alterations in ROS-related metabolism were observed in *Catharanthus roseus* plants that were under osmotic stress. These changes are mostly in lipid peroxidation, H_2O_2 content, and free-radical removal mechanisms, including both enzymatic and non-enzymatic antioxidants, and can result in higher ajmalicine content (Zhou et al. 2009).

9.7 The Effect of Nutrients

Nutrients are other factors that can influence plant's growth and productivity. For several reasons, studying the impact of nutrients on the formation of secondary metabolites in the *Senecio* plant family, specifically pyrrolizidine alkaloids (PA), is interesting. There are some invasive weeds that are included in the *Senecio* family (Radford and Cousens 2000). Not only that these invasive weeds are problematic for native species, but they are also toxic for livestock (Mattocks 1986). Pyrrolizidine alkaloids can also impact the growth of insects and microorganisms (Hol and van Veen 2002). Nutrients can be used as ways of manipulating *Senecio* family member's growth because the amount of nutrients in soil is a major determining factor that determines plant's responses towards competition. For example, high levels of potassium in soil help *Chenopodium album* plants to succeed in their competition with weed *Senecio vulgaris* (Qasem and Hill 1995). There are other advantages of studying the relationship between *Senecio* family and soil nutrients. Knowing the impact of nutrients on pyrrolizidine alkaloid concentrations in *Senecio* plants renders us the ability to predict the vegetation toxicity of these plants in cattle feeding (Brown and Molyneux 1996). Also, this allows the prediction of results of plant-herbivore interactions under different levels of nutrient availability.

In managing *Senecio* plants in natural areas, manipulation of nutrients can be helpful except when the invasive species are adapted to a spectrum of nutrient deficiency situations such as *Senecio inaequidens* (Lopez-Garcia and Maillet 2005). There are contrasting predictions of the effects that nutrients have on pyrrolizidine alkaloids concentrations. There are two main predictions: (1) pyrrolizidine alkaloid accumulation is predicted when excess amount of nutrients is available, which leads to the rest of the nitrogen to be spent on nitrogen-containing secondary metabolites such as alkaloids. Hence, *Senecio* plants would develop better protection against herbivores in nutrient-rich soils compared to the situations where the amount of nutrients is insufficient (van der Meijden et al. 1984). (2) It is predicted that when secondary metabolite's biosynthesis cannot keep up with the increased biomass, the pyrrolizidine alkaloid concentrations diminish (Koricheva 1999). Pyrrolizidine alkaloids are synthesized in the roots, and because of that the total amount of pyrrolizidine alkaloids is highly dependent on the root's biomass (Hol et al. 2003). Anything that influences the shoot-to-root ratio can alter the content of pyrrolizidine alkaloids. It is important to mention that nutrients mostly increase the shoot biomass rather than the root biomass (Poorter and Nagel 2000).

Naturally, soils are different in their nutrient type. For example, sandy soils are poor in terms of nutrients, but in contrast clay soils are rich. Except for mineral nitrogen, availability of other nutrients in those soils is unknown (Frischknecht et al. 2001). It was reported that the species *S. jacobaea* and *S. vulgaris* responded almost similarly to fertilization as both had equal or reduced pyrrolizidine alkaloid concentrations. Joosten et al. (2009) studied the effects of soil-borne microorganisms and soil type on PAs in roots and shoots of *Jacobaea vulgaris*. Soil-borne microorganisms and soil type affected the composition of PAs. By changing the composition rather than the total concentration below and above ground, plants have a more complex defense strategy than formerly thought. Interestingly, a stronger negative effect on plant growth was observed in sterilized soils inoculated with their "own" microbial community suggesting that pathogenic and/or other plant inhibiting microorganisms were adapted to their "own" soil conditions. An experiment suggested that except under very wet conditions, adding nutrient to the soil of *S. jacobaea* plants decreased pyrrolizidine alkaloid content in both shoots and roots. Another study also reported that the concentration of pyrrolizidine alkaloids in the shoot and in the roots of *S. jacobaea* was reduced by adding nutrients (Kirk et al. 2009). However, it is not yet proven that *Senecio* plants prefer to concentrate more PAs in the roots when nutrients are inducing lower PA levels (Hol et al. 2003).

In addition to pyrrolizidine alkaloid concentration, nutrients can also affect the composition of pyrrolizidine alkaloids within a plant. It might be because of the impact of the nutrient addition on flowering plants (Prins et al. 1990). This can be related to PA specification (Hartmann and Zimmer 1986). Plants that were in vegetative stages had differences in the accumulation of individual PAs in response to nutrients. Macel and Klinkhamer (2010) reported a decrease in erucifoline levels in plants grown in nutrient-rich soil, while the concentrations of jacobine were barely influenced in *S. jacobaea* plants. Also, it was observed that in comparison to other pyrrolizidine alkaloids, jacobine concentration in *S. jacobaea* plants was hardly influenced by alternation in the nutrients' level (Hol et al. 2003). It was revealed that PA composition in *S. jacobaea* is highly related to the soil-type, but the total concentration of pyrrolizidine alkaloids was unaltered. In *S. vulgaris* plants, no association was found between habitats differing in fertility and alkaloid pattern (Frischknecht et al. 2001).

9.8 Conclusion

Alkaloids are precious compounds, and their importance in our daily life cannot be ignored. Various types of stresses and harsh conditions can modify secondary metabolite and alkaloid levels in plants. By altering these compounds' concentration, plants somewhat assure their well-being in such harsh conditions. In this chapter, the impact of various stresses such as PTEs, heat, salinity, oxidative stress, and nutrient scarcity effects on concentration of different alkaloids have been reviewed. Moreover, we can utilize different stresses in various plants in order to elevate their secondary alkaloid production.

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Chapter 10

Regulation of Expression of Transcription Factors for Enhanced Secondary Metabolites Production Under Challenging Conditions

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Abstract Secondary metabolites are synthesized by plants when they are developing or responding to biotic and abiotic environmental stimuli, such as pathogen attack, light, stress, etc., that are used for pharmaceutical production or as nutritional ingredients. Research on secondary metabolites has received particular attention due to their benefits to health and in other industries. The study on plant genetics revealed transcription factors as important molecular tools for increasing the production of useful compounds by the metabolic engineering. In response to internal or external signals, transcription factors bind to the specific gene promoters, activating or suppressing the enzyme coding gene expression, and often form complexes that include others. By modifying genes that encode biochemical enzymes or regulatory proteins, different pathways could be altered. In addition to reducing the time-consuming process of investigating all the enzymatic steps of a poorly understood biosynthetic pathway, specific transcription factors would also reduce the costs of product development. This review includes regulation of the biosynthetic pathways of some important secondary metabolite classes with the knowledge of specific genes, transcription factors, and their modifications in different model plant species for the enhancement of the secondary metabolite contents.

Keywords Transcription factors · Secondary metabolites · Metabolic engineering · Biosynthetic pathway · MYB · bLWL · Jasmonic acid

10.1 Introduction

Plants synthesize a huge array of phytochemicals which are generally divided into two major classes: primary and secondary metabolites. Primary metabolites are very essential for the growth and the development of the plant, whereas secondary metabolites are used as defense molecules to protect themselves from various adverse conditions and were once thought to be non-essential for plant growth and development (Patra et al. 2013). These secondary metabolites play multiple functions throughout the plant's life cycle as mediators in the plant–environment interactions, such as plant–plant interactions, plant–microorganism and plant–insect interaction, etc. (Harborne 2001; Dixon 2001). For maintaining the defense system, plants constitutively produce antifeedants and phytoanticipins, and toxins like phytoalexins which guard the plant body against pathogens and herbivores, and also protect plants from abiotic stresses such as ultraviolet (UV) light (Vogt 2010; Vranová et al. 2012). Furthermore, humans have been widely utilizing secondary metabolites as a source of natural fragrances and pharmaceutical medicines (He and Giusti 2010; Kroymann 2011; Duan et al. 2012). Secondary metabolites also play a key role in pollination by attracting pollinators with showy flower, particularly with the help of the pigment anthocyanin and essential oils like terpenoid, and play a role in other advantageous interactions with other organisms (Gantet and Memelink 2002).

Structurally secondary metabolites are highly diverse from each other, but biosynthetically secondary metabolites are common in their basic pathways. Most secondary metabolites start from glycolysis or shikimic acid pathways and subsequently diversify to different types of phytochemicals (Patra et al. 2013). The diversification largely depends on cell types, developmental stage, and environmental cues. On the basis of their chemical structure and biosynthetic pathways, secondary metabolites are mainly classified into three major groups: phenolics, terpenoids, and nitrogen-containing compounds (Yang et al. 2012). Phenolic compounds are very diverse, such as flavonoids, coumarins, phenylpropanoids, and stilbenes. Phenylpropanoids are synthesized via alone the shikimate pathway or in combination with the mevalonate pathway. Terpenoids are synthesized via cytosolic mevalonate pathway or the plastidial methylerythritol phosphate pathway and contain one or more C5 units. The nitrogen-containing compounds include alkaloids, non-protein amino acids, and amines (Yang et al. 2012).

The protective properties of secondary metabolites are generally induced by external stress signals, though tissue-specific expressions are noticed in other secondary metabolites, such as flower pigments. Transcription factors (TFs) are sequence-specific DNA-binding proteins which regulate this coordination via interaction with the *cis*-acting promoter regions of target genes and modulation of the initiation rate of mRNA synthesis by RNA polymerase II. TFs control the coordinated expression of genes necessary for normal development and functional physiology and involved in the regulation of metabolism (Gantet and Memelink 2002). These proteins regulate gene transcription depending on tissue type and/or in response to internal signals, such as plant hormones, and to external signals like microbial elicitors or UV light. External signals may induce the internal signal production (Memelink et al. 2001a). TFs control the rate of recruitment of the preinitiation complex components, thus modifying the rate of transcription and also the rate of transition of the preinitiation complex from the closed to the open configuration, or via other mechanisms (Nikolov and Burley 1997). Genetic engineering is used to modulate the TFs of a secondary metabolic pathway that either increase or decrease the quantity of a certain compound or group of compounds in the normal producing plant species under normal or stress conditions (Verpoorte and Alfermann 2000; Verpoorte et al. 2000; Dixon and Steele 1999; DellaPenna 2001). Also, there is interest in the production of novel compounds not yet produced in nature by plants (Verpoorte and Memelink 2002). To decrease the production of a certain unwanted (group of) compound(s), one or few enzymatic steps in the pathway can be knocked out, for example, the level of the corresponding mRNA via antisense, co-suppression, or RNA interference technologies can be reduced, or an antibody against the enzyme can be overexpressed. The antisense gene approach has been successfully used for changing flower colors (Mol et al. 1990). These processes can change the expression of one or a few genes that can overcome specific rate-limiting steps in the pathway, can shut down competitive pathways, and can decrease catabolism of the product of interest. Researchers have also taken attempt to change the expression of regulatory genes that control multiple biosynthesis genes (Verpoorte and Memelink 2002). This book chapter summarizes various secondary metabolite classes, their importance, and biosynthetic pathway regulations at different stages; important transcription factors; and genes encoding catalytic enzymes, their modification by molecular engineering, and the outcomes in secondary metabolite contents after modifications.

10.2 Regulation of Phenolic Compounds

The important and major group of secondary metabolites is the phenolic compounds having a phenyl ring with one or more acidic hydroxyl groups attached to it (Achakzai et al. 2009). More than 1000 soluble and non-soluble compounds belong to this heterogeneous group which relies on the two biosynthetic pathways, the shikimic acid pathway and the malonic acid pathway (Taiz and Zeiger 2006). The phenolic compounds are divided into different groups like lignans, lignins, coumarins, tannins, stilbenes, flavonoids, styrylpyrones, and arylpyrones (Fang et al. 2011). Simple phenolic compounds are known as phenylpropanoids such as trans-cinnamic acid and p-coumaric acid and its derivatives. Lignin and cellulose are very complex phenolic compounds having highly branched polymers of phenylpropanoid groups which are very much abundant in plants (Taiz and Zeiger 2006). The amino acid phenylalanine is the key compound for the synthesis of phenylpropanoids which are derived from the phenylpropanoid pathway. Different branches of the pathway produce different compounds such as lignans, lignins, stilbenes, and flavonoids such as anthocyanins, proanthocyanidins (condensed tannins), flavonols, and isoflavonoids (Davies 2000; Winkel-Shirley 2001). The flavonoid branch of the phenylpropanoid pathway is responsible for the production of anthocyanin pigments, UV-absorbing flavones and flavonols, and antimicrobial phytoalexins, while the other branches produce lignin precursors and soluble phenolics such as the signaling compound salicylic acid (Holton and Cornish 1995).

10.3 Flavonoids (Regulation of Anthocyanin and Proanthocyanidin)

Flavonoids belong to the phenylpropanoid group, which possess antioxidant, anti-allergenic, anti-inflammatory, hepatoprotective, anticarcinogenic, antiviral, and anti-thrombotic activities. Inhibitory effects of several flavonoids were studied on the replication of HIV and tumor cell proliferation (Middleton et al. 2000). Flavonoids are naturally present in various fruits, vegetables, and grains, and also in tea and red wine, and have pleiotropic health-promoting and disease-preventing activities (Nijveldt et al. 2001). The biosynthesis of flavonoids starts with the amino acid phenylalanine, and the end products are anthocyanins, flavones or isoflavones, and condensed tannins (proanthocyanidins, PAs). Initial step of the phenylpropanoid pathway is the conversion of phenylalanine to hydroxycinnamic acids by phenylalanine ammonia lyase (PAL). Cinnamate 4-hydroxylase converts cinnamate to

4-coumarate which is then transformed to coumaroyl-CoA by 4-coumarate: CoA ligase. Coumaroyl-CoA with three malonyl-CoA molecules forms naringenin chalcone which is catalyzed with the help of chalcone synthase (CHS). Chalcone isomerase (CHI) helps chalcone to form flavanone. Flavanones are converted to dihydroflavonols by flavanone 3-hydroxylase (F3H). Dihydroflavonol reductase (DFR) catalyzes the reduction of dihydroflavonols to flavan-3,4-diols (leucoanthocyanins), which are then converted to anthocyanins through a series of enzymatic steps (Patra et al. 2013; Davies and Schwinn 2003).

10.4 Regulatory Genes

Flavonoid and anthocyanin biosynthesis was the first target for genetic engineering because the biosynthetic pathways are well known and the changes in flower color are easier to observe (Dixon and Steele 1999; Davies 2000). The structural flavonoid genes depending on the cell type and environmental conditions are regulated within a single plant (Memelink et al. 2001a). Tissue-specific expression of the flavonoid structural genes is controlled by a distinct transcription factor encoded by two families of regulatory genes, R/B and C1/Pl with WD repeats. The C1/Pl family is similar to the protein encoded by the vertebrate proto-oncogene c-Myb and the R/B family similar to vertebrate helix–loop–helix (bHLH) protein encoded by the proto-oncogene c-Myc (Grotewold et al. 2000; Ramsay and Glover 2005; Hichri et al. 2011). These transcription factors bind to specific sequences in the promoters of the target genes. The specificity of binding of plant MYB proteins with DNA varies considerably (Martin and Paz-Ares 1997). The bHLH proteins recognize the G-box (CACGTG) and interact with it (de Pater et al. 1997; Martínez-García et al. 2000).

10.5 Genetic Regulation in *Maize*

In maize, the anthocyanin biosynthesis pathway is regulated coordinately by the R2R3 MYB factors, C1/PL with R/B (bHLH) and PAC1 (WD40) in the aleurone (epidermal layer of the kernel endosperm), and by homologous genes in other plant parts (Mol et al. 1990). In in vitro maize cell cultures that are normally unpigmented, the biosynthesis and accumulation of anthocyanins are observed when R and C1 are expressed ectopically because most of the structural genes are expressed coordinately (Grotewold et al. 1998; Bruce et al. 2000). A MYB-type transcriptional regulator, P, is required in maize for the production of 3-deoxy flavonoids, which acts independently of R and C1 and has been associated with insecticidal or antifungal activity. When P is expressed ectopically in maize cells, a subset of biosynthesis genes for the accumulation of flavonoids is induced coordinately, but these genes are different from those regulated by C1/R (Grotewold et al. 1998; Bruce et al. 2000). The overexpression of the R and C1 transcription factors in undifferentiated in vitro maize cells cultured is able to achieve the induction of the complete flavonoid pathway (Grotewold et al. 1998). Viviparous 1 (Vp1) is the best characterized TF gene for an upstream regulation of the anthocyanin. A unique type of TF that was identified in maize is encoded by Vp1, along with ABA-regulated TFs, and is required for seed maturation (McCarty et al. 1991; Hattori et al. 1992).

10.6 Gene Regulation in *Arabidopsis*

Three functionally redundant R2R3 MYB TFs in *Arabidopsis*, viz., MYB11, MYB12, and MYB111, are involved in the regulation of the early biosynthetic pathway genes, such as CHS, CHI, and F3H respectively. A complex MBW (MYB/bHLH/WD) composed of four R2R3 MYBs (PAP1/MYB75, PAP2/MYB90, MYB113, MYB114 or GL1/MYB0), bHLH factors (glabrous3 [GL3])/enhancer of glabrous3 [EGL3]/transparent testa8 [TT8]), and the WDR protein (transparent testa glabra1 [TTG1]) are associated in the regulation of the late pathway genes, viz., dihydroflavonol 4-reductase (DFR), anthocyanidin synthase (ANS), and UDP-glu:flavonoid 3-O-glucosyltransferase(UFGT), including NADPH-dependent dihydroflavonol reductase (DFR) (Borevitz et al. 2000; Stracke et al. 2001, 2007; Tohge et al. 2005; Gonzalez et al. 2008). The late anthocyanin biosynthesis genes are mainly activated by RGL3, while partial redundant roles in controlling of the anthocyanin pathway are played by bHLH TFs, GL3, EGL3, and TT8 (Gonzalez et al. 2008). When MYB transcription factor PAP1 (production of anthocyanin pigment 1) is expressed ectopically in *Arabidopsis,* it was found that the expression of flavonoid biosynthesis genes is strongly enhanced, which are responsible for the purple pigmentation of most plant organs (Borevitz et al. 2000). PAP1 and a related gene, PAP2, have sequence similarity with C1, and those may be C1 orthologs from *Arabidopsis*. Proanthocyanidins are controlled by TT2 by regulating BANYULS (BAN) expression in the seed coat, which encodes nicotinamide adenine dinucleotide phosphate-dependent leucoanthocyanidin reductase (Nesi et al. 2001). The key regulatory role in anthocyanin accumulation induction at high light and sucrose conditions is played by MYBL2. It induces the PAP1 and TT8 expression by forming an active MBW complex for the upregulation of the anthocyanin biosynthesis pathway genes. Under low-light or stress-free conditions, the pathway gene expression and subsequent anthocyanin accumulation are suppressed by the induction of MYBL2 that interacts with bHLH factors (GL3/EGL3/TT8) and forms an inactive complex (Dubos et al. 2008; Matsui et al. 2008). Transparent testa glabrous1 (TTG1) is responsible for both anthocyanin biosynthesis and trichome development. The ttg1 mutant *Arabidopsis* is formed as glabrous that lose the ability to produce pigments (Walker et al. 1999). Other factors, viz., bHLH factors, GL3/EGL3, and the R3MYB CPC, are also involved in both processes (Ramsay and Glover 2005). In *Arabidopsis*, HY5, a bZIP protein, is also involved in phenylpropanoid pathway that interacts with ACE-containing light-responsive promoters (Chattopadhyay et al. 1998; Ma et al. 2002). The ANL2 (anthocyaninless2) gene that encodes the HD-GLABRA2 homeobox protein in *Arabidopsis* is required for anthocyanin production in the subepidermal cells, and the anl2 mutant affects anthocyanin production in the seedling and other vegetative tissues (Kubo et al. 1999).

10.7 Gene Regulation in *Petunia*

Involvements of several genes have been identified to be associated with the anthocyanin biosynthesis in *Petunia* floral tissue in many experiments. In Petunia also, four TFs—AN2 (ANTHOCYANIN2) or AN4 (R2R3MYB), AN1 (bHLH), and AN11 (WDR)—form the MBW complex which regulates the late anthocyanin biosynthesis genes (Spelt et al. 2000, 2002; Quattrocchio et al. 1999; de Vetten et al. 1997). In the petal limb of *Petunia*, the color is expressed by AN2, whereas AN4 develops the petal tube and the anther's color (Spelt et al. 2000). Another bHLH TF (JAF13) is also involved in flower color development in *Petunia* (Quattrocchio et al. 1998). JAF13 and AN1 are different in their amino acid sequence, and they are not functionally redundant in the regulatory cascade. An1 gene expression is dependent on the activity of An2, but not on the activity of Jaf13 (Spelt et al. 2000). In transgenic *Petunia,* the AN1–glucocorticoid receptor is found to have a direct activity in the late biosynthesis genes activation, and it also activates a myb gene (Pmyb27) (Spelt et al. 2000). The DEEP PURPLE (DPL) and PURPLE HAZE (PHZ) are the R2R3 MYB factors that coordinately work with AN1 and AN11 and regulate anthocyanin biosynthesis in vegetative and floral tissues, isolated from leaf tissues (Albert et al. 2011). DPL regulates the vein-associated pigmentation of the flower tube in *Petunia*, whereas PHZ exhibits its role in exposed petal surface as it is associated with light-induced anthocyanin accumulation (Albert et al. 2011). PhMYB27, a R2R3 MYB transcription factor having an EAR-type repression motif at the carboxy terminus, can bind to the bHLH factor AN1 and exhibit negative regulatory role in anthocyanin biosynthesis (Albert et al. 2011; Aharoni et al. 2001). PhMYB27 is highly expressed in shade-grown leaves and is repressed by high-light expressions of PHZ, DPL, and AN1 resulting in higher anthocyanin accumulation in vegetative tissues (Albert et al. 2011). The PhMYBx in *Petunia* also a R3-MYB TF was found to have negative regulatory role in the anthocyanin accumulation (Albert et al. 2011).

10.8 Gene Regulation in *Nicotiana*

In tobacco also, there are some important transcription factors such as bHLH and R2R3 MYB TFs, NtAN1 and NtAN2, that are involved in the accumulation of anthocyanin in flowers. The late pathway genes are strongly activated by NtAN1–NtAN2 complex, while moderate activation activity is shown on the early pathway genes. When NtAN2 is overexpressed in tobacco, anthocyanin accumulation is increased in floral and vegetative tissues, while NtAN1 overexpression increases the pigment accumulation only in the flowers (Bai et al. 2011; Pattanaik et al. 2010). The MYBJS1 transcription factor is identified in tobacco which was also found to be involved in phenylpropanoid regulation (Gális et al. 2006). Some MYB proteins were also found to have light-responsive repressors activity on the early phenylpropanoid biosynthesis. When AmMYB308 or AmMYB330 are overexpressed in tobacco, dramatic reductions were observed in the levels of lignin and HCA derivatives in transgenic tobacco (Tamagnone et al. 1998). The AmMYB305 gene and the equivalent protein from tobacco have been shown to enhance the expression of genes in phenylpropanoid pathway by binding and activating the P-box MRE that is expressed in petal (Sablowski et al. 1994).

10.9 Gene Regulation in *Antirrhinum*

In *Antirrhinum*, the two important genes that encode the bHLH factors are Delila (Goodrich et al. 1992) and Mutabilis (Martin et al. 2001). Rosea1, Rosea2, and Venosa are the members in the MYB gene family which are associated in anthocyanin biosynthesis (Martin et al. 2001). These genes act coordinately by forming a complex that control spatial and temporal production of anthocyanins in the petals. Delila is active in both petal lobes and tube, while Mutabilis is active only in the lobes. All these genes control the intensity of pigments and are specific for different parts of the flower. Rosea1 and Rosea2 exhibit two different actions; they are respectively associated in strong pigment and weaker pigment production. Venosa produces striking venation pattern by producing pigment only in epidermal cells overlying the veins (Martin et al. 2001).

10.10 Gene Regulation in Other Fruits

Anthocyanin and anthocyanidin glycosides are the main reason of the colors of different fruit skin and pulp which are very beneficial for human health. Various fruits like apple, peach, plum, pear, strawberry, grapes, etc. were studied extensively to identify the transcription factors involved in the biosynthesis of anthocyanin and were found to have TFs that are orthologous to the MBW complex of *Arabidopsis* which are responsible for the fruit skin and flesh color. It was found that MdMYB1, MdMYB10, and MdMYBA, when co-expressed with bHLH factors MdbHLH3 and MdbHLH33, activate the anthocyanin synthesis in the skin and flesh of apple (Takos et al. 2006; Espley et al. 2007; Ban et al. 2007). Recently, MdMYB9 and MdMYB11 were also reported in apple (*Malus* × *domestica* Borkh.) that form a MBW complex by interacting with MdbHLH3 and MdTTG1 which binds to the promoters of structural genes and regulates the JA-mediated anthocyanin and proanthocyanidin accumulation (An et al. 2015). In grapes also a MBW complex made of R2R3 MYBs (VvMYBA1/2/5a/5b), bHLH (VvMYC1, VvMYCA1), and WD40 (VvWDR1 and VvWDR2) proteins was found to be involved in anthocyanin biosynthesis. The UDP-glu:flavonoid 3-O-glucosyltransferase (UFGT) regulation is the key step for triggering anthocyanin production in *Vitis vinifera* (grape) berries during berry ripening (Boss et al. 1996; Kobayashi et al. 2002). In strawberry, the ripening and color change of fruit by the accumulation of anthocyanin are associated with the intensity of the expression of two R2R3 MYBs TFs, FaMYB10 and FaMYB1. FaMYB10 and FaMYB1 show completely different activity: FaMYB10 induces anthocyanin biosynthesis, while FaMYB1 represses (Aharoni et al. 2001).

10.11 Regulation of Terpenoid Indole Alkaloid Biosynthesis

Plants have been proposed to produce alkaloids as part of their defense mechanisms against herbivores and pathogens. They are small, heterocyclic nitrogen-containing molecules (Gantet and Memelink 2002). Pharmacological properties of several alkaloids have been exploited for years. Alternatively, alkaloids can also be derived from their natural precursors chemically or biochemically. Numerous attempts have been made to manipulate the terpenoid indole alkaloid pathway because about 15 terpenoid indole alkaloids are industrially important, including vinblastine, vincristine, and camptothecin, and pharmaceutically, these anticancer medications including Taxol, colchicine is a gout suppressant, tubocurarine is a muscle relaxant, sanguinarine is an antibiotic, and scopolamine is a sedative (Facchini 2001; Memelink et al. 2001a, b).

A few plant species belonging to the plant families Nyssaceae, Rubiaceae, Loganiaceae, and Apocynaceae are known to produce terpenoid indole alkaloids (TIAs). In recent years, the Madagascar periwinkle or *Catharanthus roseus* of the Acaciaceae family now serves as a model for understanding the biosynthesis of TIA and regulation of its different steps (Facchini 2001; Memelink et al. 2001a, b). There are two separate pathways involved in generating the tryptophan and terpene precursors that produce terpenoid indole alkaloids (TIAs) (Roberts and Strack 1999). There are common pathways for the development of these alkaloids, but once they reach the intermediate strictosidine, they diverge in the production of alkaloids in the various plant species (Verpoorte et al. 1998). Researchers have focused many efforts on identifying the initial phase of the pathway and on overexpression of early genes to boost the metabolic flux into alkaloid pathway compounds. There are currently only a few structural genes isolated from tropanes and benzylisoquinoline alkaloid pathways, both of which are often more complex than flavone biosynthesis pathways (Facchini 2001; Memelink et al. 2001b). It is believed that some steps are located in the plastid, while others occur in the endoplasmic reticulum or within the vacuole, and that there also obvious signs for transport of intermediate compounds between types of specialized cells. Thus, regulation of the pathway is likely to be complex (Memelink et al. 2001a, b).

Vinblastine and vincristine are major bioactive TIAs that are made of geraniol and tryptophan, respectively, by more than 20 biosynthetic steps. There are two components, ajmalicine and serpentine, used pharmaceutically for the treatment of hypertension and are present in some varieties of Indian serpentwood (*Rauwolfia serpentina*). TIA is produced when jasmonates [jasmonic acid and methyl jasmonate (MeJA), the volatile derivative] are present; fungi also elicit TIA production (Roberts and Strack 1999; Memelink et al. 2001a, b). Promoter analysis of strictosidine synthase (STR) and tryptophan decarboxylase (TDC) genes reveals that both encode proteins regulated by stress signals such as UV light or fungal elicitors. In the promoter region of the strictosidine synthase (STR) gene, a promoter element was identified that may be involved in both the jasmonate and the elicitor response (JERE, jasmonate and elicitor responsive element), close to the TATA box, and may have functions in both the jasmonate and elicitor response, consisting a GC core of 24 bp (Menke et al. 1999; Memelink et al. 2000). Additionally, the sequence contains a G-box (CACCGG) found in a number of genes induced by abiotic and biotic stress and upstream "BA" region containing quantitative enhancers (Memelink et al. 2000). With JERE as bait, a cDNA that encodes ORCA2 (octadecanoid-responsive *Catharanthus* AP2-domain protein 2) was isolated from yeast one-hybrid screening. The transcription factor ORCA2 belongs to the plant-specific AP2/ERF (APETALA2/ethylene-responsive factor) family and that is characterized by the presence of an AP2/ERF DNA-binding domain. Only OCRA 2 is activated by the MeJA transcription factors and elicitors and, through interaction with JERE, stimulates STR expression (Menke et al. 1999). T-DNA tagging was used to isolate ORCA3 as the third member of the family (van der Fits and Memelink 2000). Upon binding to the promoter region of Str and other TIA biosynthetic genes, ORCA3 promoted their transcription, in both transient assays, and stably transformed cell cultures. As a result of yeast one-hybrid screenings, a MYB-like protein (CrBPF-1) was also identified that interacts with the BA-region and whose transcription is influenced by fungal elicitors (van der Fits and Memelink 2000). It was recently found that CrMYC2, the bHLH family protein, binds to the JA-responsive element in ORCA3 promoter and activates gene expression and regulates alkaloid biosynthesis (Zhang et al. 2011a). Due to the lack of control of cytochrome P450 enzyme G10H which are involved in secologanin biosynthesis, overexpression of ORCA3 didn't increase alkaloid production. By feeding secologanin precursor loganin to cells, alkaloids were produced threefold more than control cells. Aside from TIA precursors, ORCA3 regulates two other genes, α-subunit of anthranilate synthase and D-1-deoxyxylulose 5-phosphate synthase, respectively involved in primary metabolism indicating that ORCA3 is a central regulator of TIA biosynthesis that acts pleiotropically on other steps of the TIA pathway (van der Fits and Memelink 2000). Using the G-box as bait, a yeast one-hybrid screen isolated G-box-binding factors (CrGBFs) from the basic leucine zipper family and MYC-type bHLH transcription factors (CrMYCs) (Pré et al. 2000). It has been shown that CrGBF1 and CrGBF2 repress STR expression by binding to a G-box-like element in the TDC promoter in vitro, suggesting that CrGBFs are consciously controlling many TIA biosynthesis genes (Sibéril et al.

2001). Yeast one-hybrid screening isolated three zinc finger proteins, ZCT1, ZCT2, and ZCT3, which are members of the Cys2/His2 type (transcription factor IIIA-type), bind at the TDC and STR promoters, and repress activity of ORCAs (Pauw et al. 2004). CrWRKY1, a phytohormone-responsive WRKY TF, bind to the W-box in the TDC promoter, regulated by several other TFs, including Box P-binding factor 1 (BPF-1) and G-box-binding factors (GBF1 and GBF2). When CrWRKY1 was overexpressed in *C. roseus* hairy roots, TIA pathway genes and ZCT1, ZCT2, and ZCT3 were upregulated, but ORCA2, ORCA3, and CrMYC2 were repressed. Thus, serpentine accumulation increased by threefold, but catharanthine and tabersonine were decreased suggesting a key role of CrWRKY1 in determining the root-specific accumulation of serpentine in *C. roseus* plants (Suttipanta et al. 2011). In a yeast one-hybrid assay, it was discovered that BPF-1, a MYB-like protein, is highly homologous to the P-binding factor involved in JA-independent elicitor-dependent signaling pathways in *Catharanthus*. Vindoline, a precursor to vinblastine and vincristine, is produced by the help of deacetylvindoline-4-O-acetyltransferase (DAT) which is regulated by JA and light. In a recent study, Wang et al. identified three TGACG motifs and an inverted motif (CGTCA) within the DAT promoter that are involved in MeJA-mediated transcription (Aerts and De Luca 1992; Wang et al. 2010). Figure 10.1 presents the schematic representation of terpenoid indole alkaloid biosynthesis producing anticancer biomolecules vinblastine and vincristine with catalytic enzymes at different steps.

10.12 Regulation of Tropane Alkaloid

Genetic engineering of tropane alkaloid compounds with pharmaceutical value has already been studied a fair amount (Oksman-Caldentey and Arroo 2000). Basically, the goal of these studies is to convert hyoscyamine into scopolamine, which has significantly of more value, catalyzed by hyoscyamine-6β-hydroxylase (H6H). A hundredfold increase in scopolamine levels can be attained in *Hyoscyamus muticus* hairy root cultures when the scopolamine gene is overexpressed in comparison with controls that produce hyoscyamine as chief alkaloid (Jouhikainen et al. 1999). Transgenic and control cells showed the same level of hyoscyamine (about tenfold higher than scopolamine in the transgenic roots). Increased flux has been sought through the biosynthetic pathways in recent years (Sato et al. 2001). *Atropa belladonna* and *Nicotiana sylvestris* were enhanced by overexpressing the putrescine N-methyltransferase (PMT) gene in order to increase tropane alkaloids and pyrrolidine alkaloids, respectively. During the synthesis of both the alkaloids, methylputrescine is initially formed by taking putrescine from the pool of biosynthetic metabolites. Transgenic belladonna plants also exhibited a modest increase in PMT levels of up to 3.3-fold, but only the amount of methylputrescine was increased, and all alkaloids level remained unchanged. Plants expressing PMT were found to be four- to eightfold more active in some transgenic *N. sylvestris* producing 40% more nicotine, while other plants exhibited a co-suppression and produced 2% nicotine (Verpoorte and Memelink 2002).

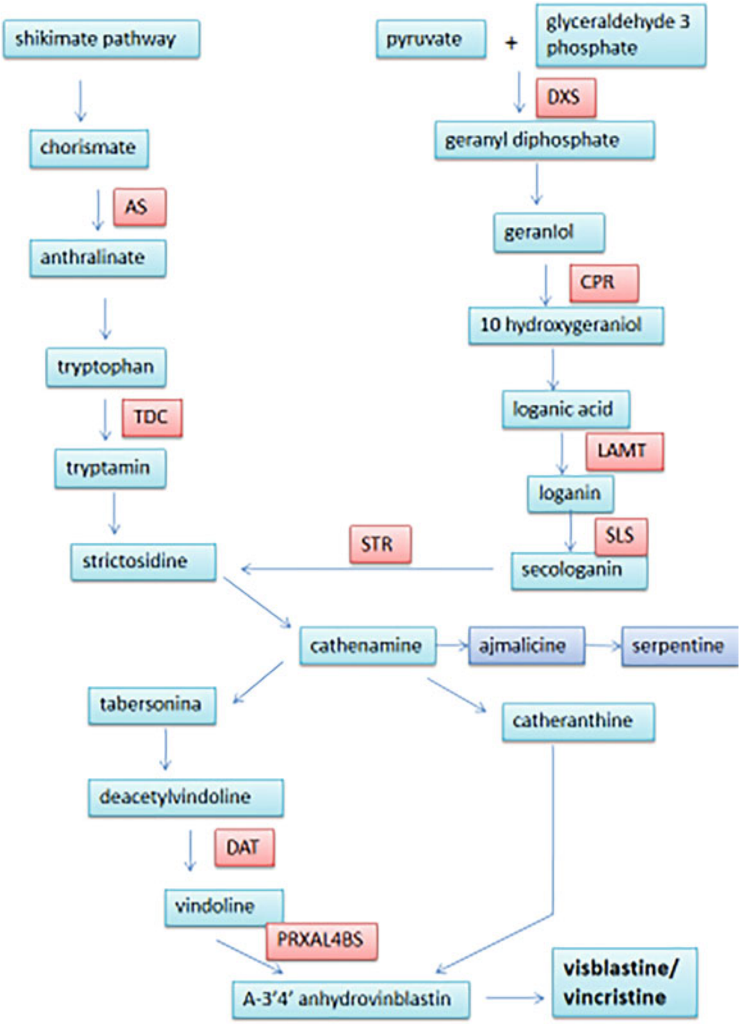

Fig. 10.1 The schematic representation of terpenoid indole alkaloid biosynthesis producing vinblastine and vincristine

10.13 Regulation of Isoquinoline Alkaloids

Isoquinoline alkaloids, including morphine and codeine, comprise another group of secondary metabolites that have therapeutic value in plants. Recently, metabolic engineering has been made possible for a number of these alkaloids by elucidating the pathways they follow (Verpoorte and Memelink 2002). A branchpoint in a pathway affected by overexpression of an enzyme should have an increased flux. The enzyme (S)-scoulerine 9-O-methyltransferase (SMT) might play a role in controlling the ratio of coptisine/berberine along with columbamine in *Coptis japonica* in berberine biosynthesis pathway (Sato et al. 2001; Yamada et al. 2011). A 20% increase in enzyme activity was observed in transgenic cells by overexpressing this gene that regulates the enzyme, and berberine and columbamine went from 79% to 91% of the total alkaloid content in content relative to wild-type cells. The plant *Eschscholzia californica*, lacking SMT enzyme, when overexpressed with the *C. japonica* SMT gene produces columbamine, which is not present in this species naturally. As a result of the new pathway being created, the flow away from the sanguinarine branch was much lower. Creating heterodimeric enzymes by recombining subunits of *Thalictrum tuberosum*, O-methyltransferase was proposed as a way of generating new alkaloids (Frick et al. 2001). In *Coptis japonica*, CjbHLH1, the bHLH TF was reported that is important in the regulation of isoquinoline alkaloid which is induced by MeJA. When the expression of the CjbHLH1 gene was suppressed by RNAi, the expression of berberine biosynthesis genes significantly decreased. The regulation of CjbHLH1 is JAZ-independent and depends on the stimulatory activity of Jas; thus, there is an interaction between CjbHLH1 and CjJAZ (Yamada et al. 2011).

10.14 Regulation of Nicotine Alkaloid

Nicotiana tabacum or tobacco is the primary source of nicotine, which is an insect antifeedant compound. Nicotine is synthesized in tobacco roots and transported to leaf tissue upon mechanical or herbivore wounding. Nicotine levels are influenced by two unrelated loci, NIC1 and NIC2 (Hibi et al. 1994). Seven AP2-ERF TFs located in the tobacco NIC2 locus influence the expression of all nicotine biosynthesis genes which are induced by JA. Two AP2/ERF-domain proteins known as NtORC1/ERF221 and NtJAP1/ERF10 are required to activate the putrescine N-methyltransferase (PMT) gene in nicotine biosynthesis from JA-treated cultured tobacco Bright Yellow-2 (BY-2) cells (Goossens et al. 2003; De Sutter et al. 2005). When tobacco cells overexpress NtORC1/ERF221 without MeJA, they produce more nicotine, and other pyridine alkaloids prove its key role in nicotine alkaloid biosynthesis (De Boer et al. 2011). Based on microarray analysis, nic1 nic2 double mutant showed the most repressed gene to be NtERF189 (Shoji et al. 2010). In the modulation of nicotine biosynthesis genes, ERF221 (ORC1) and ERF189 work in a nonredundant, but overlapping, manner. GCC-box in the PMT promoter (putrescine n-methyltransferase) is recognized by ERF221. Without JAs treatment, NtERF189 overexpression activated PMT and QPT expression in tobacco roots, resulting in higher nicotine production. When NtERF179 was overexpressed, PMT and QPT promoters were transactivated, and alkaloids were produced more abundantly (Shoji et al. 2010). Nicotine biosynthesis within MeJA-responsive loci depends also on NtERF1 and NtERF121, which do not belong to the NIC2 gene family. Three of these transcription factors bind specifically to the GCC-box-like element required for the transactivation of NtPMT1a induced by MeJA (Sears et al. 2014). In addition to regulating specific nicotine biosynthesis genes directly, the bHLH transcription factor, NtMYC2, also regulates nicotine synthesis via transcription factors NIC2 AP2-ERF. Numerous bHLH TFs associated with nicotine alkaloid biosynthesis in *N. tabacum* and *N. benthamiana* are JAs-responsive (Todd et al. 2010; Shoji and Hashimoto 2011; Zhang et al. 2012). A study using virus-induced gene silencing (VIGS), which was conducted on *N. benthamiana* roots treated with MeJA, identified NbbHLH1 and NbbHLH2 as functional regulators of nicotine biosynthesis (Todd et al. 2010). Using NbbHLH1, the MYC2 homolog of *N. benthamiana* binds to both the PMT1 and quinolinate phosphoribosyltransferase (QPRT2) promoters at G- and GCC-boxes and activates them. NIC2-locus AP2/ERF genes and MeJA-responsive nicotine biosynthesis genes, including PMT and MPO, were reduced by suppression of NtMYC2a and NtMYC2b (Shoji and Hashimoto 2011; Zhang et al. 2012). The roles of other five NIC2locus ERFs in nicotine biosynthesis regulation needed further investigations.

10.15 Regulation of Cyanogenic Glucosides

Cyanogenic glucosides are another group of nitrogen-containing alkaloids present in various forage plant species including *Sorghum* spp, *Lotus* spp, *Trifolium* spp, *Medicago*, and *Melilotus albus* (Aikman et al. 1996). In *Arabidopsis*, cyanogenic glucoside biosynthesis genes from sorghum bicolor are expressed, demonstrating the expression of a complete secondary metabolite biosynthesis pathway. Dhurrin, a cyanogenic glucoside synthesized by sorghum, is obtained from tyrosine, and the synthesis of dhurrin involves the action of two multifunctional cytochrome P450 enzymes (CYPs) and a specific UDPG-glucosyltransferase. In *Arabidopsis*, CYP79A1, CYP79E1, and UGT85B1, three genes essential for synthesis of dhurrin in sorghum, were overexpressed, and consequently, dhurrin is accumulated, making it resistant to *Phyllotreta nemorum*, the flea beetle (Tattersall et al. 2001; Kristensen et al. 2005). Dhurrin accumulation was observed in hairy roots overexpressing three enzyme genes, but no defense against insects was observed (Franks et al. 2006). In homozygous mutations in CYP79A1, phenotypically normal sorghum was produced, except that seedlings grew slightly more slowly at the beginning of the season (Blomstedt et al. 2012). Tcd2 mutant plants with homozygous mutations in the UGT85B1 gene, on the other hand, were devoid of dhurrin and exhibited reduced stature and vigor (Blomstedt et al. 2016).

10.16 Regulation of Benzylisoquinoline

Benzylisoquinolines are other alkaloids generally synthesized in opium poppy (*Papaver somniferum*) and goldthread (*Coptis japonica*), but their transcriptional regulators are even poorly defined (Kato et al. 2007). CjWRKY1 is a gene from

C. japonica that positively regulates all biosynthetic steps apart from the final one and controls the activation of the benzylisoquinoline alkaloid berberine. In addition, CjbHLH1, a member of the bHLH family from *C. japonica*, regulates all genes relevant to berberine biosynthesis except the last gene (Yamada et al. 2011). The expressions of heterologous transcription factors from *Arabidopsis*, maize (*Zea mays* ssp *mays*), and soybean (*Glycine max*) are being studied to identify genes related to alkaloid biosynthesis from opium poppy and California poppy (*Eschscholzia californica*). In both opium and California poppies, AtWRKY1 was identified as a gene that regulates multiple alkaloid biosynthesis genes (Apuya et al. 2008).

10.17 Tryptophan Regulation

An additional important pathway involves the biosynthesis of tryptophan, which provides a variety of secondary metabolites derived from indole as well as high levels of anthranilate, indolic phytoalexins, and glucosinolates (Radwanski and Last 1995). The ASA1 gene encodes the α-subunit of the enzyme anthranilate synthase which is the first and main active enzyme of the biosynthetic pathway in *Arabidopsis*, induced by several environmental factors including infection by pathogens and amino acid deficiency. The β-subunit of the enzyme is encoded by the ASB gene, which also plays a role in the upregulation of the ASA1 gene, and controlled by the environmental signals. A mutant *Arabidopsis* plant with altered tryptophan regulation (atr) can provide insight into how transcription is regulated in tryptophan metabolism. The ASA1 transcript abundance is increased by the atr1D mutation in transgenic *Arabidopsis* by the overexpression of ATR1 (MYB gene allele), while atr2D gene overexpression has indirect regulation of tryptophan biosynthesis genes by ATR2 (bHLH gene allele) which is evident from its pleiotropic phenotypes, such as dark pigmentation and sterility (Smolen et al. 2002). Based on the effects of double mutant atr1D atr2D on yeast two-hybrid assays, it appears that MYB and bHLH function independently (Smolen et al. 2002).

10.18 Glucosinolate Regulation

Glucosinolate biosynthesis is regulated in aerial tissues by the two AtMYB29 and At-MYB76 genes, which are involved in the aliphatic glucosinolate biosynthesis (Gigolashvili et al. 2008). As well as regulating the metabolizing P450 genes CYP79B2, CYP79B3, and CYP83B1 in roots and late-stage leaves, AtMYB34, AtMYB51, and AtMYB122 contribute to indolic glucosinolate synthesis from tryptophan where, indole-3-acetaldoxime (IAOx) acts as the precursor (Glawischnig et al. 2004; Celenza et al. 2005; Gigolashvili et al. 2007; Dubos et al. 2010). When OBP2 a DNA-binding-with-one-finger (DOF) transcription factor is overexpressed, an increase in CYP83B1 expression is observed, and the opposite—decreased expression—is observed when OBP2 is silent, suggesting that OBP2 may be part of a regulatory network that regulates the synthesis of glucosinolates in *Arabidopsis* (Skirycz et al. 2006). There are three AtMYCs (AtMYC2, AtMYC3, and AtMYC4) that are positively regulated in indolic glucosinolate biosynthesis. Met-derived GSLs (aliphatics) are biosynthesized by specific genes activated by MYB28, MYB29, and MYB76 (Hirai et al. 2007; Sønderby et al. 2010; Gigolashvili et al. 2008). Triple mutants of myc2/myc3/myc3 had significantly reduced levels of glucosinolate, as well as reduced expression of glucosinolate biosynthesis genes, including BCAT4 (branched chain aminotransferases) and CYP79B3 (Schweizer et al. 2013; Frerigmann and Gigolashvili 2014). Glucosinolate production in *Arabidopsis* is regulated by ATR1 (AtMYB34), a MYB transcription factor which was identified in an analysis for altered metabolism of tryptophan metabolism. There was an increase observed in indolyl glucosinolates and IAA accumulation but not aliphatic glucosinolates, after overexpression of ATR1. Those with the recessive atr1–2 mutation had reduced expression of genes, and less indolyl glucosinolates had been accumulated (Celenza et al. 2005).

10.19 Camalexin Regulation

Camalexin is a sulfur-containing molecule also synthesized from tryptophan which acts as a defense molecule in response to a variety of pathogen- and reactive oxygen species-induced stresses and is one of the most influential phytoalexins in *Arabidopsis* (Glawischnig 2007). Gene expression in the camalexin biosynthetic pathway is controlled by MYB, bHLH, NAC, DOF, and WRKY TFs (Celenza et al. 2005; Skirycz et al. 2006; Dombrecht et al. 2007; Qiu et al. 2008). IAOx is produced from tryptophan and with the help of CYP71A13 and CYP71A12 converted to indole-3-acetonitrile (IAN) and then by glutathione S-transferases conjugated to glutathione and subsequently converted to camalexin via dihydrocamalexic acid by hydroxylation are catalyzed by P450 CYP71B15/PAD3 (Nafisi et al. 2007; Böttcher et al. 2009; Millet et al. 2010; Chen et al. 2012a, b). WRKY33, a WRKY transcription factor after infection with *Pseudomonas syringae* pv. *tomato* and flagellin, a bacterial elicitor, interacts with a kinase cascade, phosphorylates MKS1 (MAP kinase substrate 1), and binds to the promoters of CYP71B15/PAD3 (phytoalexin deficient 3) (Qiu et al. 2008). A wrky33 mutant was found to be prone to infection by *B. cinerea* and *A. brassicicola*, and when overexpressed, the infection resistance increased (Zheng et al. 2006). WRKY33 is regulated by mitogen-activated protein (MAP) kinase and in response to *P. syringae* infection; MPK4 phosphorylates MKS1 and releases WRKY33 from the MKS1/WRKY33 complex, which stimulates the production of camalexin via the CYP71B15 promoter (Andreasson et al. 2005; Qiu et al. 2008). In addition, in response to *B. cinerea* infection, MPK3/MPK6 phosphorylates WRKY33 (Raina et al. 2012; Mao et al. 2011). Diverse pathogen-induced pathways are likely to trigger respective MAP cascades and result in WRKY33 activation, promoting camalexin synthesis. In recent years, researchers found that ANAC042 is a NAC transcription factor that regulates the biosynthesis of camalexin in *Arabidopsis*. Plants expressing the anac042 mutant exhibited reduced expression of CYP71A12, CYP71A13, and CYP71B15/PAD3 and failed to accumulate camalexin at a much lower level than in wild type and were much more susceptible to infection with *A. brassicicola*. Analyses with kinase inhibitors and mutants suggested that ANAC042 may work independently of WRKY33-mediated signalling and also showed that infection with pathogens can induce ANAC042 expression (Saga et al. 2012).

10.20 Regulation of Terpenes

By far the most abundant class of plant secondary metabolites, terpenoids contain more than 50,000 chemicals. A number of genes of the MEP (2-C-methyl-D-erythritol-4-phospate) pathway have been cloned and have been implicated in the biosynthesis of plastidial terpenoids, such as carotenoids, monoterpenes, and diterpenes (Broun and Somerville 2001; Mahmoud and Croteau 2001; Lange and Croteau 1999; Lange et al. 2000). The discovery of a number of transcription factors that cause sweet wormwood (*Artemisia annua*) to biosynthesize artemisinin, a sesquiterpene lactone employed to treat malaria, is revealing. ADS (amorpha-4,11-diene synthase) and CYP71AV1 (sesquiterpene oxidase) are modulated by the transcription factors AaERF1 and AaERF2 that respond to jasmonate and are members of the B3 subfamily of AP2-ERF TFs (Yu et al. 2012). 3-Hydroxy-3-methylglutaryl-CoA reductase (HMGR) and artemisinic aldehyde δ11(13) reductase (DBR2) are gelulated by WRKY TF member, AaWRKY1. AaORA1, an AP2/ERF TF, was recently found to control the accumulation of artemisinin and plays a critical role in the defense of *A. annua* against *Botrytis cinerea*, which is a necrotrophic plant pathogen (Lu et al. 2013). All the early steps of artemisinin biosynthesis take place under the control of AaERF1, AaERF2, AaORA1, and AaWRKY1. It occurs before branching into either dihydroartemisinic acid and artemisinin or artemisinic acid and arteannuin B. AaORA1 and AaWRKY1 function as transcriptional regulators of DBR2, which converts artemisinic aldehyde into dihydroartemisinic acid that leads to the formation of artemisinin (Roth and Acton 1989). A transgene for S-linalool synthase was overexpressed in tomato plants, which produced many times as much monoterpenoids as control plants, but did not differ in the amounts of other terpenoids (Wang et al. 2001). DBAT (10-deacetylbaccatin III-10 β-O-acetyltransferase) is an enzyme essential for limiting the rate of synthesis of paclitaxel in *Taxus* and increasing paclitaxel accumulation in cell suspension when it is overexpressed (Walker and Croteau 2000). TaWRKY1, a WRKY TF, is also identified in *Taxus chinensis* cells, which regulates DBAT expression (Li et al. 2013). GaWRKY1 regulates the expression of CAD1 {(+)-δ-cadinene synthase} in a temporal and spatial manner, which forms gossypol during sesquiterpene synthesis in *Gossypium arboreum* (cotton) and, additionally, in cell suspension cultures in response to fungi and jasmonates (Xu et al. 2004). In *Hevea brasiliensis* (rubber tree), the latex production is regulated by HbEREBP1 and HbWRKY1. HbWRKY1 is induced by ethylene, abscisic acid, jasmonate, *Oidium heveae* infection, wounding, and osmotic stress, while HbEREBP1 acts as a negative regulator of early induction by jasmonate and wounding (Zhang et al. 2011b; Chen et al. 2012a, b). Mint plants overexpressing the gene encoding deoxyxylulose phosphate reductoisomerase (DXR) had a four- to sixfold increase in the activity of the enzyme (Mahmoud and Croteau 2001).

10.21 Carotenoid Regulation

Plant carotenoid pigments inhibit the oxidation of the chloroplast during photosynthesis, and they also provide flowers and fruits with bright colors. It is surprising that there hasn't been any report of transcription factors involved in carotenoid biosynthesis so far (White 2002). However, Rin (ripening inhibitor) is a genetic locus identified in *Lycopersicon esculentum* (tomato) which encodes the MADS-box protein LeMADS-RIN essential for the development of carotenoid pigments in fruit (Vrebalov et al. 2002). This is therefore an important step forward in introducing β-carotene biosynthesis by overexpression of phytoene synthase, lycopene β-cyclase, and phytoene desaturase into rice (Ye et al. 2000). Overexpressing a phytoene desaturase in tomato plastids resulted in the increase in β-carotene content by over threefold (Römer et al. 2000). A specific promoter was used to increase the amount of β-carotene sevenfold after overexpression of the lycopene β-cyclase gene (βLcy) in tomato (Rosati et al. 2000). Using a gene encoding the algal enzyme β-carotene ketolases, it has been possible to produce astaxanthin, mainly in the nectaries and within tobacco chromoplasts, and the total carotenoid content was increased (Mann et al. 2000).

10.22 Regulation of Benzoic Acid Derivatives

A signal molecule in plants that plays a crucial role in the acquisition of systemic resistance when plants are challenged with plant pathogens is salicylic acid (SA) which is formed through the conversion of chorismate to isochorismate, catalyzed by isochorismate synthase (ICS) in response to pathogen infection (Wildermuth et al. 2001). In the bacteria, SA is made from chorismate, which is converted to isochorismate by isochorismate pyruvate lyase (IPL) (Verberne et al. 2000). A rate-limiting step in the formation of SA is the IPL. *Arabidopsis* has been introduced with a protein constructed from two bacterial enzymes, and the plant exhibited a 2–3-fold increase in SA levels and a 20-fold increase in plastid (Mauch et al. 2001). To avoid negatively affecting the availability of precursors for other pathways, the activity of overexpressed enzymes in a pathway must be adjusted correctly by engineering two or more enzymes (Nugroho et al. 2001; Wildermuth et al. 2001). The ubiC gene encodes chorismate pyruvate lyase, which usually is produced by the phenylalanine pathway in *Lithospermum erythrorhizon* hairy root cultures which normally produce the naphthoquinone shikonin. As a result of chorismate production, the hairy roots produced 20% of the total 4-hydroxybenzoic acid (4HB) salicylic acid (Sommer et al. 1999). Table 10.1 presents the transcriptions factors regulating the secondary metabolite biosynthesis in different plants.

10.23 Conclusion

Studies of secondary metabolic pathways have found transcriptional regulation to be the most significant control point. Recent studies have shown that several genes involved in secondary metabolism are overexpressed in the original plant or other plant species. Overexpression in some cases led to an improvement in production, whereas in others it has led only to an enhanced amount of the overexpressed enzyme's direct product. It is not only gene expression levels that determine metabolite content through biosynthetic pathways but also posttranslationally regulated enzyme activity and compartmentalization and transport of enzymes and metabolites. Improvement in yields in metabolites is possible by using more diverse TFs. A diverse array of transcription factors positively and negatively regulates the expression of multiple DNA sequences encoding key enzymes, thus controlling the metabolic flow along the biosynthetic pathway. AP2/ERF, bHLH, MYB, and WRKY are all known to be factors the plant uses to produce a variety of secondary metabolites, including phenol, flavonoids, terpenoids, etc. In this article we looked at some examples of how such regulators' activity can be controlled.

The most studied pathway is the phenylpropanoid pathway which is responsible for the production of flavonoids including anthocyanin and proanthocyanidin. Several information about the transcription factors involved in many steps of the pathway and their regulation mechanisms are reported. The promoter genes of the pathway lack codons that are conserved or canonically binding to TFs that regulate their activity, so specific cis-elements that participate in responses to environmental factors and developmental factors were identified. Several tissue-specific transcription factors are involved in the modification of the phenylpropanoid pathway, and MYB and bHLH and their homologs and orthologs were used to modify the pathway. Transcription factors can be engineered to produce flavonoids by overexpressing genes encoding MYB and bHLH proteins. It is also possible to engineer plants to produce anthocyanin through ectopic expression of C1 and R. Currently, the majority of knowledge revolves around light-induced anthocyanin production in leaves and floral tissues and proanthocyanidin production in seeds. *Catharanthus* has only been studied extensively in terms of regulation of two TIA pathway genes, TDC and STR. A jasmonate-responsive transcription factor with an AP2/ERF-domain was used to modulate the TIA biosynthesis pathway. A major bottleneck in finding potential regulators of the alkaloid biosynthesis pathway is the lack of genetic tools that will allow us to identify the potential regulators involved,

Table 10.1 Transcription factors regulating the secondary metabolite biosynthesis in different plants

Metabolite	Protein	Type	Plant species	Reference
Anthocyanin	AtMYB113	MYB	*Arabidopsis thaliana*	Gonzalez et al. (2008)
	AtMYB114	MYB	*A. thaliana*	Gonzalez et al. (2008)
	PAP1 (AtMYB75)	MYB	*A. thaliana*	Borevitz et al. (2000)
	PAP2 (AtMYB90)	MYB	*A. thaliana*	Borevitz et al. (2000)
	TT2	MYB	*A. thaliana*	Nesi et al. (2001)
	TT8	bHLH	*A. thaliana*	Nesi et al. (2001)
	TTG1	WD40	*A. thaliana*	Walker et al. (1999)
	GL3	bHLH	*A. thaliana*	Gonzalez et al. (2008)
	eGL3	bHLH	*A. thaliana*	Gonzalez et al. (2008)
	HY5	bZIP	*A. thaliana*	Ma et al. (2002)
	ANL2	HD ZIP	*A. thaliana*	Kubo et al. (1999)
	C1/PL	MYB	*Zea maize*	Mol et al. (1990)
	R/B	bHLH	*Z. maize*	Mol et al. (1990)
	PAC1	WD40	*Z. maize*	Mol et al. (1990)
	VP-1	ABI3	*Z. maize*	McCarty et al. (1991)
	AN2	MYB	*Petunia*	Spelt et al. (2000, 2002); Quattrocchio et al. (1999)
	AN4	MYB	*Petunia*	Spelt et al. (2000, 2002); Quattrocchio et al. (1999)
	AN1	bHLH	*Petunia*	Spelt et al. (2000, 2002); Quattrocchio et al. (1999)
	AN11	WDR	*Petunia*	Spelt et al. (2000, 2002); Quattrocchio et al. (1999)
	JAF13	bHLH	*Petunia*	Quattrocchio et al. (1998)
	DPL	MYB	*Petunia*	Albert et al. (2011)
	PHZ	MYB	*Petunia*	Albert et al. (2011)
	PMYB27	MYB	*Petunia*	Spelt et al. (2000)
	NtAN1	bHLH	*Nicotiana*	Bai et al. (2011)
	NtAN2	MYB	*Nicotiana*	Bai et al. (2011)
	MYBJS1	MYB	*Nicotiana*	Gális et al. (2006)
	AmMYB308	MYB	*Nicotiana*	Tamagnone et al. (1998)
	AmMYB330	MYB	*Nicotiana*	Tamagnone et al. (1998)
	ROSEA$_1$	MYB	*Antirrhinum majus*	Martin et al. (2001)
	ROSEA$_2$	MYB	*A. majus*	Martin et al. (2001)
	VENOSA	MYB	*A. majus*	Martin et al. (2001)

(continued)

Table 10.1 (continued)

Metabolite	Protein	Type	Plant species	Reference
	DELILA	bHLH	*A. majus*	Goodrich et al. (1992)
	MUTABILIS	bHLH	*A. majus*	Martin et al. (2001)
	MYB10	MYB	*Malus* × *domestica*	Takos et al. (2006)
	MYB1/ MYBA	MYB	*M.* × *domestica*	Takos et al. (2006)
	MdbHLH3	bHLH	*M.* × *domestica*	Takos et al. (2006)
	MdbHLH33	bHLH	*M.* × *domestica*	Takos et al. (2006)
	MdMYB9	MYB	*M.* × *domestica*	An et al. (2015)
	MdMYB11	MYB	*M.* × *domestica*	An et al. (2015)
	MdbHLH3	bHLH	*M.* × *domestica*	An et al. (2015)
	MdTTG1	WD40	*M.* × *domestica*	An et al. (2015)
	VvMYC1, VvMYCA1	bHLH	*Vitis vinifera*	Kobayashi et al. (2002)
	VvMYBA1/ 2/5a/5b	MYB	*V. vinifera*	Kobayashi et al. (2002)
	VvWDR1, VvWDR2	WD40	*V. vinifera*	Kobayashi et al. (2002)
	FaMYB10	MYB	*Fragaria* × *ananassa*	Aharoni et al. (2001)
	FaMYB1	MYB	*Fragaria* × *ananassa*	Aharoni et al. (2001)
Indole alkaloid	CrMYC2	bHLH	*Catharanthus roseus*	Zhang et al. (2011a, b)
	ORCA2	AP2/ ERF	*C. roseus*	Menke et al. (1999)
	ORCA3	AP2/ ERF	*C. roseus*	van der Fits and Memelink et al. (2000)
	CrGBF1	bZIP	*C. roseus*	Suttipanta et al. (2011)
	CrGBF2	bZIP	*C. roseus*	Suttipanta et al. (2011)
	CrWRKY1	WRKY	*C. roseus*	Suttipanta et al. (2011)
Tropane alkaloid	CjbHLH1	bHLH	*Coptis japonica*	Yamada et al. (2011)
Nicotine	NtORC1/ ERF221	AP2-ERF	*Nicotiana tabacum*	De Boer et al. (2011)
	NtJAP1/ ERF10	AP2-ERF	*N. tabacum*	De Boer et al. (2011)
	ERF221	ERF	*N. tabacum*	Shoji et al. (2010)
	NtERF1	ERF	*N. tabacum*	Sears et al. (2014)
	NtERF121	ERF	*N. tabacum*	Sears et al. (2014)
	NtMYC2	bHLH	*N. tabacum*	Todd et al. (2010)
	NbbHLH1	bHLH	*N. benthamiana*	Todd et al. (2010)
	NbbHLH1	bHLH	*N. benthamiana*	Todd et al. (2010)

(continued)

Table 10.1 (continued)

Metabolite	Protein	Type	Plant species	Reference
Cyanogenic glucosides	CYP79A1	CYP	*A. thaliana*	Kristensen et al. (2005)
	CYP79E1	CYP	*A. thaliana*	Kristensen et al. (2005)
	UGT85B1		*A. thaliana*	Kristensen et al. (2005)
Benzylisoquinolines	CjWRKY1	WRKY	*Coptis japonica*	Yamada et al. (2011)
	CjbHLH1	bHLH	*C. japonica*	Yamada et al. (2011)
	AtWRKY1	WRKY	*Eschscholzia californica* and *Papaver somniferum*	Apuya et al. (2008)
Tryptophan	ATR1	MYB	*A. thaliana*	Smolen et al. (2002)
	ATR1	bHLH	*A. thaliana*	Smolen et al. (2002)
Glucosinolate	AtMYB29	MYB	*A. thaliana*	Gigolashvili et al. (2008)
	AtMYB34	MYB	*A. thaliana*	Celenza et al. (2005)
	AtMYB51	MYB	*A. thaliana*	Gigolashvili et al. (2007)
	AtMYB122	MYB	*A. thaliana*	Gigolashvili et al. (2007)
	CYP79B2	P450	*A. thaliana*	Celenza et al. (2005); Gigolashvili et al. (2007)
	CYP79B3	P450	*A. thaliana*	Celenza et al. (2005); Gigolashvili et al. (2007)
	CYP83B1	P450	*A. thaliana*	Celenza et al. (2005); Gigolashvili et al. (2007)
	AtMYB76	MYB	*A. thaliana*	Gigolashvili et al. (2008)
	AtMYB28	MYB	*A. thaliana*	Gigolashvili et al. (2008)
	AtMYC2	bHLH	*A. thaliana*	Gigolashvili et al. (2008)
	AtMYC3	bHLH	*A. thaliana*	Gigolashvili et al. (2008)
	AtMYC4	bHLH	*A. thaliana*	Gigolashvili et al. (2008)

(continued)

Table 10.1 (continued)

Metabolite	Protein	Type	Plant species	Reference
Camalexin	CYP71A13	P450	*A. thaliana*	Nafisi et al. (2007)
	CYP71A12	P450	*A. thaliana*	Nafisi et al. (2007)
	CYP71B15	P450	*A. thaliana*	Nafisi et al. (2007)
	WRKY33	WRKY	*A. thaliana*	Qiu et al. (2008)
	ANAC042	NAC	*A. thaliana*	Saga et al. (2012)
Terpene	AaERF1	AP2-ERF	*Artemisia annua*	Yu et al. (2012)
	AaERF2	AP2-ERF	*A. annua*	Yu et al. (2012)
	AaORA1	AP2-ERF	*A. annua*	Lu et al. (2013)
	TaWRKY1	WRKY	*Taxus chinensis*	Li et al. (2013)
	GaWRKY1	WRKY	*Gossypium arboreum*	Xu et al. (2004)
	HbEREBP1	EREBP	*Hevea brasiliensis*	Zhang et al. (2011a, b)
	HbWRKY1	WRKY	*Hevea brasiliensis*	Zhang et al. (2011a, b)
	LeMADS-RIN	MADS-box	*Lycopersicon esculentum*	Vrebalov et al. (2002)

making it challenging to understand the regulation of the pathway. When ORCA3 was overexpressed in response to jasmonate, tryptophan and tryptamine levels were elevated, as well as certain TIA levels, upon intake of a terpenoid precursor. As of today, genes encoding direct regulators of biosynthesis pathways for tryptophan and TIA have been cloned, but none are known for major pathways such as those for glucosinolates, terpenoids, and carotenoids. Figure 10.2 presents the schematic representation of phenylpropanoid pathway producing anthocyanin and proanthocyanidin with the catalyzing enzyme and regulation transcription factors for each step.

Currently, there are only a few genes identified that encode the pathways that produce secondary metabolites in plants, which is a major constraint in engineering secondary metabolite production. On the other hand, a few plant species are studied for their genome and can be used as model plants, and cloning the secondary metabolite pathway genes from the plants is of limited value. We urgently need to uncover more data on posttranscriptional gene regulation, their modification, enzyme regulation, and compartmentalization and subcellular trafficking in the coming years. The combination of proteomics and metabolomics with genetic information of the plant metabolite pathway and in-depth analysis of the enzymes will allow us to achieve successful strategies for altering the accumulation of certain compounds in order to map the pathway and better understanding that metabolic network.

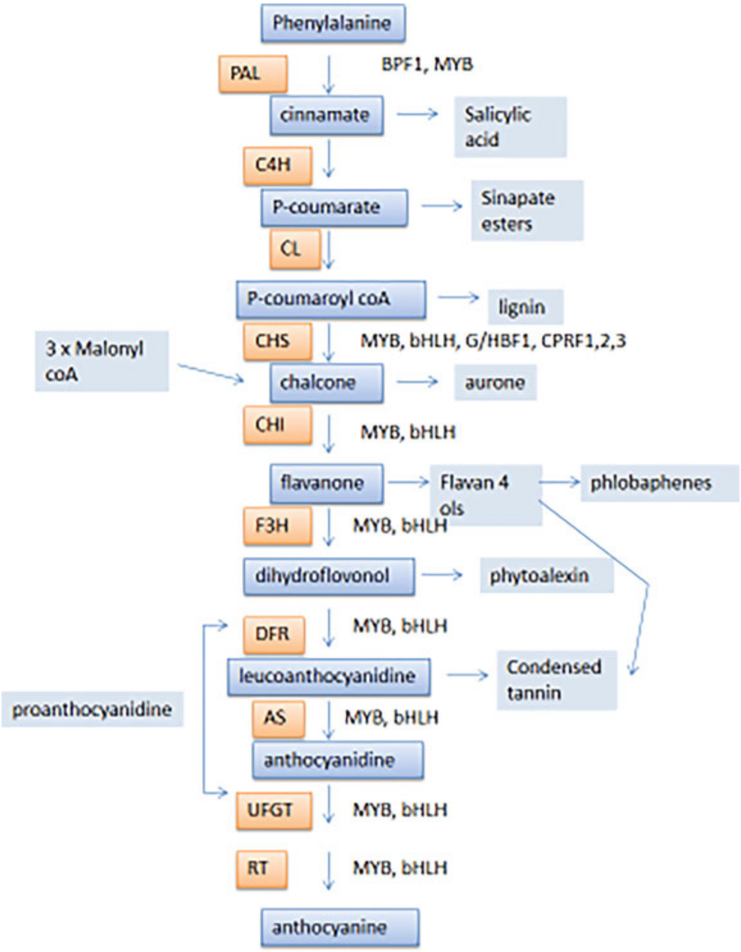

Fig. 10.2 Schematic representation of phenylpropanoid pathway producing anthocyanin

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Chapter 11
Sustainable Use Practices of Medicinal Plants and Environmental Challenges: A Case Study in Pakistan

Hassan Sher and Haidar Ali

Abstract Environmental challenges in various forms are posing a great threat not only to humans but also to the entire biodiversity. These environmental challenges are directly affecting the wild habitats. On the other hand, unsustainable use practices, overexploitation etc. are threatening the medicinal and aromatic plants (MAPs) in particular and biodiversity in general. The current study attempted to increase livelihood opportunities for plant collectors through sustainable production of MAPs and also to adopt new strategies for climate change mitigation. Methodology of the study was based on a series of consultative meetings with key resource persons and stakeholders of MAPs. It covered series of interventions including awareness-raising campaigns, capacity-building trainings on sustainable collection, control of post-harvest losses, marketing, introduction of standardized in situ and ex situ production techniques, fixing of harvest quotas for high-value MAPs. The authors generally observed climate base shifting of MAPs from low altitude towards alpine and subalpine regions. It was observed that sustainable production of MAPs will not only enhance local income generation opportunities for communities living in fragile ecosystems of Pakistan but will also act as an engine of economic development for the country's economy and will also be used as a tool in the development of climate smart agriculture to address the global issues of climate change and food security. This study proved that sustainable MAP production is an appropriate approach for addressing the issues of climate change and dimensions of food security. It is recommended that with the active involvement of local community a species-specific conservation strategy be devised based on allocation of collection quota for further enforcement and proper management. Further study is required to evaluate the level of climate change effect on MAPs production based on supply and demand and according to the agro-ecological conditions of the country.

Keywords Medicinal and aromatic plants · Sustainable use practices · Environmental changes

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11.1 Introduction

It is widely accepted that our environment is in continuous state of change. This change in environment has been badly affected by various anthropogenic activities. The most important among these is the climate change. Climate change has been causing a number of detrimental effects, and it is not restricted to melting of polar ice caps, but is also accompanied by spread of new diseases, periodic floods (Figs. 11.1 and 11.2) and variations in the whole weather conditions (Demeritt 2001).

Global warming is considered as the main cause of climate change, which in turn is considered as mainly caused by the burning of fossil fuels and the increase in the temperature of the atmosphere due to the emission of hazardous gases by industries (Kazemi and Ghorbanpour 2017). Global warming may be defined as the change in global air temperature, which is believed to have been caused by increasing environmental concentration of carbon dioxide, produced as a result of industrial revolution, in the eighteenth, nineteenth and twentieth centuries.

In 1765, the concentration of carbon dioxide in the atmosphere was c. 280 ppmv, in 2000 it was c. 364 (ppmv), whereas it was c. 410 ppmv in 2019 (46% increase) (Bereiter et al. 2015). Almost all of this increase is due to human activities (USGCRP 2017).

At the end of the eighteenth century, unprecedented increase in the use of fossil fuels as a source of energy has been observed, which ultimately increased the concentration of carbon dioxide in the atmosphere.

Fig. 11.1 Human settlement is affected by the devastating effects of flood on 3 April 2016 in Mingora City

Fig. 11.2 Roads and highways are destroyed as a result of floods

It is widely accepted that during the past one and a half century, the global temperature has risen from 0.31 to 0.61 °C (Hansen et al. 2002). As a result of these complex and multidimensional phenomena, distribution, abundance and occurrence of plants along the altitudinal gradient are badly affected (Suggitt et al. 2019).

According to Kelly and Goulden (2008), c. 65 m shift from the lower altitude to the upper altitude was recorded in the dominant plant species between the surveys carried out for plant cover in 1977 and 2006–2007 along a 2314 m elevation gradient in Southern California's Santa Rosa Mountains.

Rise in atmospheric temperature and carbon dioxide not only affects various physiological activities inside the plant but also affects productivity, flowering, fruiting and abundance (Tisserat 2002). Medicinal plants in this connection are with no exceptions. Since human populations especially in the rural areas depend on medicinal plants for the treatment of various ailments and use plants both directly and indirectly, therefore, these medicinal plants are directly affected.

According to Humphreys et al. (2019), c. 600 plant taxa have become extinct during the last 250 years. Beside climate change, quite few of the anthropogenic activities are also responsible for this species extirpation, especially habitat destruction and fragmentation (Skole and Tucker 1993; Haddad et al. 2015).

According to Ali et al. (2012), annual production of medicinal plants in Swat District and its share to the trade were 8.056 and 6.644 million kg in the years 2004–2005 (Rs.4475 million) and 2005–2006 (Rs.5084.70 million). As a result of the unsustainable collection in the wild, the quantity traded reduced, while the amount circulated grew due to an increase in per kg price of individual species, which in turn is considered to be due to the increased demand from local/national and worldwide markets. The study also indicated that the abundance of medicinal plants has been decreasing day by day for the past two decades (Ali et al. 2012).

According to the discussions with local elderly people, most of the plants described as having therapeutic properties were plentiful in the area c. 20 years ago. However, due to overexploitation, expansion of agricultural fields, rising market demand, lack of alternative income options in the area and unsustainable harvesting practices, their number has significantly decreased. All of these problems had a very bad impact on the wild medicinal plant populations.

The current studies were therefore conducted to document the current situation of sustainable utilization of medicinal plants and to promote optimal benefits to collectors and other stakeholders and to adapt strategies contributing to prevent the depletion of medicinal plants in the study area. According to the medicinal plant market surveys conducted by SDC (2000), some MAPs are of higher market value, beside certain species which were imported in the national market from other countries and were available in the selected districts.

The market channels of some high-value and potential MAPs in selected Union Councils were studied for planning their sustainable harvest and production from their natural habitats. Most of MAPs are over harvested because of which some of these plants have become rare and sparse in the wild habitats. It has become an urgent need for the local people to think and make decisions about the sustainable harvesting of the precious MAPs; otherwise, these precious resources will be depleted from wild habitats.

To put any species to a sustainable use, it is necessary to quantify the current growing stock and carrying capacity within its natural habitat as compared to its harvesting quantity. It has been generally observed that on certain sites, the plants in demand are collected beyond their harvest limits and regeneration rates, but on certain other sites, these are underutilized (Sher et al. 2010d).

Every plant species has its specific time of collection, which is based on their phenological status, maturity of fruits/seeds, root-shoot growth ratio etc. For the collection of roots, corms, rhizomes or bulbs, the collectors should wait for the plants to become dormant, whereas in some cases until the onset of winter season. Similarly, leaves and barks may be collected during the summer, flowers may be collected at the peak of its bloom time and seeds should be collected when properly mature (Ali et al. 2011). However, a majority of the local collectors are uneducated and are therefore unaware of these phenomena; therefore, they violate these simple rules. Since, in most of the cases, the plants have not yet completed their biological life cycle, therefore, they do not give sustainable and desired production (Sher and Al-Yemeni 2011).

There is a need to know the current growing stock based on the calculation of carrying capacity and the rate of their natural regeneration of selected potential MAPs in the field in order to maintain the population size over the long term. The available stock of a species in the field is based on the time required for reproducing the required part by the plant after the harvest; it is important to determine the harvest limits for its sustainable production. A study was undertaken to prepare resource inventory of MAPs and to assess the growing stock of selected market-oriented medicinal plant species and to recommend the harvest limits from the respective sites.

11.2 Approach and Methodology

Field studies were arranged during summer, 2020, for data collection regarding MAP inventory preparation and sustainable utilization in three districts, viz. Swat, Shangla and Upper Dir. Methods such as rapid rural appraisal (RRA) and participatory rural appraisal (PRA), focus group discussion (FGD), consultative meetings with the primary stakeholders of MAPs and interviews with key informants were employed. For obtaining data from local residents, forest owners, MAP collectors, local traders and sale agents, PRA and semi-structured interviews were adapted. The purpose of all these meetings and discussions was to understand the existing resource and also to document the opinion of various interest groups and stakeholders for information collection and, additionally, to obtain feedback and information about the particular site and compartment to be surveyed.

Whenever possible, the FGD was conducted separately in a local Hujra in each union council of the three districts. It was emphasized that a maximum number of stakeholders should participate in the FGDs such as MAP collectors, local traders, village elders, representatives from forest department and Directorate of NTFPs. These types of interactive meetings are considered as very important, because during these types of meetings, experts and stakeholders sit together and share their experiences and find out solutions for their common problems.

About 600 farmers and forest owners from ten union councils have shown interest to play their role in preventing the depletion of medicinal plants wealth.

A survey of the 17 selected medicinal plants was carried out twice in a year i.e. before harvesting and after harvesting in 3 districts, i.e. Swat, Shangla and Upper Dir. Data was collected in consultation with community members of each Union Council, associated with MAPs collection. The local field assistants had the knowledge and experience with the plants and the sites of their occurrence and also had some literacy for recording data. These local field assistants were initially trained on the procedure of field data collection, format and on the use of field equipment. In each focus group discussion (FGD), information was obtained about the sites with reasonable occurrence and seasonality of any of the concerned species. Since the sites are located in very rugged topography with high altitudinal gradient, and consist of insignificant area on a map, therefore, a crude method, but understandable by the community and field workers, was adopted for calculation of the area of the sites in the field. However, for the current studies, area of the sites was calculated through a map (Fig. 11.3).

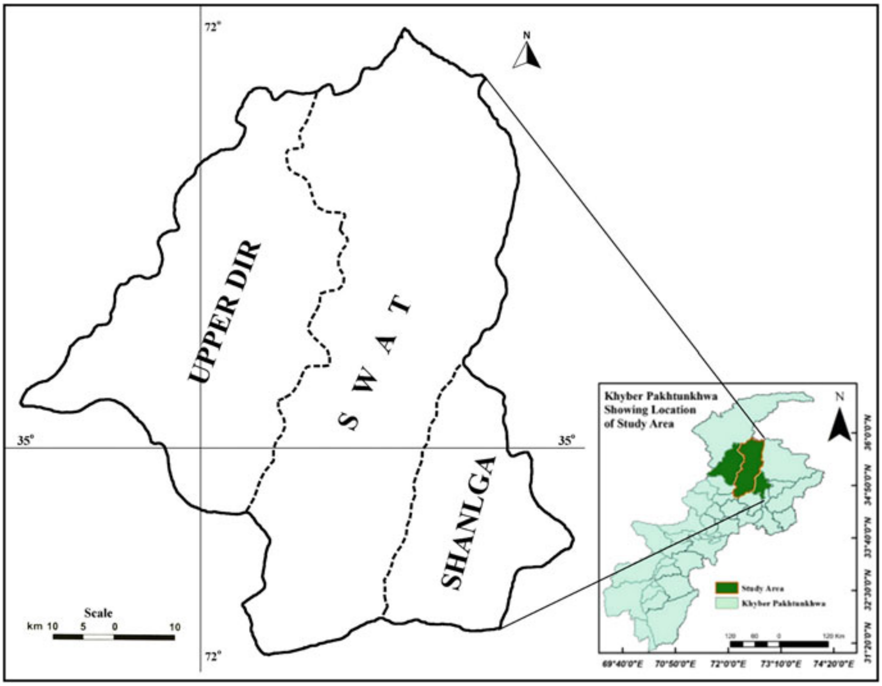

Fig. 11.3 Location map of three districts, i.e. Swat, Shangla and Upper Dir

11.3 Interpretation of Results

11.3.1 Harvesting of Selected MAPS

The harvest quantity at which MAPs may be harvested is determined by the plant's biological response. When the corm or rhizome of a plant is collected, then the whole plant is lost. For example, corms or rhizomes of *Podophyllum emodi*, *Dioscorea deltoidea*, *Bistorta amplexicaulis*, *Valeriana jatamansi* and many other rhizomatous plants in the field have all died as a result of harvesting of rhizomes and corms (underground stems).

Harvesting will have no effect on above-ground vegetative parts, such as leaves and flowers in case of *Viola canescens* and fruits in case of *Trachyspermum ammi*, and their rejuvenation will be determined by the rate of recovery and harvesting technique. Plants usually recover very quickly from the tissue damage caused by harvesting of leaves and flowers, when proper harvesting techniques and precise time of collection are followed. This can result in a net increase in biomass production when compared to an undisturbed plant. Based on their phenological status, the best moment to collect the plant parts is mentioned in Table 11.1.

The catastrophic effects of current environmental degradation have badly affected not only the human settlement but the biodiversity as well (Yousaf et al. 2014).

Table 11.1 Harvesting information for selected MAPs

S. No.	Botanical name	Plant part collected	Flowering period	Required seed germination period	Mature during the months	Recommended harvest gap
1.	*Aconitum heterophyllum*	Rhizome	May–June	2 years	Aug–Sept	3 years
2.	*Berberis lycium*	Root bark	May–June	1 year	Aug–Sept	2 years
3.	*Bistorta amplexicaulis* (Fig. 11.4)	Rhizome	May–June	1 year	Aug–Sept	1 year
4.	*Corydalis govaniana* (Fig. 11.5)	Rhizome	May–June	1 year	July–Aug	2 years
5.	*Dioscorea deltoidea*	Rhizome	May–June	2 years	Aug–Sept	3 years
6.	*Geranium wallichianum*	Rhizome	April–May	1 years	June–July	2 years
7.	*Hypericum perforatum*	Flower	April–May	6 months	July–Aug	1 year
8.	*Jurinea himalaica*	Rhizome	April–May	1 year	July–Aug	2 years
9.	*Paeonia emodi* (Fig. 11.6)	Rhizome	May–June	1 year	July–Aug	2 years
10.	*Podophyllum emodi* (Fig. 11.7)	Rhizome	May–June	1 year	July–Aug	2 years
11.	*Polygonatum multiflorum*	Rhizome	May–June	1 year	Aug–Sept	2 years
12.	*Primula denticulata*	Leaves	April–May	1 year	July–Aug	2 years
13.	*Thymus serpyllum*	Leaves	May–June	6 months	Aug–Sept	1 year
14.	*Trachyspermum ammi*	Seed	May–June	6 months	Aug–Sept	1 year
15.	*Trillium govanianum*	Rhizome	March–April	1 year	July–Aug	2 years
16.	*Valeriana jatamansi*	Rhizome	April–May	1 year	July–Aug	2 years
17.	*Viola canescens* (Fig. 11.8)	Flowers/ leaves	March–May	6 months	July–Aug	1 year

11.3.2 Sustainable Collection of Medicinal Plants

MAPs used as per traditional medicine system are usually in crude form and are collected in the wild, mostly in forests and rangeland regions. These plants can be

Fig. 11.4 *Bistorta amplexicaulis*

found in a variety of habitats, including subalpine, temperate and sub-tropical mountainous locations, as well as deserts in the plains. A majority of the MAPs are collected from their natural habitats by labourers hired from the local community, most among which are paid on a daily basis. Small and marginal farmers make up a large number of rural households and professional collectors of MAPs (Sher et al. 2010a). These labours are not properly trained for MAP collection, and they are not being supervised by any technical person. Very few among these are sometimes aware of the identification of few plant species, which is being inherited from their forefathers. Collection of MAPs from the wild habitats, in most cases, surpasses the permissible quantity (sustainable quantity) extractable from natural habitats, putting plant species at risk. To ensure the survival of species in nature for future generations, nearly all developing countries have enacted rules governing and monitoring the production of MAPs. As a result of the haphazard collection, lack of proper grading system and inadequate drying and storage, many MAPs have become rare and highly threatened. Adulteration with apparently alike, but unwanted, plants is sometimes done for the sake of greater profits and financial benefits (Sher et al. 2010b).

Fig. 11.5 Habit of *Corydalis govaniana*

11.3.3 Regenerative Capacity of MAPs as a Result of Overgrazing

It is widely accepted that there is a correlation between plant growth and the effect of overgrazing. Despite the fact that most MAPs are unpalatable even then mature plants are grazed and browsed over a specific period of time, however their regeneration response is very poor. This way the individual plants never reach to maturity to complete their life cycle. Overgrazing contributes to the degradation of existing vegetation by limiting the expansion of species not only through direct use but also by drastically altering their habitats. To establish such a relationship in multiple sites with varied intensities of grazing on various plant species, a rigorous study is required. Soil compaction, mechanical damage to seedlings and occurrence of soil organisms are all indirect impacts of overgrazing. These methods make the soil more susceptible to erosion and loss of fertility. Fungus infestation is possible in the grazed section of the plant. As a result, most of the plants are now considered rare in most grazing areas (Sher et al. 2010c).

Fig. 11.6 *Paeonia emodi*: A flower; B close-up of fruit

The biological response of MAPs to different levels of collection, during different time periods, greatly varies from species to species. During summer, the locals harvest the rhizomes of selected rhizomatous plants, and at this time, these plants use the chemistry and nutrients of the roots to build aerial portions, particularly to produce stronger reproductive growth (i.e. to give fruits and seeds). The locals collect the entire plant of *Viola canescens* (as much as they can find) during its blooming condition. The rhizomes and the flowers are both considered as essential components of the plant for regeneration and long-term viability in the plant community (Sher and Yousaf 2014).

11.3.4 Socio-economic Factors

The targeted MAPs (Table 11.1) are found in wild habitats located at faraway places from the human settlements. These localities are not accessible by vehicle; therefore, the collectors have to walk for many hours along the rugged mountainous terrains, in selected union councils of the three districts, for collection of MAPs.

Most of the MAPs collectors are farmers with low land holdings. Sometimes, children and women also collect MAPs in their leisure time. Since, shepherds are directly involved in these habitats, therefore, they collect MAPs, whenever found, on their way through pastures. Collectors mostly take digging tools, pruners, large

Fig. 11.7 *Podophyllum emodi*: A habit; B close-up of fruit

shopping bags, extra clothes and food items whenever they go for collection of MAPs.

They return with a bundle of expensive MAPs after a successful harvest. One complete load (c. 40 kg) per collector takes at least 2 to 5 days of extensive field excursion in the wild habitat. Therefore, the collection of MAPs is considered as really a hard job. Despite these difficulties, the money earned by the collection of MAPs is greater than the daily wage of a labour. Wholesalers or local shopkeepers will sometimes pay collectors in advance.

The profits from the sale of MAPs are used to buy clothing and food and fulfil other needs of life. Farmers, on the other hand, are not interested in growing MAPs on their land. This might be because of their limited knowledge regarding the agronomic techniques of MAP cultivation.

11.3.5 Government Policy on Collection, Processing and Trade of MAPs

As per the forest law, for gathering of rhizomatous plants or roots from natural forests, a collection permit must be obtained in advance, from the local District Forest Offices (DFO). It is prerequisite for the collector to mention quantity and species of the plant which has to be collected.

Fig. 11.8 *Viola canescens*: A habitat; B close-up of flower

However, in general, the MAPs collectors never obtain a collecting permit before beginning to collect. Traders must release the permits they have obtained after paying a royalty at the rate of 20 Pakistani rupees per kilogram to the DFO. Nobody is allowed to transport the roots of plants like *Valeriana jatamansi* and *Podophyllum emodi* from the area of its collection, unless the permissions are released. It is worth mentioning that no such policy exists for the collection of leaves, flowers and seeds; secondly, transportation permit is also not required for the transportation of MAP leaves and flowers. For example, *Viola canescens* and *Hypericum perforatum* are free from such duty (Sher et al. 2013).

After paying a fee to the DFO, traders are legally allowed to take rhizomatous plants like *Trillium govanianum* and *Valeriana jatamansi* outside from their respective districts of collection during 15–20 days. If dealers are unable to organize transportation by air or any other mode of conveyance during this time, they must request and receive approval from the concerned DFO to extend the validity of permit. Therefore, a majority of the dealers are against this policy. Nevertheless, no vibrant policy is available about the export of MAPs.

11.3.6 Opportunities and Threats

Non-timber forest products such as MAPs are quite valuable. By engaging local people in the production through cultivation, processing and trading of some valuable MAPs, there is a greater chance of improving their economic conditions.

The production of MAPs would be among the most viable strategies to generate money for local people of the study area. The market price fluctuates from year to year. The involvement of middlemen has an impact on the selling price of MAPs in crude form at the collection locality. In addition, there is a greater risk of adulteration, beside the issue of market price variation. The roots of *Cymbopogon schoenanthus* and *Selinum tenuifolium*, for example, are detected as adulterants in rhizomes of *Valeriana jatamansi* (Sher et al. 2011).

11.3.7 Management System

The selected MAPs are mostly found in the natural coniferous forests of the studied sites in the three districts. There is a scarcity of data about the current stock levels/ volume of selected MAPs in the whole Pakistan in general and Khyber Pakhtunkhwa in particular.

As a result, comprehensive studies regarding these MAPs are urgently needed in order to create a management plan based on sustainability principles. Prior to harvesting, a resource inventory of high-value MAPs should be required in order to create site-specific information that may be used to establish the optimal harvest level.

11.3.7.1 Harvesting Techniques

Depending on phenology of the plant, there is an appropriate time to collect it. Part of the plant being collected has a direct relationship with appropriate time of collection. Active chemical ingredients present in plants vary in quantity and nature throughout the year and, during their life cycle, have a definite association with harvesting time which affects plant propagation. Each portion of a plant has distinct chemical components; collecting the wrong part for a certain purpose will result in an erroneous result.

The local collectors collect well-developed fruits of *Trachyspermum ammi*, the leaves and flowers of *Viola serpens* and the leaves or young shoots of *Thymus serpyllum*, when they are morphologically mature, i.e. in late summer. Inappropriate collection procedures will threaten these MAPs by inflicting excessive loss.

Uprooting a plant for utilization of its aerial parts only can result in the severe depletion of its population. Similarly, collecting excessive amounts of a tree’s bark may result in its death.

11.3.8 Processing Techniques

Once collected, drying is the next technical step in the practical process. The loss of vital constituents can occur if the drying process is not carried out correctly. Drying properly aids in preserving and fixing the essential components. Currently, residents of the study area dry all plant material in the direct sun. The plants are placed on the open floor, which is contaminated by dust particles and harmful agents, because the community lacks an appropriate drying facility. The loss of vital chemical contents occurs as a result of the unhealthy drying of plant material. The chemical constituents of some plants are not affected by direct sunlight, e.g. *Colchicum luteum* can be sundried as a general rule (Evans 2009).

Plants with volatile components and oil, such as *Valeriana jatamansi*, *Viola serpens* and *Trachyspermum ammi*, should be dried in the shade, as should all other target species. Plants that have been dried in the sun or in the shade must be suspended in wooden trays to allow air to circulate freely. It is also necessary to turn over the leaves 3–4 times every day. It's imperative to ensure that the true value of herbal material is found in its scientific processing (extraction, standardization, quality control and correct packaging) and not merely in its gathering and growing (Jan et al. 2020).

It is, therefore, critical to build centrally controlled processing facilities in places where wild MAPs are prevalent. These may consist of:

- Units for cleaning, drying, grading and packing
- Technical facility for marketing and transportation
- Units for the preparing of "mother tinctures" and "standardized extract of herbal medicines"
- Units for the extraction of essential oil (aromatic compounds) from fresh plants

11.3.9 Demonstration

In order to monitor sustainable level of harvesting in the study area, some research plots may be established, and different levels of utilization (harvesting) practices may be employed by using both scientific and local indicators based on perception of plant life cycles and plant population dynamics. Wild plant population may be monitored for few years over a range of harvesting intensities. This study will assist the semi-technical and technical workers engaged in the field (Sher and Barkworth 2015).

11.3.10 MAP Trade and Its Linkages with Conservation

There are c. 0.422 million plant taxa in the world, among which 12.5 per cent are being designated as MAPs (Shinwari 1996). Pakistan has ratified the UN's CBD, the FAO's 2002 resolution and the CITIES, as well as other multilateral agreements (Khan 2012). As a result, Pakistan has made attempts to conserve its biological diversity, including MAPs. The Biodiversity Action Plan (BAP) was prepared by the Ministry of Environment in coordination with all partners. The BAP has recommended in situ and ex situ biodiversity conservation strategies, particularly MAPs. The Ministry of Environment has provided a list of endangered and vulnerable MAPs in Pakistan in the goals and objectives section of its strategic plan, as well as provisional list of indicator species for monitoring progress of MAP conservation (Sher et al. 2014).

MAPs' genetic diversity has been under constant threat of extinction owing to environmentally unsustainable collection methods. The value of MAPs for their environmental function and the value of products developed from these must be viewed in balance. Furthermore, this sector needs focus as it is facing problems of diversified nature. The majority of MAPs are collected from the wild habitats, which makes maintaining a supply difficult. The international demand and supply for MAPs has become market-driven, and the number of wild collectors is outnumbering natural populations. As a result, this trade must be established on a scientific and long-term basis. Since, the MAPs are more vulnerable to overexploitation and extinction, therefore, these issues must be addressed in a practical way.

No doubt that the policy of Pakistan on Conservation of Biodiversity of MAPs is concentrated on in situ and ex situ conservation. Although the country's conservation policy is focused on in situ and ex situ conservation measures, it still has a long way to go in terms of enacting laws and policies to protect all of its genetic diversity. This can be accomplished through bilateral and multilateral plant germplasm exchange, collaborative research among breeders and farmers, community rights protection and the formulation of a complete action plan to fulfil the goals of trade sustainability and conservation. Growth, exploitation and environmentally unfriendly harvesting processes, non-equitable distribution of profits emerging from the collection and trading of MAPs, loss of MAP growth habitats and unmonitored trade of MAPs are all contributing to the extinction of genetic diversity in MAPs.

11.3.11 Challenges in MAP Management in Khyber Pakhtunkhwa, Pakistan

According to the present field studies and literature review, there are potential impediments and problems in the long-term management and development of MAPs in Khyber Pakhtunkhwa. These are grouped into the following categories.

11.3.12 Medicinal Plants at the Policy Level

MAPs play a vital role in the region's rural livelihoods by supplying essential subsistence products and monetary revenue, which is especially crucial to the community and, in particular, the poorest households. Medicinal plants, though a key component of rural livelihood strategies, have seldom gained the much-needed practical attention by the policymakers and are often a low priority at the policy level (Sher et al. 2012).

- The related policies are either weak, or medicinal plants are tucked in with timber management without a policy of its own. This way they lose importance and are mostly left out unmanaged.
- There is lack of policy direction. Whatever paper policy directions are available lack adequate implementation.
- Conventional forest management by the relevant government departments mainly focuses on trees and wood production rather than other goods and services of the forests. There is inadequate of understanding of medicinal plants' ecology, which contributes to loss of the much-needed focus at the policy level.
- The importance of handling medicinal plants, as a subsector, is not understood at the policy level.

11.3.13 Silviculture and Governance-Related Shortfalls in Management

There is limited silviculture management of medicinal plants for a variety of reasons.

- The medicinal plants are normally found in far-flung inaccessible areas and do not receive proper silviculture management in natural occurrence.
- The constant unregulated unsustainable harvesting is resulting in degradation of ecosystem, escalating people vulnerability to poverty, especially in cases where livelihood of local people depends on these MAPs.
- Local/indigenous knowledge is orally transmitted, mostly remains undocumented and is seldom disseminated.

- Formal research is confined to academic institutions, and the mechanism to share the results with collectors, regulating bodies and traders is not practised.
- Sufficient and authentic data on the regenerative capacity of the medicinal plants are lacking.
- Rehabilitation of medicinal plant resources has not been practised.
- There is insufficient capacity building on the management of medicinal plants.
- There is a lack of understanding of medicinal plants' ecology which makes it difficult to take management decision regarding what, when and how much to harvest.
- In most cases the much-needed scientific research-based knowledge at the ecosystem level about the proper stage of the physiological growth in natural habitats is very limited.

11.3.14 Governance

Medicinal plant reserves have seldom received the much-needed attention of sustainable management for affording enhanced opportunities for sustainable livelihoods and healthy ecosystems. Some salient elements that contribute to the weak governance in the medicinal plants subsector are as follows:

- The regulatory focus of management is on royalty generation and not on what is best for medicinal plants reserves.
- Quota allocations for MAP collection are not based on sound scientific grounds.
- Weak capacities of the management authorities to apply and implement regulations framed on the basis of relevant international conventions.
- The requisite capacity building initiatives by the government for sustainable management of MAPs are generally absent.
- The officials, traders and processors do not understand the importance of management of MAPs as a safety net and livelihood strategy for the associated rural communities.

11.3.15 Trade, Tenure, Equity and the Missing Link of Knowledge Sharing

Having significant economic and commercial value, MAPs are threatened due to unsustainable collection practices by the communities, and they themselves are weakening their own resource base. The significant contributing factors being:

- Absence of the knowledge about the regenerative capacities of the resource base.
- The gap between production and supply is widening exerting additional pressure on the resource extraction to meet the market demand.

- The resource ownership is not clear, and, in most cases, medicinal plants are considered as an open-access resource. Mostly the poor collect them from the natural occurrence to a degree that certain biomes are unable to meet the demand and face increasing and non-sustainable harvest pressure.
- The medicinal plants are found in areas where the land tenure is not clear and, in many instances, makes the ownership contentious that leads to accelerating resource depletion.
- Very little has been done to pass on scientific knowledge in simple language to the communities; therefore, they lack awareness regarding sustainable use of medicinal plants.
- The harvest is unmanaged and uncoordinated from the natural occurrence to a degree that certain biomes are unable to meet the demand and face increasing and non-sustainable harvest pressure.
- The tenure system of non-timber forest products is unclear. It gives rise to rights and equity issues and becomes one of the main limiting factors in the management of medicinal plants. The moment a common natural resource starts yielding a high value return, the more powerful tend to step in to reap the benefits and limit the access of the weaker ones to the resource. This in turn tends to further marginalize the already marginalized sections of the population.
- The illegal trans-border trade is lucrative. For want of a little additional cash, the poor collectors are tempted to unsustainably harvest medicinal plants.

11.3.15.1 Market Information and Local-Level Business Service Providers

The harvest and trade of medicinal plants involves a complex group of stakeholders. Most of the officials and traders lack the understanding regarding the perspectives of the highly vulnerable rural communities and the various coping strategies adopted by them. The distribution of benefits in the medicinal plant trade is inequitable. The trade of medicinal plants is informal, uncoordinated and mostly unregulated.

- The secretive nature of the MAP traders blocks the flow of market information to the collectors. There is lack of market intelligence that makes the distribution of benefits unfair.
- The local MAP collectors lack the capacity to sustainably manage the resource base and negotiate with market to derive optimum benefits.
- The absence of business service providers at the local level who have the capacity to help the rural population in disposal of the collected produce at remunerative prices and derive optimum benefits.
- The lack of post-harvest handling capacities and facilities compel collectors to hand over the harvest to local-level vendor.
- Lack of sharing of knowledge with collectors by research institutions gained through research and the absence of its felt need.

11.3.15.2 Law Enforcement and Regulatory Frameworks

Most of the medicinal plants are traded within the countries or across the borders both legally and illegally. Though the laws that govern the trade of medicinal plants do exist, they are seldom enforced, or the law enforcement is weak. This is because:

- Real value of medicinal plants is not understood by managers, private sector, communities and law-enforcing agencies.
- The communities either do not understand their rights and duties or do not consider it their obligation to report any violation.
- There hardly exists any baseline that provides information on laws and regulations that govern the management and trading of medicinal plants.
- The regulatory frameworks in different countries are highly variant which provide ample ways to the traders to get away for any offence that might have been committed.
- The organizations involved have weak capacities to observe, identify, measure and control the trade in medicinal plant throughout the region.

11.3.16 Changing Land Use Preferences

With growing population rapid changes in land use are occurring. More natural habitats are being converted into agricultural land and habitations. The rapid change of land use is directly resulting in the altering ecosystems and habitats that support medicinal plants. Many medicinal plants are on the verge of possible extinction at the local, regional or global level and directly affect the livelihood of local communities which are dependent on these plants.

11.3.16.1 Reinvestment in Management of Medicinal Plants

The private sector is involved in reaping the benefits through processing and marketing bulk of the raw material obtained from the wild resource pool. However, the government and the private sector lack the focus to reinvesting in the management of natural resource base.

11.3.17 Armed Conflicts, Natural Disasters and Rehabilitation Processes

Armed conflicts and natural disasters adversely affect the social and ecological environment and have caused serious destruction of ecosystems and habitats.

Furthermore the post-conflict and post-disaster rehabilitation processes normally do not take stock of the capacity of natural resource pools to endure the pressures as:

1. The impacts of armed conflicts and natural disasters on ecosystems have not been appropriately studied.
2. The impacts of post-conflict and post-disaster rehabilitation process on natural resources are seldom documented.
3. The potential of medicinal plants as an alternate livelihood strategy in post-conflict and post-disaster rehabilitation process has not been appropriately estimated.

11.3.18 Climate Change

The already stressed ecosystems are now faced with the need to adapt to new regimes of temperature, precipitation and other climatic extremes. The mean global surface temperature has increased by about 0.3–0.6 °C since the late nineteenth century and by c. 0.2–0.3 °C over the last 40 years. Precipitation averaged over the Earth's land surface increased from the start of the century up to about 1960, but has decreased since 1980. These changes will potentially have wide-ranging effects on the natural environment as well as on human societies and economies. Climate change contributes to the vulnerability of the ecosystems (Nawab et al. 2016b). This in turn would affect the poor who have a high dependency on natural resources for their livelihoods averse to risks and shocks. To manage the ecosystems that are habitats to medicinal plants, in a manner that they adapt to the changing climatic conditions especially at high altitudes and keep on sustainably supporting livelihoods, there is:

- Lack of knowledge on adaptability of species and habitats
- Insufficient scientific data on the response of ecosystem in terms of composition and physiological growth to diurnal variations and growing season

Several initiatives have been undertaken, which yielded good results. However, most of the initiatives were either site or species specific. It is beyond doubt that positive results could be achieved to halt the depletion of the ecosystems that support medicinal plants and livelihood of the highly vulnerable sections of the society (Nawab et al. 2016a).

11.3.19 Conclusion and Recommendations

The current chapter is produced as a result of field studies, meetings with local communities in three districts of Khyber Pakhtunkhwa, Pakistan. It will also contribute to the development of sustainable use management plan for selected MAPs with the potential to contribute to reduce poverty and its continuing practicability in the study area. The key challenges and opportunities were identified, and broader governance issues were outlined. Seventeen MAPs were identified: these are most commonly collected and traded MAPs in the country. In this report, a comprehensive description of biology, management problems, availability, distribution and socio-economic importance are deliberated for each species. The current volume/stock of these species in the wild was determined, and sustainable harvest quotas for each species were given.

It also highlights certain areas, particularly those related to the establishment, operation and long-term viability of chosen MAP-based businesses, which need more research and development funding, not only as a source of income but also as a means of saving in community funds. Part-time and full-time work in company management, raw material collecting, processing and marketing has been achieved by men and women in the community.

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Chapter 12
Profiling of Trace Elements and Regulatory Landscape of Dietary Herbal Supplements

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Abstract Elevated growth in the therapeutic market of phytopharmaceuticals and herbal supplements was observed in the last few years. The rise in the herbal market has also attracted researchers, pharmaceutical companies, and regulatory bodies to focus on TEA. It influences all body functions in minute amounts for proper physiological function and development. Excess and deficiency of the essential trace elements (TE) resulted in several nutritional disorders and diseases. The development of cutting-edge technologies with potential advantages like high-throughput measurement, minimum test sample, and easier operational steps boosted and solved several analytical challenges of the essential TE. This book chapter listed out analytical approaches of TE with their regulatory landscape.

Keywords Essential trace elements (TE) · Phytopharmaceuticals · Herbal supplements

Abbreviations

CF-LIBS	Calibration-free laser-induced breakdown spectroscopy
ED-XRF	Energy dispersive X-ray fluorescence

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FAAS	Flame atomic absorption spectrometry
GFAAS	Graphite furnace atomic absorption spectroscopy
HGAAS	Hydride generation atomic absorption spectroscopy
HGAFS	Hydride generation atomic fluorescence spectroscopy
HP	Herbal plants
ICP-AES	Inductively coupled plasma atomic emission spectroscopy
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical spectroscopy
ID-ICP-MS	Isotope dilution inductively coupled plasma mass spectrometry
INAA	Instrumental neutron activation analysis
LA-ICP-MS	Laser Ablation inductively coupled plasma mass spectrometry
LALI-TOF-MS	Laser ablation laser ionization time-of-flight mass spectrometer
PIXE	Particle-induced X-ray emission
ppm	Part per million
ppt	Part per trillion
TE	Trace elements
TEA	Trace elemental analysis

12.1 Introduction

An escalated growth in the global herbal drug market has occurred over the past 15 years. The European and US markets alone reached $7 billion and $5 billion per year, respectively (Calixto 2000; Smith et al. 2020). The American Botanical Council reports that in the USA, herbal supplement sales increased by 8.6% in 2019, and record-breaking sales are predicted for 2020. Highlights of this prediction were found to be a steep increase in the sales of herbal supplements during the COVID-19 pandemic (American Botanical Council 2020).

Essential TE are dietary elements that the body requires in tiny fractions which influence all body functions. Around 98% of the body mass of a human being is made up of 9 nonmetallic elements, and a human adult is made up of 11 typical TE (TE 2021). Most TE mediate vital biochemical reactions. As catalysts of enzymes and hormones, TE are essential for the proper functioning of metabolic, hormonal, and immune systems in the body. The deficiency and excess levels of essential TE may cause diseases and disorders. TE underlie the etiopathogenesis of many nutritional disorders (National Research Council (US) Committee on Diet and Health 1989, Prashanth et al. 2015, TE 2021). Various classifications of TE as per Prashanth et al. (2015) are given in Table 12.1.

Therapeutic herbal plants (HP) are known to the world as an option for the treatment of several disease conditions from the ancient period. It has played a prime role in the uncovering of present-day therapeutic interventions. The success of herbal medications for healing purposes is often credited for their organic bioconstituents. Prolonged intake of phytotherapeutics may lead to their

Table 12.1 Various classifications of TE

Essential elements for the human body	**Biological classification of TE**
• Four organic basic elements: H, C, N, O • Quantity elements: Na, Mg, K, Ca, P, S, Cl • Essential TE: Mn, Fe, Co, Ni, Cu, Zn, Mo, Se, I • No specific identified biochemical functions: Li, V, Cr, B, F, Si, As	• Essential TE: B, Co, Cu, I, Fe, Mn, Mo, Zn • Probable essential TE: Cr, F, Ni, Se, V • Physically promotive TE: Br, Li, Si, Sn, Ti
Categorical classification of TE	**Essential TE**
• Group I: C, H, O, N • Group II: Na, K, P, Ca, Mg, Na, K, P, Cl, S • Group III: Cu, Fe, Zn, Cr, Co, I, , Mo, Se • Group IV: Cd, Ni, Si, Sn, V, Al • Group V: Au, Hg, CN, Pb	• Macroelements: Ca, Mg, Na, K, P, Cl, S • Trace or microelements: Fe, Zn, Mn, Cu, I, Co, Ni, F, V, Cr, Mo, Se, Sn, Si

accumulation and toxicity (Benzie and Wachtel-Galor 2011; Ekor 2014). Essential metals will also produce few toxic effects when they're taken in excess amount, and nonessential metals are toxic even at minimal levels in human beings.

Osamu, W.A.D.A. published a detailed review on TE functions and deficiencies. A summary of TE with their functions and toxicity (National Research Council (US) Committee on Diet and Health 1989, Osamu 2004) is tabulated in Table 12.2. These TE show several symptoms depending upon the deficiency and excess states. The article also incorporated in-depth clinical aspects of TE with surveys and studies.

The latest technologies provide potential advantages over the conventional elemental profiling techniques with the advantages like high-throughput measurement, less sample, and less tedious operational steps with limitations. It incorporates unbiased findings suitable for trace element-based biomarkers for herbal supplements and products (Sium et al. 2016).

This chapter is a summary of the role of the essential TE from herbal supplements and their profiling approaches. This chapter also appraises the selected works on the profiling of TE from dietary herbal supplements and their regulatory landscape.

12.2 Analytical Approaches for Profiling of TE

The estimation and characterization of known or unknown trace metals with sophisticated approaches are highly sensitive and complex methodologies. So it is therefore essential to have a proper sample preparation process. An overview of the workflow on trace metal analysis is provided in a pictorial format in Fig. 12.1. The sample processing will vary according to the specific trace element under study. Most of the trace elemental analytical procedures require the sample to undergo sample treatment or digestion depending on the complexity of the sample preferably

Table 12.2 Summary of TE with their functions and toxicity (NIH fact sheets combined 2021)

Essential elements	Functions	Toxicity
Iron	Growth and development, production of red blood cells	Gastric upset, constipation, nausea, abdominal pain, severe cases: committing fitness, convulsions, multisystem organ failure, coma and even death, hemochromatosis
Zinc	Maintain the integrity of the skin and mucosal membranes, develop and activate T-lymphocytes	Acute nausea, vomiting, cramps, diarrhea and headache, altered iron function, reduced immune function and reduced levels of high-density lipoproteins
Manganese	Increase bone mineral density, bone formation, glucose, carbohydrate, and lipid metabolism	Tremors, muscle spasms, tinnitus, hearing loss, mania, insomnia, depression, delusions, anorexia
Copper	Hemopoiesis, bone metabolism, connective tissue metabolism	Liver damage, diarrhea, nausea, vomiting, cramps, abdominal pain; people with Wilson's disease have a high risk of copper toxicity
Iodine	Fetal and infant development, fibrocystic breast disease, thyroid hormone production	Goitre, elevated TSH levels, and hypothyroidism Burning of the mouth, throat, and stomach; fever; abdominal pain; nausea; vomiting; diarrhea; weak pulse; and coma
Cobalt	Production of red blood cells, metabolism of fats and carbohydrates, synthesis of proteins in the nervous system prevents demyelination	Cardiomyopathy, goitre, reduced activity of the thyroid, increased blood sugar levels
Fluorine	Dental caries and bone fractures	Nausea, vomiting, abdominal pain, diarrhea, periostitis, and even death in rare cases
Chromium	Impaired glucose tolerance and diabetes, metabolic syndrome, polycystic ovary syndrome dyslipidemia, and weight and lean body mass.	Weight loss, anemia, thrombocytopenia, liver dysfunction, renal failure, rhabdomyolysis, dermatitis, and hypoglycemia
Molybdenum	Amino acid metabolism, uric acid metabolism	Achy joints, gout-like symptoms, and abnormally high blood levels of uric acid
Selenium	Cancer, cardiovascular disease, cognitive decline, and thyroid disease	Gastrointestinal and neurological symptoms, acute respiratory distress syndrome, myocardial infarction, hair loss, muscle tenderness, tremors, facial flushing, kidney failure, cardiac failure, and death in rare cases

in liquid form. The most important decision criteria regarding the analytical methodology are the detection limit of the application, the objective of the application, and the complexity of the samples to be analyzed. Digestion by the microwave

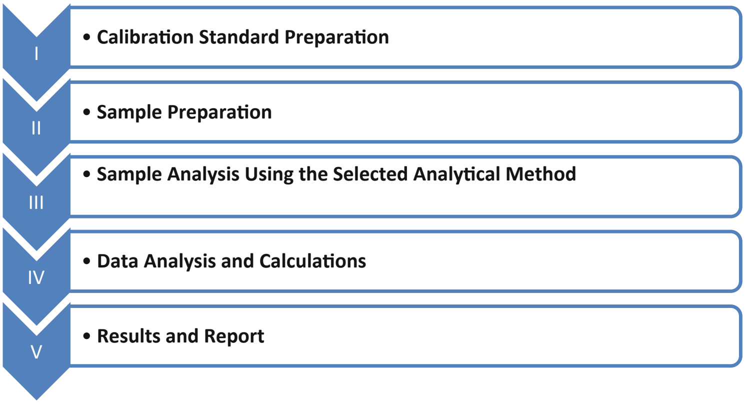

Fig. 12.1 General workflow of trace metal analysis

method and acid digestion are widely used to ensure TE are completely dissolved before jumping to the profiling techniques or instrumental approaches (Mettler Toledo 2021**;** Yang et al. 2020) (Table 12.3).

12.2.1 Flame Atomic Absorption Spectrometry (FAAS)

FAAS is based on element-specific wavelength light absorption by ground state atoms in the flame for rapid analysis of TE. AAS is a reasonably priced TE analytical technique and provides a better degree of accuracy and precision for the estimation at ppm level (Podraza and Jellison 2017; Helaluddin et al. 2016). Subramanian et al. performed the quantification of some minerals and TE in selected tropical medicinal plants (Subramanian et al. 2012). Shirin et al. studied and determined the major and trace elements (Fe, Cu, Ni, Mn, and Zn) in *Withania somnifera* using FAAS (Shirin et al. 2010).

Hydride generation atomic absorption/fluorescence spectroscopy (HGAAS/HGAFS) is available via an add-on option for the recent models of AAS/AFS instruments and requires only the hydride generation h. The atomization efficiency is multiplied with a parallel decrease in sensitivity (Li and Guo 2005). Liu et al. estimated the total selenium in Chinese medicinal herbs by HGAFS approach (Liu et al. 2005).

12.2.2 Graphite Furnace Atomic Absorption Spectroscopy (GFAAS)

In GFAAS, chemical interference is comparatively minimal with the usage of chemical modifiers, which makes the matrix volatile and results in sensitive analysis

Table 12.3 Recent research works on TE with their methodology

Methodology	Elements identified/estimated	Reference
ICP-OES and ICP-MS	K, Ca, Rb^{85}, Sr^{87} Zn^{64} Cu^{63}, Ni^{60}, Cr^{52}/Cr^{53}, and Co^{59}	Khan et al. (2021)
ED-XRF and PIXE	P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Se, Br, Rb, Sr, Ti, and V	Jyothsna et al. (2021a)
INAA	Ca, Mg, Cl, K, Al, Na, and Fe	Melkegna and Jonah (2021)
ICP-MS	Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Cd, and Pb	Taşkin et al. (2021)
ED-XRF	P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Se, Br, Rb, and Sr	Jyothsna et al. (2021b)
X-ray fluorescence spectrometry	Br, Cr, Cu, Fe, K, Mn, P, Rb, Sr, Yb, and Zn	Nedjimi (2021)
ICP-MS	Cu, K, Ca, Mg, Fe, Mn, Zn, Ni, Pb, Cr, Co, As, and Cd	Parvathy et al. (2021)
CF-LIBS and ICP OES	Ca, K, Mg, S, Si, Al, Fe, P, Na, Ba, Ti, Mn, B, Se, Cu, Cd, Pb, and Zn	Aldakheel et al. (2021)
ICP-OES	Zn, Cu, Ni, Co, Fe, Mn, Cr, Pb, and Cd	Fagbohun et al. (2020)
ICP-OES	Ca, K, Mg, Si, Al, Fe, P, Na, Ni, Mn, Se, Cu, Zn, and Sr	Durante et al. (2021)
PIXE	K, Ca, Mn, Fe, Cu, Zn, As, Se, and Pb	Swain and Rautray (2021)
PIXE	P, S, Cl, K, Ca, Sc, Ti, Mn, Fe, Cu, Zn, Rb, Br, and Sr	Singh et al. (2020)
EDXRF	Fe, Cr, Mn, Co, Ni, Cu, Zn, As, and Pb	Kulal et al. (2021)
ICP-OES	Cd, Pb, Zn, Cu, Ni, Cr, Fe, and Mn	Georgieva et al. (2020)
INAA	Al, Ca, Cl, K, Mg, Mn, and Na	Soliman et al. (2020)
FAAS	V, Zn, Cr, Cu, Fe, K, Na, and Ni	Gholamhoseinian et al. (2020)
EXDRF	P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Se, Br, Rb, and Sr	Jyothsna et al. (2020)
ENAA	K, Ca, Mg, Fe, Mn, Cu, Zn, Co, Mo, Cr, Al, Ba, V, Se, Ni, Sr, and Cd	Tkachenko et al. (2020)
LIBS	Na, Mg, Ca, K, Ti, Fe, Li, Al, Ba, and Si	Shahida et al. (2020)
INAA	Al, As, Ba, Br, Ca, Ce, Cl, Co, Cr, Cs, Fe, Hf, K, La, Mg, Mo, Mn, Na, Rb, Sb, Sc, Sr, Sm, Th, U, and Zn	Zinicovscaia et al. (2020)
LIBS and ICP-OES	Al, Ba, Cu, Cr, Ca, Fe, K, Mn, Mg, Mo, Ni, Si, Zn, Sr, and Na	Sharma et al. (2020)
FAAS ICP-OES	Fe, Mn, Zn, Cu, Pb, Cd, and Cr	Konieczynski et al. (2020)
ED-XRF	Pb, K, S, Ca, Fe, Zn, Cu, Co, Ni, Mn, and Se	Agbo et al. (2020)
AAS	Na, K, Ca, Mg, Cu, Mn, Fe, and Zn	Ceccanti et al. (2020)
ICP-AES	Al, As, B, Ca, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, P, Si, Ti, and Zn	Du et al. (2020)
PIXE	Al, Si, P, S, Cl, K, Ca, Ti, Mn, Mn, Zn, Cu, Fe, and Sr	Elayaperumal et al. (2020)
ICP-MS	V, Cr, Mn, Co, Ni, Cu, and Zn	Suleiman and Brima (2020)

(continued)

Table 12.3 (continued)

Methodology	Elements identified/estimated	Reference
ICP-MS	Ca, Mn, P, Ti, Mg, Pb, Na, Cr, K, Sr, Fe, Cu, Al, Ni, B. Ba, Zn, Mo, Co, As, Se, Cd, and Sn	Yabalak et al. (2020)
ICP-MS	K, Ca, Mg and Ni, Na, Se, Cd, Hg, Pb, and Mn	Potortì et al. (2020)
ED-XRF	Mg, K, Ca, Al, Si, P, S, Ti, Sr, V, Cr, Mn, Na, Fe, Cu, Zn, As, Rb, and Pb	Aksoy et al. (2020)
INAA	Ba, Br, Ca, Co, Cr, Fe, K, La, Na, Rb, Sc, Sm, and Zn	Datta et al. (2020)

(Holcombe and Borges 2017). It has enhanced detection limits with minimal sample volume. This analytical technology works with restricted working range, slow analysis, and high cost (GFAAS 2021). Jasha et al. determined 10 TE (Mg, Ca, V, Cr, Mn, Fe, Cu, Zn, Mo, and Cd) using GFAAS from selected medicinal plants (Anal and Chase 2016).

12.2.3 Inductively Coupled Plasma Optical/Atomic Emission Spectroscopy (ICP-OES/AES)

ICP spectroscopy is a technique used for elemental analysis and trace analysis. The main advantage of the ICP-OES technique is lower detection limits from parts per million (ppm) to parts per billion (ppb). Pytlakowska et al. in 2012 explored 12 minerals and TE (Al, B, Ba, Fe, Zn, Mn, Mg, K, Na, P, Cu, Sr, and Ca) from herbs and infusions using ICP-OES (Pytlakowska et al. 2012). Niu et al. used ICP-AES for mineral estimation of nutrients in *G. macrophylla* (Niu et al. 2014). Yan et al. established ICP-AES-based methodology for the simultaneous determination of major and trace elements like Na, K, Cu, Fe, Zn, Mn, Ca, Mg, Cr, Ni, Pb, Se, As, and Cd from six herbal drugs (Qing-hua et al. 2012; YAN et al. 2011).

12.2.4 Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

ICP-MS is also a powerful analytical method for TEA, but at this point, it is much expensive when compared with AAS or HGAAS with the ability to detect individual elemental isotopes. An ICP-MS instrument is hyphenated with a high-temperature ICP source with a mass spectrometer which is relatively sensitive and free from interferences (Helaluddin et al. 2016, (Wilschefski and Baxter 2019). Serife et al. performed the estimations of Cr, Mn, Fe, Co, Ni, Cu, Zn, Rb, Sr, and Pb elements in 30 herbal medicines using ICP-MS approach (Tokalıoğlu 2012). James et al.

performed the estimation of Pb in Chinese herbs using isotope dilution inductively coupled plasma mass spectrometry (ID-ICP-MS) methodology. The strategies involved include the addition of enriched isotopic solutions to the herbal samples while flowing towards the plasma (Lam et al. 2010).

12.2.5 Instrumental Neutron Activation Analysis (INAA)

In INAA technique, the sample is exposed to a neutron flow after which radioactive isotopes decay to a lower energy state and emit delayed gamma rays with characteristic energies for each element proportional to the amount of the element in the sample. It has the capability to estimate several elements simultaneously without destroying the sample and offers high sensitivity for different TE (Hamidatou et al. 2013; Helaluddin et al. 2016; INAA 2007, 2019). Oladipoa et al. utilized INAA for the multi-trace elemental determination of 6 medicinal plants, and they estimated 21 TE similar to the herbs under study (Oladipo et al. 2012). Bouzid et al. carried out an investigation in *A. herba-alba*, and Ca, K, Fe, Zn, and Cr were detected by using INAA (Nedjimi and Beladel 2015).

12.2.6 Energy Dispersive X-Ray Fluorescence (ED-XRF)

ED-XRF has a small, compact instrument design with one of the two general types of X-ray fluorescence techniques. In ED-XRF spectrometers, all of the elements in the sample are excited simultaneously, and a multi-channel analyzer is used to simultaneously collect the fluorescence radiation emitted from the sample. The different energies of the characteristic radiation from each sample elements are then separated. Resolution of ED-XRF systems is dependent upon the detector, and it is a minimal maintenance instrument. The ED-XRF systems offer simplicity, fast operation, non-movable parts and high source efficiency. The ED-XRF will be helpful in small feature analysis and enhanced performance for the measurement of TE micro ED-XRF applications (Markowicz 2011; Yao et al. 2015).

Beheraa et al. carried out the multi-elemental estimation in vitro-proliferated root tissues of *Andrographis paniculata* and of the naturally grown in vivo plants. TE, namely, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Rb, Sr, and Pb, in addition to two macroelements K and Ca were identified and estimated using the ED-XRF technique (Behera et al. 2010).

12.2.7 Particle-Induced X-Ray Emission (PIXE)

PIXE involves the measurement of X-rays emitted from a sample due to high-energy ion bombardment. PIXE analysis is a nondestructive applicable technique for multi-

TE analysis and is now widely engaged in many fields where the detection and estimation of TE are required. It also offers better signal-to-background ratios for TE analysis (Ishii 2019; PIXE 2021).

Raju et al. performed the TEA of some anti-diabetic HP (Raju et al. 2006). Bhanisana et al. utilized this methodology for the estimation of major and trace elements from ten medicinal plants (Devi and Sarma 2013).

12.2.8 Calibration-Free Laser-Induced Breakdown Spectroscopy (CF-LIBS)

CF-LIBS is an in situ quantitative method for the direct measurement of line intensities and plasma properties without sample preparation. It utilizes highly energetic laser pulses to induce optical sample excitation. The full exploitation of the CF-LIBS techniques is not carried out due to the lacunae in its experimental constraints (Anabitarte et al. 2012; Dong et al. 2015; Tognoni et al. 2010; Yang et al. 2018). Quantitative TEA results are reported in the literature in a wide range of experimental conditions (Rai et al. 2013). In 2012, Santos et al. conducted a detailed review of the LIBS approach for the estimation from plant materials (Santos Jr et al. 2012). Recently in 2020, Legnaioli also reviewed the industrial applications of LIBS (Legnaioli et al. 2020). Sanja et al. performed the estimation of Mn and Ba in peppermint tea using CF-LIBS (Zivkovic et al. 2018).

12.3 NOVEL Techniques in Trace Element Analysis

12.3.1 LA-ICP-MS (Laser Ablation Inductively Coupled Plasma Mass Spectrometry)

LA-ICP-MS is a microanalytical technology that enables a highly sensitive elemental and isotopic direct analysis of solid samples. LA-ICP-MS begins with a laser beam focused on the sample surface to generate fine particles by a process known as laser ablation. The ablated particles are then carried to the secondary excitation source of the ICP-MS instrument to be digested and ionized in the plasma torch and then subsequently introduced to a MS detector for both elemental and isotopic analysis (Applied Spectra 2021). This technique was applied to take the images to study the specific elemental distribution of the biological sample brain. The estimation of copper, zinc, and other minor elements were found in thin slices of human brain samples (Becker et al. 2007).

12.3.1.1 Laser Ablation Laser Ionization Time-of-Flight Mass Spectrometer (LALI-TOF-MS)

LALI-TOF-MS is a novel development that can be utilized for the simultaneous assessment of the entire periodic table of elements which requires a direct analysis of solid samples at its MS detection limits. LALI avoids conventional sample digestion approaches and bypasses the limitations like matrix suppression effects, polyatomic spectral interferences, and signal instability exhibited in other approaches (Putman and Williams 2020).

12.4 Regulatory Aspects of Essential TE in Herbal Supplements and Phytopharmaceuticals

The majority of the phytochemical research targets the role of TE metabolic function and their toxicity. Recent regulatory platforms thrust on the need to analyze these herbal supplements to detect their components including TE which are essential for the normal healthy functioning of the human body (Bhattacharya et al. 2016). The WHO has set guidelines, which are the golden standard for the assessment of herbal medicines with regard to contaminants and residues. It recommends about TE mineral intake of 1.5 grams for normal healthy people (WHO 2007).

Each country has its regulations for herbal medicines, and its chronology varies across jurisdictions of individual countries. Mostly developed countries have started the regulatory platform for herbal products and supplements that have been in existence for a long time, whereas some countries recently started to strictly enforce the rules and regulations for the same. The rise in the herbal market had resulted in the marketing of these products with claims and without proper education, and it may be potentially dangerous to the consumer (Thakkar et al. 2020). The USFDA had recommended a series of tests and controls to ensure the quality and therapeutic standards of the botanical products (USFDA 2016) in botanical products.

12.5 Conclusion

The knowledge on the traditional medicine practices had resulted in increased development of dietary supplements and herbal medicine. Many of the products contain ingredients marketed under the claim of several health benefits. The number of humanoids having the intake of these products has increased in recent years. Although having some TE is essential for our well-being, many of it can be toxic and result in negative impacts. Trace metal analysis of herbal supplements is used to ensure compliance with regulatory requirements and quality standards. The rapid advancements and emerging technologies will play a vital role in the research and quality element together involving in its regulatory functions.

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Chapter 13
Sustainable Economic Systems Against Biotic and Abiotic Stress in Medicinal Plants: Aeroponics, Hydroponics, and Organoponics

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Abstract Plants are sessile organisms and the constant monitoring of environmental changes is needed for them to modify, adjust, and adapt their development and metabolism accordingly. The response to these environmental stimuli requires a multi-integral mechanism where internal and external signals are detected and cause an appropriate reaction in the plant. According to the medium in which the roots develop, soilless cultivation systems are classified into three groups: cultivation in (1) substrate, (2) water (hydroponics), and (3) air (aeroponics). In particular, aeroponics is the most modern hydroponic system. The main advantage that aeroponics offers is the excellent aeration it provides to roots. Aeroponics has been used with great success in plant propagation and, more specifically, in the

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propagation of cuttings of herbaceous or woody species that are difficult to root. This is an agricultural production technique in which plants are grown without the use of soil and nutrients are delivered through a liquid solution. In soilless cultivation, soil is replaced by an inert substrate, and the nutrients needed by the plant to grow are delivered via irrigation. Organoponics is a form of urban agricultural production with one of the greatest impacts in terms of production volume per farm area. The variety of fresh vegetables and condiments at lower prices marketed based on supply and demand, together with the jobs generated and the productive use of otherwise wasted space, are key advantages that the use of this technique provides. On the other hand, this technology represents a form of intensive production based on the extensive use of organic fertilizers, which are applied in areas with infertile soils or with serious limitations for their exploitation, and even on artificial surfaces created specifically for this activity. Therefore, this chapter aims to (a) describe the background of aeroponics, hydroponics, and organoponics, (b) indicate the main medicinal plants that have been identified as candidates for biotic and abiotic stress tolerance, and (c) understand how the incursion of sustainable economic systems are efficient and effective methods to counteract the effects of biotic and abiotic stress in medicinal plants.

Keywords Aeroponics · Climate change · Hydroponics · Phloem transport · Organoponics

13.1 Introduction

Aeroponic, hydroponic, and organoponic cultures are techniques that allow the sustainable production of medicinal plants and food. In these circumstances, fertile soil is not indispensable, and any space, inside or outside the garden, becomes a favorable place for the cultivation of plants. Because they are intensive systems, it is possible to grow medicinal plants and food in acceptable quantity and quality, since by having greater control of environmental conditions, pests, and nutritional requirements, the option of producing medicinal plants is greater than in an open field. To obtain satisfactory results in all three systems, it is advisable to grow short-cycle medicinal plants to harvest them as soon as possible. Among these plants are some fruit species, medicinal plants, and vegetables. The Earth temperature will increase between 3 and 5 °C in the next 60–100 years. The increase in temperature and the irregularity of precipitation cause changes in droughts and floods to occur. Salt stress is caused by the exploitation of metal resources, inadequate irrigation, and excess fertilizers derived from anthropogenic activities. Plants face these forms of biotic and abiotic stresses with great frequency. Food and farmers' security must be ensured through the development of stress-tolerant crops. Also, molecular work should be carried out at the genetic level for the development of mechanisms in plants to prevent them from different types of biotic and abiotic stresses.

Plants produce primary metabolites (carbohydrates, proteins, and fatty acids) and secondary metabolites, which are biosynthesized directly from primary metabolites and certain secondary metabolites, produced only in specific species or genera. Currently, approximately 200,000 secondary metabolites have been isolated (Schwab 2003) and classified into three major groups: phenylpropanoids, isoprenoids, and alkaloids (Frey et al. 1999). Secondary metabolites are involved in defense against biotic and abiotic stresses as well as in adaptation but are not directly involved in plant development and growth (Jaillais and Chory 2010). Secondary metabolites accumulate in specific anatomical structures of the plant, such as laticiferous cells, canals, ducts, or glandular trichomes, or in large amounts in vacuoles in glycosidic form (Santner et al. 2009). Secondary metabolites serve as antioxidants compounds and protective compounds in unfavorable environments such as drought. Jenks and Hasegawa (2007) mention that in tobacco and bean a high concentration of phenylamides and polyamines accumulate under abiotic stress conditions. Drought as an abiotic stress generates a higher accumulation of flavonoids (Alpert 2005) and phenolic acids (Hirt and Shinozaki 2004), anthocyanins, and polyamines (Alpert 2006; Pathak et al. 2014). Therefore, this chapter aims to (a) describe the background of aeroponics, hydroponics, and organoponics, (b) indicate the main medicinal plants that have been identified as candidates for biotic and abiotic stress tolerance, and (c) understand how the incursion of sustainable economic systems are efficient and effective methods to counteract the effects of biotic and abiotic stress in medicinal plants.

13.2 Organoponics System

The organoponics system is an open-space cultivation technique, established on substrates prepared by mixing organic materials with plant layers, which are co-located in containers or beds and installed in empty spaces in densely populated areas, where the soil is unproductive for various reasons (Vega-Ronquillo et al. 2006).

Organoponics is an ancient technique derived from hydroponics, also known as semi-hydroponics or geoponics. This method consists of placing solid substrates that cover the nutritional requirements necessary for plant development. It is practiced with great success in different regions of the world, and nowadays, with the use of worm humus, the technique has been improved. Organoponics can be practiced at all levels, being able to be established at home in gardens, plots, rooftops, and anywhere where there is a small suitable space that can be used, to larger spaces, such as a plot or a farm. This method can also be used to grow medicinal plants and some fruit species, as well as ornamental plants. Unlike hydroponics, this technique is less intensive, which means that it can be slower to obtain the products. However, it is feasible to develop it in small-scale farms at low cost and without the dependence on expensive agrochemicals that are difficult to acquire. The word organoponics refers not only to the use of organic substrates but also to the use of practices compatible with organic or natural agriculture.

The production of organoponic cultivation or cultivation on organic substrates must be adapted to the conditions of each locality, although its principles and cultural practices have general features that make it a form of cultivation practicable in the most diverse conditions of climate and soil. In this sense, this technique is gaining ground and followers at present, for the following reasons: it prevents erosion, improves drainage, and facilitates irrigation and pest and disease control, obtaining products free of toxins (Gómez 2019).

Regarding the specific technique of organoponics, this is selected as the most favorable alternative to the physical conditions and for its ease in the management of substrates, among others. OAS (2012) suggests the idea of favoring agriculture in reduced spaces and using natural substrates.

13.3 Hydroponic System

Hydroponic agriculture consists of growing plants using mineral solutions that are dissolved in water with essential elements or nutrients, either directly in water with mineral elements or in inert agricultural substrates such as washed sand, perlite, vermiculite, sawdust, coconut fiber, and rock wool among many other substrates, in which the roots receive a balanced nutrient solution (Zacchini et al. 2009). Hydroponic system can also be performed in a closed and fully controlled environment. Nowadays, hydroponics is becoming very popular in different countries around the world, where conditions for agriculture are adverse, combining hydroponics with a good greenhouse management and obtaining yields much higher than those obtained in open-air crops, which is why it is so important to opt for this technique.

Hydroponic system allows meeting food needs without thinking about large enterprises, since we can achieve hydroponic crops at home, in the garden or on the rooftop, whether vegetables, flowers, and even shrubs and medicinal plants, allowing to obtain products for a healthy diet and with a good form of therapy as it helps to lower the high levels of stress. In this sense, it is good to remember that a stress situation (lack of irrigation) in a hydroponic crop can have more serious consequences than in a soil crop, due to the different inertias of the cultivation systems. Hence, a good water and nutritional status of the plant would minimize these carbon losses. On the other hand, the reduction of stress factors, caused by inadequate thermal levels, pests, diseases, or weeds, would minimize the alteration of plant structures that will later demand carbon expenditures in maintenance respiration to replace them. Another strategy is the selection of genotypes with low maintenance respiration costs (due to an increase in the efficiency of photoassimilate transport and in the half-life of their proteins). Genotypes with an inverse relationship between growth and respiration have also been detected, i.e., genotypes with a faster growth rate have lower maintenance respiration rates, so most of the energy produced by respiration is used for active growth.

13.4 Aeroponic System

The term aeroponics is derived from the Greek terms *aero* and *ponos*, which, respectively, mean air and work, this being a process for growing plants in an aerial or water mist environment without using the soil. Aeroponics is a modern, advanced, and high-tech type of agriculture, in which plants grow suspended in the air, using the recycling of nutrients from the mist that sprays them, through the irrigation system, which is supplied periodically in the form of mist or spray, thus allowing greater growth and development of crops. The basic principle of aeroponics is that it is a safe and ecological method for the production of healthy plants and crops, which allows greater access to oxygen, and in making plants grow in a closed, semi-closed, or uncovered environment, irrigated with a nutrient-rich solution to the roots that are suspended in the air, which are supported by various conduction or support structures.

13.5 Advantages of Aeroponic System

By using aeroponic systems, the presence of fungal diseases is reduced, which allows crop plants to maintain constant growth and development, with more vigorous and fast-growing roots. On the other hand, crops in the air allow optimizing the benefits unlike traditional horticultural crops on soil or substrates, which can damage production with poor soil quality, inadequate texture, lack of nutrients, or presence of pests and diseases. Another advantage of opting for the aeroponic technique is the supply of water and fertilizers that are generally found in the nutrient solutions of salts and minerals, and also if a plant becomes diseased, it can be removed quickly and easily from its support structure without destabilizing the rest of the plants.

13.6 Current State of Knowledge in Aeroponic System

The aeroponic system is one of the techniques which currently has become very popular in protected agriculture and is implemented very easily according to the large and multiple benefits it brings; one of the benefits is that it achieves higher growth than any other systems on the market, because the air is the best medium that is of greater use, which offers an adequate and balanced nutrient solution that is oxygenated to the roots of the plants and allows them to grow much faster and stronger, more resistant, and of better quality (Sivakumar et al. 2010). As a result of the above, aeroponics is implemented for gardening, vegetables, forage crops, grasses, fruit trees, and medicinal and aromatic plants, resulting in feasibility and profitability for most producers. Due to the rapid growth that this technique provides, the plants manage to reach their optimum size, bloom, and bear fruit before time unlike other methods of cultivation; therefore, with this method it is possible to obtain better clones and yields in the same system instead of the typical transplant. Aeroponics is ideal for advanced studies on plant water stress, because it allows supplying the amount of water and moisture levels at the root. It is considered a good tool to further study root morphology, inhibiting any kind of damage that may be caused, and therefore it is the best system that produces more adequate and normal roots than any other (Weathers et al. 2004).

Plasticulture system with aeroponic techniques in protected agriculture is nowadays of great importance for small and large producers in several countries, because they allow an adequate use and management of natural and environmental resources, as well as fertilizers. This leads to produce in a small environment with large productions if the corresponding and adequate management use, resulting economical for the farmer. To carry out this system, the grower needs to obtain the seedlings in vitro to guarantee the production, yield, and maximum quality of the crop, as well as the necessary use of nutrient solutions, which will be supplied by the appropriate person who frequently monitors the greenhouse.

13.7 Economic Importance of Aeroponic System

This novel form of cultivation of numerous plant species turns out to be very important from a strategic and economic point of view since it allows a considerable reduction in the amount of water and fertilizers required for plant production. These factors turn out to be of utmost importance, since, in large semi-desert areas of the world, surface water reserves reduce and subway aquifers deplete, providing fossil water that has been stored in the aquifers for thousands of years.

In addition, because aeroponics makes it possible to precisely provide the amount of nutrients needed by the plant at each stage of its development, it is possible to achieve a more sustainable production from an environmental and economic point of view. At the present time, there has been a significant increase in the price of essential raw materials in the production of fertilizers, especially natural gas, potash, sulfur, etc. For example, gas tariffs paid by Spanish industry have increased by almost 50% since 2005. On the other hand, phosphate rock, which is mainly purchased from North Africa, costs today 90% above the figure paid for the same rock in 2009. Potash prices on the international market are also rising sharply. It should be borne in mind that both natural gas and rock phosphate account for more than 60% of the manufacturing costs of nitrogen and phosphate fertilizers, respectively. Due to the above, an agricultural production method such as aeroponics becomes more relevant since it allows significant savings in the consumption of synthetic fertilizers of high economic value.

13.8 Aquaponics

Plants in aquaponics are subject to many of the same pests and diseases that affect field crops, although they appear to be less susceptible to attack by soil pests and diseases. Instead, non-chemical methods were used, i.e., biological control, resistant crops, predators, antagonistic organisms, barriers, traps, environmental manipulation, etc. It also appears that plants in aquaponic systems may be more resistant to diseases that affect those in hydroponic systems. This resistance may be due to the presence of organic matter in the water, creating a stable, ecologically balanced growth environment with a high diversity of microorganisms, some of which are antagonistic to pathogens affecting plant roots. Common plants that do well in aquaponic systems include any lettuce, spinach, arugula, basil, mint, watercress, chives, and the most common houseplants, among others.

13.9 Sustainable Economic Systems Against Biotic and Abiotic Stress in Medicinal Plants

Currently, there are different ways of growing medicinal and/or aromatic plants under greenhouse conditions to preserve the freshness and culinary fragrance they possess. To validate this, a research was carried out at the Humboldt University of Berlin, in which several authors worked with four species of the Lamiaceae family: Thai basil (*Ocimum basilicum*), holy basil (*Ocimum sanctum*), perilla (*Perilla frutescens*), and Vietnamese balsam (*Elsholtzia ciliata*). These species are grown in hydroponics as substrate and aeroponic cultures, comparing the quality of each of them. However, the use of the aeroponic system showed the best results, because it guarantees a quick reaction to changes in nutrient solution, high cleanliness, and comfortable handling of the plants. On the other hand, when establishing an aeroponic culture system based on calcined clay, a substrate that allows the feeding of herbivorous insects on *Arabidopsis thaliana* roots, it was found that when infested with *Bradysia* larvae, the growth and yield of that plant were positively affected.

13.10 Tolerance Mechanisms

Tolerance to abiotic and biotic stress is a multigene trait and highly regulated by the effects of loci effects of hundreds of genes that control physiological and morphological responses of plants (Hu and Xion 2013) (Fig. 13.1).

Plants possess different mechanisms to survive under high temperatures, including short- and long-term adaptations, phenological or morphological adaptations, and adjustment mechanisms such as changes in leaf orientation (paraheliotropism), allowing leaf lamina temperature to be maintained closer to the thermal optimum for

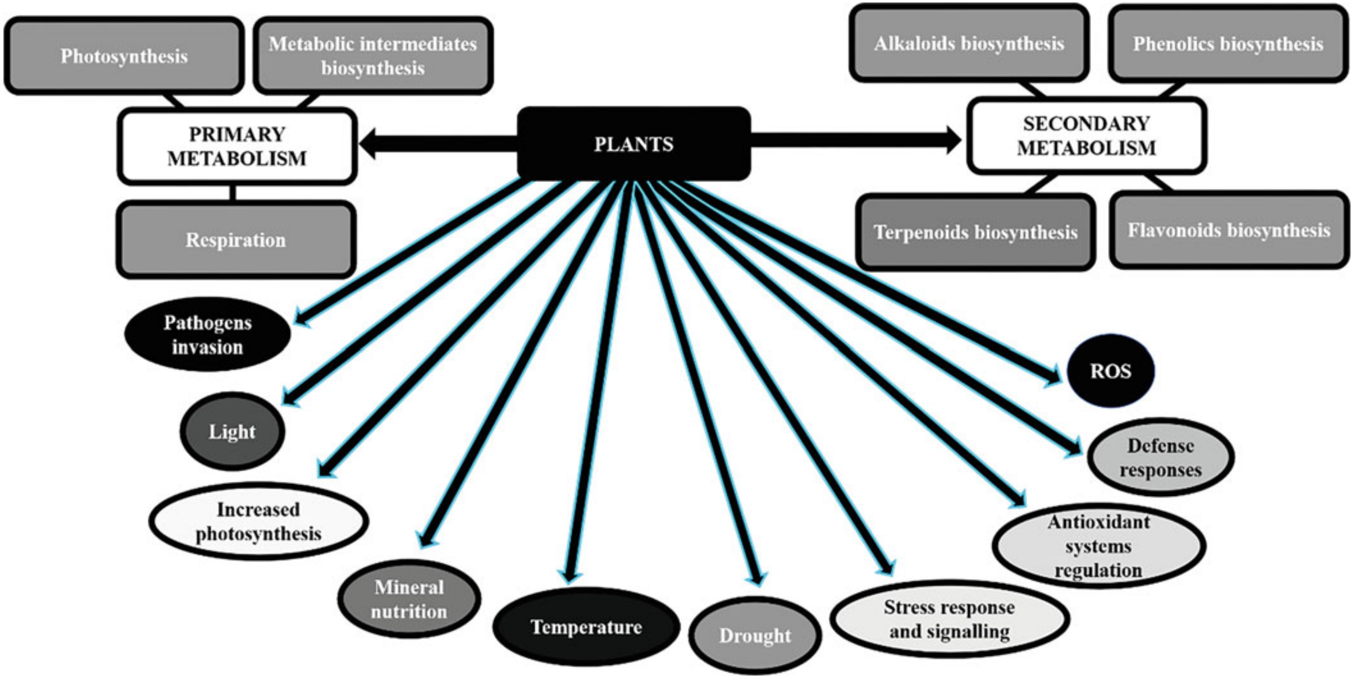

Fig. 13.1 Types of abiotic stress and their interaction with primary and secondary plant metabolism

photosynthesis on hot days, as occurs in beans (Fu and Ehleringer 1989). Other tolerance mechanisms include modifications in membrane lipid composition (Wahid et al. 2007), production of antioxidants, accumulation of compatible osmolytes and pigments, and synthesis of heat shock proteins (HSPs) (Iba 2002; Wang et al. 2004; Almeselmani et al. 2006; Lincoln and Zeiger 2006).

An ideotype of an annual crop tolerant to high temperatures and multiple associated stresses, such as drought, high radiation, and nutritional disorders, must bring together a combination of morphophysiological characters involving canopy, growth habit, phenology, and root-stem ratio, through a flexible and plastic root system that can vary from dimorphic with deep and shallow components (Kell 2011) to specializations for diverse situations such as soilless agriculture and hydroponics.

It has also been observed that one of the main adaptations that allow crops to tolerate high temperatures is the ability to produce and maintain a greater amount of viable pollen. This was demonstrated in tolerant genotypes of tomato and bean, able to maintain anther dehiscence, higher pollen viability, and lower floral abortion, resulting in higher yields in high temperature environments (Porch and Jahn 2001; Firon et al. 2012).

One of the primary effects of temperature stress is an increase in plasma membrane fluidity and electrolyte loss (Wahid et al. 2007; Porch and Hall 2013), so to maintain the integrity of cell membranes, plants must repair and remodel them (Upchurch 2008), through adjustments to the degree of lipid saturation to increase their thermostability (Falcone et al. 2004; Larkindale and Huang 2004; Upchurch 2008; Benning 2009; Su et al. 2009).

> Acquired thermotolerance is another mechanism of tolerance to high temperature stress and refers to the ability of plants and other organisms to acquire tolerance rapidly, within hours even, and survive otherwise lethal temperatures. The acquisition of thermotolerance is a cell-autonomous phenomenon, usually resulting from prior exposure to high but sub-lethal temperatures, which prepare plants to withstand subsequent periods of high temperature stress of a lethal nature (Vierling 1991). Acquired thermotolerance in plants coordinates through signaling by substances such as abscisic acid (ABA), ROS, ethylene, and the expression of HSPs. This process occurs when there is an increase of 5–10 °C above the optimum temperature or above normal temperature conditions for crop growth (Iba 2002; Firon et al. 2012).

Medicinal plants are exposed to various extreme and adverse environmental conditions in the forms of biotic and abiotic stresses. Biotic stress is caused by bacteria, fungi, and viruses. Abiotic stress is a condition associated with various atmospheric changes, such as droughts, frosts, extreme temperatures, increased ultraviolet radiation, and high energy blue light. Recordings of higher ozone levels, the application of fertilizers, as well as the increase of soil and water salinity, together with mineral deficiency (a condition referred to as oxidative stress or heavy metal stress), have led to the study and analysis of other conjunctural aspects (Fig. 13.2).

Negative impacts have been seen on the physiology, biochemistry, morphology, growth, development, and survival of large areas of land around the world. The

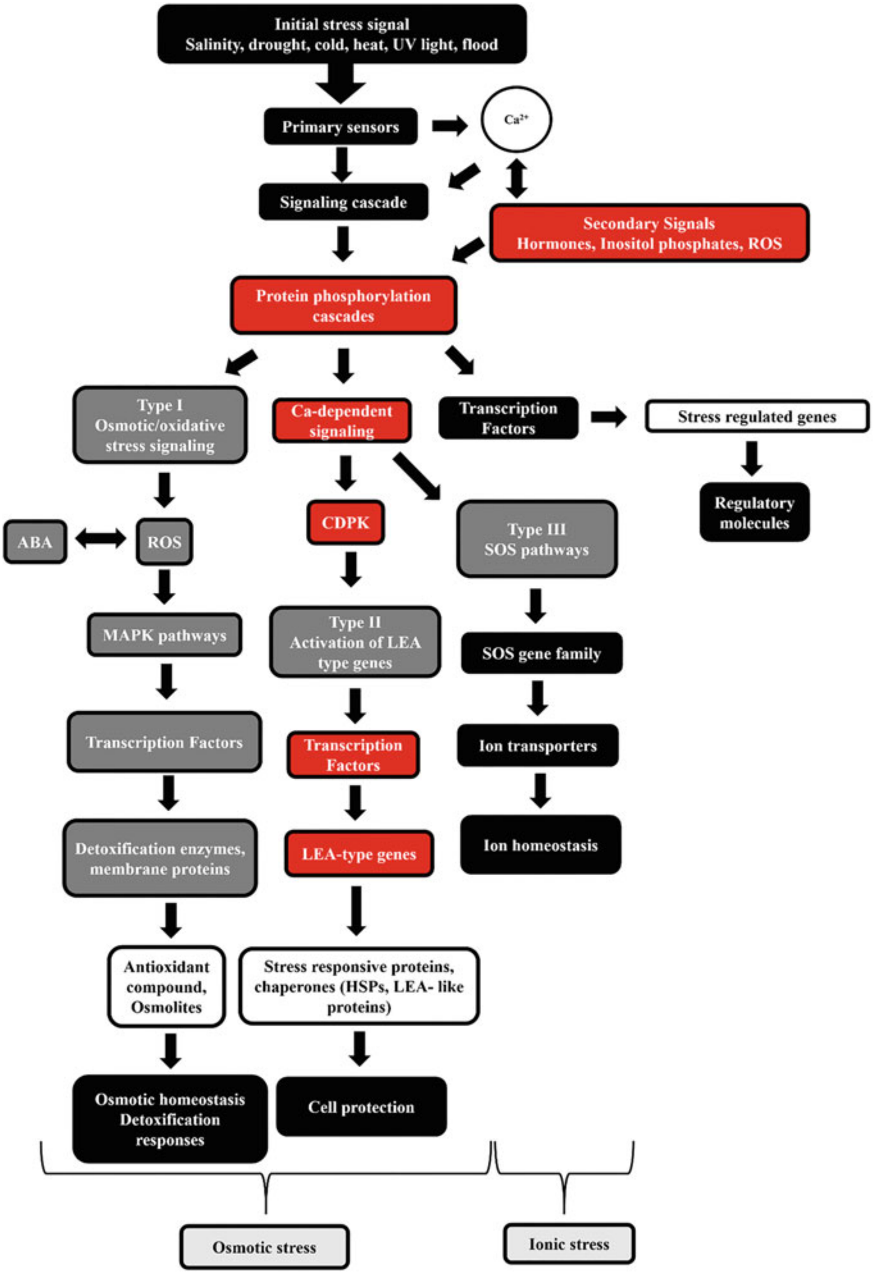

Fig. 13.2 Mechanisms of plant response to initial stress signals

damage caused by these two types of stresses represents a material concern for producers, consumers, and governments.

13.11 Oxidative Stress in Medicinal Plants

Rodriguez et al. evaluated the antioxidant enzyme activity of five edible aromatic medicinal plants: peppermint (*Mentha sativa*), mint (*Mentha piperita*), parsley (*Petroselinum crispum*), basil (*O. basilicum*), and French oregano (*Plectranthus amboinicus* L.).

In common basil, the highest anti-radical activity was found, while common basil showed the highest reducing power, that is, the highest total antioxidant capacity, while oregano had the lowest antioxidant enzyme activity. Based on the values obtained by the ferric reducing antioxidant power (FRAP) method, basil stood out compared to the rest of the plants studied with more than double the antioxidant activity.

Tarchoune et al. (2012) studied the effect of different sodium salts on physiological parameters and antioxidant response in basil (*O. basilicum*) cultivar "Fine" after 15 days of treatment with equimolar concentrations of 25 mM Na_2SO_4 and 50 mM NaCl; no changes were observed in dry biomass, leaf area, and number of leaves.

However, at 30 days of treatment dry matter and leaf area decreased similarly under both treatments compared to the control, while no effect observed by exposure to these salts on leaf number (Tarchoune et al. 2013). Hydrogen peroxide levels under NaCl treatment increased in plants after 15 and 30 days (Tarchoune et al. 2012).

In plants treated with Na_2SO_4, they remained unchanged compared to the control (Tarchoune et al. 2012). These results suggest that under NaCl exposure, antioxidant defenses against H_2O_2 are lower than the production of this ROS (Tarchoune et al. 2012). H_2O_2 produced by apoplastic polyamine oxidase influences salt stress signaling in tobacco (*Nicotiana tabacum*) and plays a role in the balance of tolerant stress response and cell death (Moschou et al. 2008).

The activity of the antioxidant enzyme ascorbate peroxidase (APX) remains constant after 15 days of exposure to Na_2SO_4, while under NaCl exposure it increases twofold. After 30 days, APX activity increases in plants treated with 25 mM Na_2SO_4 and decreases in plants treated with 50 mM NaCl compared to the control (Tarchoune et al. 2013). The rate of CO_2 assimilation in basil (*O. basilicum*) leaves exposed to NaCl decreases, possibly due to a decrease in stomatal conductance constant after 15 days of Na_2SO_4 exposure, while under NaCl exposure it increases twofold. After 30 days, APX activity increases in plants treated with 25 mM Na_2SO_4 and decreases in plants treated with 50 mM NaCl compared to the control (Tarchoune et al. 2013). The rate of CO_2 assimilation in basil (*O. basilicum*) leaves exposed to NaCl decreases, possibly due to a decrease in stomatal conductance (Tarchoune et al. 2013).

Benzarti et al. (2012) evaluated photosynthetic activity and antioxidant response in salt plants (*Atriplex portulacoides*) under extreme salinity. No changes were observed in catalase (CAT) enzyme activity in leaves at 400 mM NaCl concentrations, but it decreases at salinities 800–1000 mM. Superoxide dismutase (SOD) activity increased significantly in response to both treatments, being proportional to the salt concentration (Benzarti et al. 2012). The activity of APX in leaves was higher at 400 mM NaCl than under control conditions (Benzarti et al. 2012). SOD and CAT enzymes are involved in the detoxification of (O_2) and (H_2O_2) and thus prevent the formation of (-OH) (Benzarti et al. 2012). Elouaer and Cherif (2013) conducted a study on the emergence and growth of coriander (*C. sativa*) seedlings under salinity; the results indicate that increasing salinity decreased fresh and dry weight and mineral content.

Mehr and Bahabadi (2013) analyzed the physiological and antioxidant response of coriander (*C. sativum*) under salinity at different levels (0.25 mM, 50 mM, 75 mM, and 100 mM NaCl) (Mehr and Bahabadi 2013). The results suggest that CAT activity in treated plants increases with NaCl concentration compared to the control. The highest CAT activity is observed at 75 and 100 mM NaCl.

13.12 Plant Species Sensitive to Salt Stress

Plant growth can vary within a wide range, depending on the genetic capacity of the species, phenological stage, environmental interactions, and type of ion (Ashraf and Foolad 2007).

For example, among cereals, rice (*O. sativa*) is the most sensitive and barley (*Hordeum vulgare*) is more tolerant (Munns and Tester 2008).

The effect of salinity on growth attributed to the combined effects of water stress, ionic toxicity, disturbance of mineral nutrition, and oxidative stress (Hasegawa et al. 2000; Zhu 2002).

Plants are classified into two groups: halophytes if they can grow in NaCl (3.5 M) soils and in most cases grow satisfactorily in NaCl (0.3 and 1 M). Hydroponic complexes combined with desalination units are developed to use seawater as a source of irrigation water; mainly tomato (*L. esculentum*) is grown, due to its high productive potential (Resh 1992).

The nutrient solution, the mutual relationship between anions and cations, the concentration of nutrients expressed as electrical conductivity, pH, the NO_3-NH_4 ratio, and temperature are a fundamental part of hydroponics; the magnitude and quality of production depend on it (Herrera 1999). These anion and cation ratios should be regulated according to plant demand at the phenological stage of the crop (Herrera 1999). Herb production in hydroponic production models can be an alternative under greenhouse conditions (Herrera 1999). Mexico is the main supplier of green basil (*O. basilicum*), cilantro (*Coriandrum sativum*), and parsley (*Petroselinum sativum*) to the US market (Minero 2004).

13.13 Water Deficit

Water deficit is a limiting factor for plant growth, triggering the accumulation of secondary metabolites. Marchese et al. (2010) evaluated the content of artemisinin (antiparasitic drug) in *Artemisia annua* L., under the effects of biotic and abiotic stress. *A. annua* grown in growth chambers subjected to five water deficit treatments (irrigated, 14, 38, 62, and 86 h without irrigation). Water deficits of 38 and 62 h ($\Psi w = -1.39$ and -2.51 MPa, respectively) increased artemisinin content in leaves, but only 38 h significantly increased the concentration of the secondary metabolite artemisinin in leaves as well as in the plant (29%), without damage to biomass production. These results indicate that artemisinin is part of the chemical defense system of *A. annua* against water deficit.

Barba de la Rosa et al. evaluated the incidence of biotic and abiotic stress on the accumulation of phenolic acids and flavonoids in the leaves of two cultivars of *Amaranthus hypochondriacus* differentiated by leaf color (red and green).

Phenolic compounds were isolated by accelerated solvent extraction (ESA) and analyzed by LC-MS. Rutin was the main flavonoid in amaranth leaves; the highest concentrations were found in the green-leaved species when plants were subjected to stress (9715 $\mu g\ g^{-1}$). Ferulic-type phenolic acid was the minority compound, which was found in red leaves (0.5 $\mu g\ g^{-1}$) and p-coumaric acid only in green leaves (0.7 $\mu g\ g^{-1}$).

Singh et al. (2021) evaluated the effect of various abiotic factors and seasons on *Bacopa monnieri* growth, bacoside A production, and antioxidant and acetylcholinesterase inhibitory activities of the plant extract.

Under different abiotic stresses, bacoside A content in the methanol extracts of the dried aerial parts was determined by a densitometric thin-layer chromatography (TLC) method. The in vitro 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and Ellman's method were used, to evaluate the antioxidant and acetylcholinesterase inhibitory activities of the different extracts, respectively. Plants grown in season 2 (sandy loam soil) had significantly higher bacoside A content and higher antioxidant activity and acetylcholinesterase inhibitory activities than plants grown in season 1 (clay loam soil). Therefore, these authors recommend that the plant should grow from February to May under water stress to improve the production of the marker compound and to obtain significant bioactivities.

13.14 Silicon

Although silicon was not recognized as an essential chemical element in plant growth, it has been observed that several plants accumulate it under different biotic and abiotic stress conditions, and a high accumulation of silica has been found on the surface of the tissues. Silica is effective in the control of several diseases caused by fungi and bacteria in different plants and exerts mitigating effects on various abiotic stresses, such as salt stress, metal toxicity, drought stress, radiation damage, nutrient imbalance, high temperatures, and frost. To obtain plants resistant to multiple stresses, genetic modification of the root capacity to absorb silicon has been proposed.

13.15 Drought

Water availability affects plant growth. The commercial medicinal values of an aromatic medicinal plant are a function of the concentration of secondary metabolites that could be affected by water depletion.

Tatrai et al. (2016) evaluated 2-year-old medicinal thyme (*Thymus citriodorus*) plants, which were subjected to different concentrations of polyethylene glycol (PEG-6000) under greenhouse conditions (0, 2% and 4%) for 15 days. The thyme plants reflected a morphological process of drought avoidance, the fresh weight of shoot radicles reduced, and there was a high water absorption capacity of the roots. Stressed thyme maintained water use efficiency and root-shoot ratio. Tissue dehydration was reduced by stomatal closure and improved root water uptake. The concentration of the volatile secondary metabolite's thyme oil, diisobutyl phthalate, and geraniol increased by drought stress, and pseudothymol was decreased. Thymol increased with drought stress to the extent of 4.4%, while carvacrol accumulated significantly under drought stress (+31.7%) compared to control plants. Soil and water salinity cause reduced plant growth and production (Yamaguchi and Blumwald 2005).

Drought and herbivory are two major types of stresses in terrestrial ecosystems that cause changes in biochemistry, physiology, and whole-plant growth.

Recent studies of plant under water stress (Mundim and Pringle 2018) reported that primary metabolites and secondary metabolites would give insight into the complex metabolic and structural demands necessary for plants to acclimate and maintain function when faced with multiple stresses.

13.16 Salinity

Salinity is a very important factor in soil degradation; it was estimated that, worldwide, about 800 million hectares are under irrigation and 200 million are subject to salt stress.

Salinity in plants directly affects the photosynthesis process due to stomatal closure and carbon dioxide (CO_2) assimilation, which decreases water absorption by roots and sap formation. Toxicity due to sodium (Na^+) accumulation in plant tissues constitutes an additional stress (Munns 2002). Drought and salinity effects often occur simultaneously, which is very common in arid and semi-arid areas (Morales et al. 2004).

Gengmao et al. (2014) evaluated biochemical and physiological responses under five salinity treatments (0, 25, 50, 50, 75, and 100 mM NaCl) in the medicinal plant *Salvia miltiorrhiza*. Salinity treatments <100 mM NaCl did not affect *S. miltiorrhiza* growth in a morphological sense but significantly inhibited dry matter accumulation. Salt stress significantly decreased leaf superoxide dismutase (SOD) activity compared to that of the control. Catalase (CAT) activity in leaves increased with increasing salinity of the hydroponic solution. Protein and soluble sugar contents increased with increasing salinity of hydroponic solution. The results reflected that osmolytes and antioxidant enzymes were involved in the adaptive response to salt stress, thus maintaining better plant growth under saline conditions. Aromatic grasses like palmarosa (*C. martinii*) and lemongrass (*C. flexuosus*) are reported to withstand salinity (Naeem et al. 2013).

13.17 Herbivory

Recent studies on plant-herbivore interactions have shown that shoot metabolic profiles were altered by root herbivory (Erb et al. 2009; Kaplan et al. 2008; Wondafrash et al. 2013), but it remains surprisingly rare to examine whole-plant responses to herbivory under concurrent abiotic stresses. The allocation of metabolic pathways for tolerance and protection of tissues from damage under stress affects plant fitness and competitive ability, as well as plant mediation of multispecific trophic interactions.

13.18 Extreme Temperatures

Global warming influences rising temperatures by reducing precipitation at most sites. The intermittent and simultaneous occurrence of water deficiency and temperature extremes causes heat stress damage to plants. Alhaithloul et al. (2020) evaluated metabolomic, physiological, and biochemical variations in *Mentha piperita* and *Catharanthus roseus* as a function of heat and drought stress (alone and combined). Plants were exposed to drought and/or heat stress (35 °C) for 7 and 14 days. Plant height and fresh and dry weights significantly decreased by the stress. Drought and/or heat stress triggered the accumulation of osmolytes (sugar alcohols, including inositol and mannitol, glycine, betaine, sugars, and proline), with maximum accumulation in response to the combined stress.

Total contents of saponins, phenols, and flavonoids reduced in response to drought and/or heat stress at seven and fourteen days; however, alkaloids, tannins, and terpenoids increased under stress in both plants, with maximum accumulation under combined heat and drought stress.

Mala et al. (2021) evaluated the transcriptome of the endangered medicinal plant *Rheum australe* that has anticancer properties. The species thrives in extreme environmental conditions in habitat. The transcriptome of plants growing in their natural habitat and those growing in growth chambers maintained at 4 °C and 25 °C were studied to understand the genes associated with different temperatures. A total of 39,136 transcripts were obtained. Transcriptome analysis showed 22.4% of genes differentially up-expressed and 22.5% down-expressed in species growing in natural habitat and at 4 °C compared to those at 25 °C. These adaptive advantage genes belong to secondary metabolites, phytohormones, signaling pathways, and transporters and were associated with cell protection.

13.19 Polyamines

Polyamines are nitrogen-containing metabolites; the maximum representatives are spermidine, spermine, and putrescine (Alcázar et al. 2010). Pathak et al. (2014) mention that polyamines contain low molecular weight and act in stabilizing the structure of lipids, membrane proteins, and macromolecules such as DNA and RNA (Liu et al. 2007; Takahashi and Kakehi 2010; Hussain et al. 2011). Polyamines behave as regulatory elements in biochemical, physiological processes and plant development, as well as responses to biotic and abiotic stresses. Liu et al. (2007) mentioned that the increase in polyamine concentration during drought stress is due to de novo biosynthesis or reduced degradation. The alteration of polyamines under stress conditions could be affected by the type of sensitive/tolerant plant, duration, and intensity of stress and developmental stage of tissues. Liu et al. (2004), Dhruve and Vakharia (2013), and Cruz-Ortega et al. (1998) mention that there is an increase in spermine and spermidine concentration for the tolerant cultivar compared to the sensitive cultivars of wheat, groundnut, and tomato, respectively. In these experiments, the sensitive cultivars showed an elevation of putrescine level under stress conditions. Consequently, transgenic plants with increased accumulation of putrescine, spermidine, and spermine enhanced drought stress tolerance (Capell et al. 2004; Bassie et al. 2008).

13.20 Jasmonates

Jasmonates (jasmonic acid and methyl jasmonate) correlate with the accumulation of defense systems, expressing phenolic phytoalexins, alkaloids, coumarins, and terpenoids (van der Fits and Memelink 2000). Saponin (Chan et al. 2010), melatonin (Tan et al. 2007), and serotonin (Anjum et al. 2008) function as secondary metabolites in abiotic stress tolerance through their role as antioxidant compounds. Pathak et al. (2014) mention the key role of abscisic acid, salicylic acid, jasmonic acid, and polyamines in biotic and abiotic responses.

13.21 Conclusions

Understanding the biotic and abiotic factors and their subsequent modification during cultivation/growth of medicinal plants will help to increase the production of valuable secondary metabolites. The increased accumulation of secondary metabolites observed in response to drought and/or heat stress suggests that imposition of abiotic stress may be a strategy to increase the content of therapeutic secondary metabolites associated with these plants. The production of medicinal plants under the organoponic, hydroponic, and aeroponic systems offers a solution to the food problem of people with limited economic resources in rural areas, as they can grow their food under this technique, even when there are problems of drought, flooding, salinity, acidity, or erosion.

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Chapter 14
Influence of Salinity on the Growth, Development, and Primary Metabolism of Medicinal Plants

Tawseef Ahmad Mir, Muatasim Jan, Rakesh Kumar Khare, Sandeep Dhyani, and Neha Saini

Abstract The cultivation of medicinal plants has proven to be financially useful to the pharmaceutical and medical industries. These plants are rich in active ingredients that can be turned into a wide range of medicines, some of which are life-saving. These plant products have high curing values and can naturally flourish in a variety of environments. This property can be used to make them more widely cultivated in less-than-ideal environments. However, since most cultivable lands are mostly used for the production of important food crops, it is difficult to begin large-scale cultivation of these plants. Such non-cultivable lands are often subjected to a variety of abiotic stresses, the most common of which is salinity. This chapter examines how various medicinal plants react to salinity stress in terms of growth and physiology. We emphasize medicinal plants' physiological cycles, as well as their primary and secondary metabolism, during salt stress, in order to facilitate a global cultivation map for medicinal plants in such difficult or aggressive environments.

Keywords Stress · Medicinal plants · Salinity · Growth · Physiology

14.1 Introduction

Salinity stress has long been viewed as a major limiting factor in agricultural productivity across the world. Approximately 20% of the world's irrigated soil has a high salt level, making it harmful to agriculture (Flowers and Yeo 1995). Salinity has a deleterious impact on plant growth by lowering leaf water potential, causing

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Table 14.1 Effect of salinity on the various aspects of different medicinal plant species

Plant species	Salinity effect	References
Ocimum basilicum	Germination delayed	Miceli et al. (2003)
Thymus maroccanus	Germination delayed	Ramin (2005)
Thymus maroccanus	Decrease in seedling development	Said-Al Ahl and Omer (2011)
Matricaria chamomilla	Cell division stops	Said-Al Ahl and Omer (2011)
Citronella java	Reduced number of tillers	Chauhan and Kumar (2014)
Cuminum cyminum	Vegetative phase stops	Hassanzadehdelouei et al. (2013)
Majorana hortensis	Plant growth inhibition	Aziz et al. (2008)
Thymus vulgaris	Plant growth inhibition	Said-Al Ahl and Omer (2011)
Mentha piperita var. officinalis	Reduced leaves	Tabatabaie and Nazari (2007)
Lippia citriodora	Reduced leaves	Tabatabaie and Nazari (2007)
Withania somnifera	Reduced overall growth	Jaleel et al. (2008a)
Salvia officinalis	Reduced overall growth	Ben Taarit et al. (2009)
Ammi majus	Fruit yield reduced	Nabizadeh (2002)
Trachyspermum ammi	Reduced overall yield	Ghavami and Ramin (2008)
Simmondsia chinensis	Ca2+ level decreased	Ali and Hassan (2013)
Teucrium polium	Chlorophyll content decreased	Said-Al Ahl and Omer (2011)
Catharanthus roseus	Reduced protein content	Osman et al. (2007)
Satureja hortensis	Reduction in proline oxidase activity	Muthukumarasamy et al. (2000)
Ricinus communis	Oil output decreased	Banerjee and Roychoudhury (2014)
Foeniculum vulgare	Alteration in carbohydrate balance	Abd El-Wahab (2006)
Mentha pulegium	Phenolic accumulation occurs	Queslati et al. (2010)
Mentha suaveolens	Decrease in essential oils	Aziz et al. (2008)

morphological and physiological alterations, producing reactive oxygen species (ROS), increasing osmotic stress, modifying metabolic processes, and increasing ion toxicity (Khan et al. 2014) (Table 14.1). Plants develop salt stress tolerance as a result of multilayer changes in molecular responses, as well as modifications in the transcriptome, metabolome, and proteome. Since ancient times, India has been renowned as a repository of traditional medical knowledge. The ancient literature of Ayurveda is entirely based on herbal treatment, which is useful in curing complicated problems that current medicine cannot always heal. Plant medications account for up to 25% of total medications in industrialized nations like the United States but up to 80% in developing nations like China and India (Jan et al. 2021a, b). Medicinal plant production and upkeep is thus a lucrative sector that must be safeguarded all around the world. Cultivating these plants in salt-contaminated soils, on the other hand, has resulted in serious anomalies in their general development and physiology (Thornber et al. 1977). In dry and semi-arid regions, where

25 percent of agricultural areas are affected, the impacts of salt stress are particularly noticeable. Shoot ion-independent and ion-dependent responses are the two primary stages of salinity stress-induced plant responses. The first phase, driven by sodium ions, occurs within minutes or days, resulting in the closing of stomata and the inhibition of leaf growth. Salinity has an impact on the plant-water relationship during this period. In the second phase, harmful ions accumulate, especially in older leaves, causing premature senescence, reduced output, and plant mortality. Plants have developed a variety of strategies to cope with the negative consequences of salt stress (Munns 2002). Plants have demonstrated tolerance mechanisms such as tissue tolerance, shoot ion-independent tolerance, and ion exclusion to resist salt stress. One of the most significant physiological processes of salt stress resistance in plants is osmotic adjustment. Water intake, cell turgor, and other physiological activities including cell expansion, photosynthesis, and stomatal opening are all well controlled by osmotic adjustment in salt-resistant plants' leaves and roots (Serraj and Sinclair 2002). Although the link between salt tolerance and osmoregulation is unclear, there is evidence that a small number of plant genotypes demonstrate salt tolerance as osmotic adjustment increases (Chaves et al. 2009). Plants that are resistant to salt stress will thrive by increasing the synthesis of secondary metabolites and avoiding the ion's damaging effects (Chen et al. 2009). Other physiological characteristics that affect salinity tolerance include transpiration use efficiency, transpiration, antioxidant generation, seed germination, harvest index, early seedling development, leaf area, and water status maintenance (Negrao et al. 2017). As a result, understanding the relationship between medicinal plants and salt stress in terms of productivity and sustainability is important.

14.2 Causes of Salinity

14.2.1 *Primary Cause*

The majority of saline soils form as a result of natural geological, hydrological, and pedological processes. Igneous rocks, volcanic rocks, sandstones, alluvial deposits, and lagoonal deposits are among these soils. Evapotranspiration is particularly significant in the pedogenesis of saline soils in arid and semi-arid environments. Other forms of salinity can be found in tide-prone coastal locations, and the major source of salinity is saline water incursion into rivers (Omami et al. 2006).

14.2.2 *Secondary Cause*

Secondary salt-affected soils are those that have been salinized by humans, mostly as a result of incorrect irrigation practices and poor irrigation water quality. Water logging caused by inappropriate irrigation causes anthropic salinization in arid and semi-arid environments (Ponnamperuma 1984). Human activities other than irrigation can also generate secondary salt-affected soils, for example:

(i) As a result of the consequences of salt movement in both the higher and lower layers, deforestation has been identified as a primary source of soil salinization and alkalization.

(ii) Wastewater, airborne salts from industrial pollutants or waterborne salts accumulate in soils.

(iii) Chemical pollution has resulted in salinization. Modern intensive agricultural systems, particularly greenhouses and intensive farming systems, are especially prone to salinization.

(iv) Overgrazing occurs mostly in arid and semi-arid climates, where natural soil cover is sparse and cannot provide the food needs of significant animal husbandry (Omami et al. 2006).

14.3 The Relationship Between Salt Stress and Medicinal Plant Developmental Morphology

14.3.1 Germination

Salt stress inhibits germination by either killing the embryo or significantly lowering the soil potential, causing water intake to be impeded. When *Ocimum basilicum*, *Eruca sativa*, *Petroselinum hortense*, *chamomile*, *sweet marjoram*, and *Thymus maroccanus* seeds were placed in salt polluted soil, germination was delayed (Miceli et al. 2003; Ramin 2005; Ali et al. 2007; Belaqziz et al. 2009). Salinity has two effects on seed germination: (i) there may be enough salt in the medium to reduce the osmotic potential to the point where the intake of water required for nutrient mobilization is slowed or prevented, and (ii) the salt components or ions may be poisonous to the embryo (Jamil et al. 2006). Figure 14.1 shows the germination of plant under different concentrations of salinity treatment.

14.3.2 Seedling Growth

The seedling stage of the plant's life cycle has long been thought to be the most susceptible. Salinity was found to block reserve food mobilization, stop cell division, and enlarge and injure hypocotyls, resulting in a substantial of *Thymus maroccanus*. Basil, chamomile, and marjoram all have similar reports (Said-Al Ahl and Omer 2011). In *Aloe vera* plants exposed to 2, 4, 6, and 8 ds m1 salt, the quantity of foliages, root development, and dry matter reduced. These limits were mostly caused by lower amounts of total soluble solids (TSS). In addition, the salt-stressed plants produced 30 percent fewer sprouts than the control plants (Moghbeli et al. 2012).

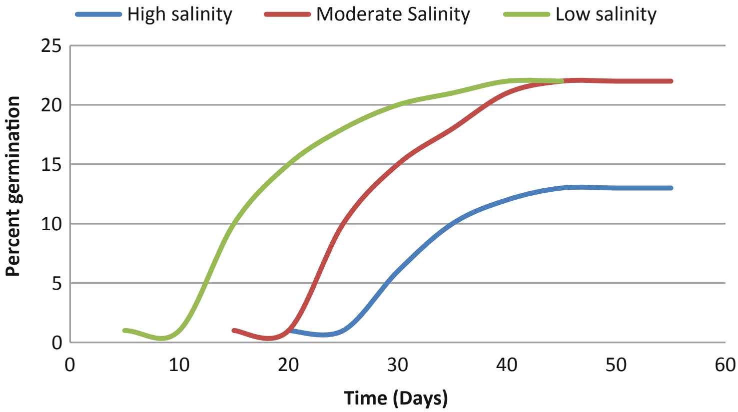

Fig. 14.1 Salinity effects on relationship between percent germination and time after water addition at low, moderate, and high salinity

High saline levels dramatically reduced the number of tillers in *Citronella java* plants, according to Chauhan and Kumar (2014). Cumin's vegetative and reproductive phases were discovered to be the most vulnerable to 5 ds m1 salt stress (Hassanzadehdelouei et al. 2013).

14.3.3 Survival and Developmental Characteristics

In medicinal plants such as *Majorana hortensis*, *peppermint*, *pennyroyal*, *apple mint*, *Aloe vera*, *Matricaria recutita*, *Thymus maroccanus*, *geranium*, *Thymus vulgaris*, *sweet fennel*, *sage*, and *Mentha pulegium*, salinity stress has been identified as a key inhibitor of plant growth (Said-Al Ahl and Omer 2011; Aziz et al. 2008). *Mentha piperita* var. officinalis and *Lippia citriodora* var. verbena subjected to salt stress had considerably less leaves, leaf area, and leaf biomass (Tabatabaie and Nazari 2007). In milk thistle, salt stress of higher than 9 ds m1 decreased plant height, number of leaves per plant, number of capitula per plant, and diameter of the main stem capitulum. Under salt stress, growth metrics of *Withania somnifera*, *Catharanthus roseus*, *Achillea fragrantissima*, *Salvia officinalis*, *thyme*, *Nigella sativa*, *Chamomilla recutita*, and basil were shown to be suppressed (Jaleel et al. 2008a, b; Abd EL-Azim and Ahmed 2009; Ben Taarit et al. 2009; Hussain et al. 2009; Ghanavati and Sengul 2010).

14.3.4 Productivity

Salinity has a significant negative influence on plant production. As previously indicated, the negative impacts of excessive salinity on plants can be seen at the whole-plant level as plant mortality and/or reduced yield (Parida and Das 2005). Increasing salt concentrations reduced the number of umbels, fruit yield/plant, and weight of 1000 seeds in fennel, cumin, and *Ammi majus* (Nabizadeh 2002; Ashraf et al. 2004; Abd El-Wahab 2006). Milk thistle and *Trachyspermum ammi* both showed similar decreases in seed output and yield components per plant (Ashraf and Orooj 2006; Ghavami and Ramin 2008).

14.3.5 Nutrient Uptake

By disturbing the plant system's nutrient absorption balance, salinity directly affects plant development. The availability of nutrients, partitioning, and transportation are all affected. This is due to Na+ and Cl− ions competing with nutritional ions such as K+, Ca2+, and NO3−. The biophysical and/or metabolic components of the plant system are directly affected by ionic imbalances generated by an excess of Na+ and Cl− ions (Banerjee and Roychoudhury 2016). In fennel, *Trachyspermum ammi*, *peppermint*, *lemon verbena*, *Matricaria recutita*, and *Achillea fragrantissima*, increased levels of Na+ and Cl− under salt stress resulted in lower levels of N, P, K+, Ca2+, and Mg2+ (Abd El-Wahab 2006; Queslati et al. 2010; Abd EL-Azim and Ahmed 2009). Different salt concentrations altered the percentages of N, P, and K+ in the leaves of *Simmondsia chinensis*, according to Ali and Hassan (2013). The three elements were significantly reduced when the salt concentration exceeded 17.2 mM. The Ca2+ level in the salt-stressed leaves decreased at a similar rate.

14.4 The Relationship Between Salt Stress and Medicinal Plant Photosynthesis

The photosynthetic mechanism of plants is the principal target of any abiotic stress. The most significant and fundamental physiological mechanism for plant development and survival is photosynthesis. To maintain this mechanism a significant amount of energy is required and is thus prone to stress, which disrupts the metabolic balance within the cell (Roychoudhury et al. 2007; Banerjee and Roychoudhury 2017). In centaury, *Teucrium polium*, *Thymus vulgaris*, *Zataria multiflora*, *Ziziphora clinopodioides*, and *Satureja hortensis*, both chlorophyll a and b, as well as total chlorophyll content, were decreased (Said-Al Ahl and Omer 2011). The reduction in chlorophyll concentration is mostly due to chlorophyll production inhibition along with increased chlorophyll breakdown. Because of the imbalance in chlorophyll metabolism, photosynthesis is hindered, and the plant eventually dies due to a lack of energy equivalents. Salt stress disrupts ion absorption, which has a deleterious impact on the growth of chloroplasts and the protein translation machinery within plastids. In fennel, this is occasionally accompanied with plastid deterioration (Abd EL-Wahab 2006).

14.5 The Relationship Between Salt Stress and Primary Metabolites in Medicinal Plants

14.5.1 Proteins

Degradation of intact proteins has also been used to explain an increase in free amino acid content in plants exposed to salt stress (Roychoudhury et al. 2011). *Catharanthus roseus* showed a reduction in protein concentration when exposed to salt (Osman et al. 2007). In salt-stressed chamomile and sweet marjoram, soluble protein levels dropped. This suggests that salt stress causes protein aggregation within cells, resulting in a decrease in the soluble protein fraction (Ali et al. 2007). In *Achillea fragrantissima* subjected to a salt concentration of 4000 ppm, there was a reduction in crude protein synthesis (Abd EL-Azim and Ahmed 2009). Protein synthesis stimulation has also been linked to the degree of salinization in other studies. This increase in protein accumulation might be to give nitrogen in a form that can be reused by plants during their post-stress recovery period (Roychoudhury and Chakraborty 2013).

14.5.2 Amino Acids

Salt-stressed plants may have higher amounts of some chemicals. Plants exposed to salt stress have been found to accumulate amino acids (alanine, arginine, glycine, serine, leucine, and valine, as well as the amino acid proline and the non-protein amino acids citrulline and ornithine) and amides (such as glutamine and asparagines) (Mansour 2000). Plants treated to salt stress have greater total free amino acids, according to *Catharanthus roseus* and *Matricaria chamomilla* (Osman et al. 2007; Cik et al. 2009). Proline is found in abundance in higher plants, and it accumulates in greater quantities than other amino acids (Abraham et al. 2003). Proline accumulates in the leaves of *Salvia officinalis*, *Trachyspermum ammi*, *spearmint*, *chamomile*, *sweet marjoram*, *Catharanthus roseus*, *Achillea fragrantissima*, *Matricaria chamomilla,* sweet fennel, and *Satureja hortensis* as a reaction to salt stress, according to the literature (Hendawy and Khalid 2005; Ashraf and Orooj 2006; Ali et al. 2007; Osman et al. 2007; Al-Amier and Craker 2007; Zaki et al. 2009; Abd EL-Azim and Ahmed 2009; Cik et al. 2009; Najafi et al. 2010). In saline circumstances, the rise in proline content might be attributed to a reduction in proline oxidase activity (Muthukumarasamy et al. 2000).

14.5.3 Lipids

The amount of fatty acids in a plant's cells is a good predictor of its vulnerability to salt stress. Abiotic stressors have an impact on plant oil and fatty acid production (Banerjee and Roychoudhury 2014). The oil output of *Ricinus communis* roots subjected to salt stress was observed to be decreased. The oil content of the plant shoots, on the other hand, rose (Ali et al. 2008). Salt stress considerably decreased the total fatty acid content of *Coriandrum sativum* leaves. Increased NaCl concentrations also resulted in reduced a-linolenic and linoleic acid levels (Neffati and Marzouk 2008).

14.5.4 Carbohydrates

Nutritional imbalances, hyper-osmotic stress, and impaired photosynthesis are all caused by salinity stress. As noted in fennel, this alters the overall carbohydrate balance (Abd El-Wahab 2006). With increasing concentrations of salt stress, however, a paradoxical rise in carbohydrates was reported in *Salvia officinalis* and *Satureja hortensis* (Hendawy and Khalid 2005; Najafi et al. 2010).

14.6 The Relationship Between Salt Stress and Secondary Metabolites in Medicinal Plants

14.6.1 Stress and Phenolic Compounds

Phenolics have been used as a salt stress sensor. Some phenolic compounds can scavenge the reactive oxygen species (ROS) created by abiotic stressors, and this category comprises of around 9000 chemicals with varied biological activities in plants. Salt stress decreases electron flow in the photosynthetic electron transport system and causes oxidative stress through the generation of reactive oxygen species (ROS) (Das and Roychoudhury 2014). To scavenge the toxic ROS, higher plants create phenolic acids, flavonoids, and proanthocyanidins. Several studies in medicinal plants have revealed that phenolics accumulate during salt stress (Waskiewicz et al. 2012). Salt-stressed spearmint and *Achillea fragrantissima* plants produced more phenolic acid and tannin (Abd EL-Azim and Ahmed 2009). Procatechuic, chlorogenic, and caffeic acids all rose dramatically in *Matricaria chamomilla* plants exposed to salt stress (Cik et al. 2009). In *Nigella sativa* and *Mentha pulegium*, phenolic accumulation was also seen when salt content increased (Bourgou et al. 2010; Queslati et al. 2010). Other phenolic substances including quercetin, apigenin, and trans-cinnamic acid production were apparently boosted in Nigella cultivated in high-salinity soil (Bourgou et al. 2010)

14.6.2 Study of Alkaloids Through Proteomic and Other Approaches

Technological advancements in the field of proteomics have opened up new avenues for ethnobotanical and phytomedical study. The control of secondary metabolism at the protein level is critical for medicinal plant development. In most medicinal plants, the natural production of secondary compounds is minimal. The secondary metabolite, on the other hand, is what defines the plant's medical and commercial value. The complex metabolic architecture of these metabolites has made it difficult to research their characteristics further. Overexpression of rate-limiting enzymes involved in the manufacture of these secondary metabolites has been proposed using cell suspension cultures and metabolic engineering approaches. As a result, a proteomic technique was used to identify the proteins involved in secondary metabolite production (Banerjee et al. 2016). *Catharanthus roseus* is a model system for secondary metabolites in medicinal plants (Verpoorte et al. 1997). Vinblastine and vincristine, two anti-cancer alkaloid medicines, have been claimed to be produced by the plant. The effects of zeatin and 2,4-dichlorophenoxyacetic acid (2,4-D) on protein patterns and alkaloid synthesis in *C. roseus* were investigated using two-dimensional gel electrophoresis. It was discovered that proteins that were lowered by 2,4-D but enhanced by zeatin exposure may play a role in alkaloid biosynthesis control (Jacobs et al. 2000). Differential expression of 88 protein locations was discovered in another proteome investigation of *C. roseus*. Strictosidine synthase, tryptophan synthase, and 12-oxophytodienoate reductase all have two isoforms, according to mass spectrometric analysis. In alkaloid biosynthesis, strictosidine synthase catalyzes the creation of strictosidine (Jacobs et al. 2005). Tryptophan synthase is required for the production of tryptamine, an alkaloid precursor, and 12-oxophytodienoate reductase catalyzes the last step in the synthesis of jasmonic acid, a nontraditional plant growth regulator (JA). JA has long been thought to be a key signaling molecule that controls plant defense during biotic stress reactions and promotes alkaloid accumulation. During salt stress, the content of reserpine in *Rauvolfia tetraphylla* rose. In the roots of salt-stressed *Ricinus communis*, however, the ricinine alkaloids were decreased. *Solanum nigrum* (solasodine) and *Achillea fragrantissima* plants growing in high saline soils showed a significant rise in alkaloid levels (Aghaei and Komatsu 2013). Hung et al. (2010) looked into the impact of *Salvia miltiorrhiza* secondary metabolites on atherosclerotic lesions and cancer. Ginsenosides are significant alkaloids found in *Panax ginseng* roots. To identify the proteins involved in the formation of ginsenoids, researchers looked at the root proteome from a culture of *P. ginseng* hairy roots. The root proteome research revealed the possible activities of 91 of the 159 proteins discovered. During stress reactions, around 20% of the proteins (with specified activities) were identified to control energy metabolism. Enolase, glyceraldehyde-3-phosphate dehydrogenase, and aldolase have all been identified as isotypes. Their relationship with secondary metabolism, however, has remained a mystery (Nam et al. 2005). *Chelidonium majus* milky sap was subjected to two-dimensional gel electrophoresis, which revealed 21 proteins involved in plant defense, nucleic acid binding, and stress signaling (Nawrot et al. 2007). Using two-dimensional electrophoresis of poppy latex, Decker et al. (2000) aimed to discover proteins involved in morphine biosynthesis in the opium poppy (*Papaver somniferum*). The most significant protein found was codeinone reductase, which may play a key role in morphine production during times of stress.

14.6.3 Essential Oils and Salt Stress

The commercial and industrial value of essential oils is enormous. Peppermint (*Mentha piperita*), pennyroyal (*Mentha pulegium*), and apple mint (*Mentha suaveolens*) are the most common mint species that contain them. Salt stress reduced the essential oils of all mint species, according to Aziz et al. (2008). In comparison to the control, the salt-induced drop in essential oil concentration was greater in peppermint and apple mint than in pennyroyal. Monoterpenes are the main component of essential oils found in mint species. With a rise in salt concentration, the proportion of menthone (another ingredient of essential oils) increased. Other compounds, on the other hand, reduced when the plant was subjected to the maximum level of salt stress. In comparison to peppermint and apple mint, the authors discovered that changes in the amounts of components in pennyroyal were far milder under salt stress. *Trachyspermum ammi*, *Thymus maroccanus*, basil, and fennel all had lower essential oil yields when exposed to salt (Ashraf and Orooj 2006). Salinity stress reduced the anethole concentration in fennel, according to Abd El-Wahab (2006). Salt stress had a varied effect on the levels of the major components in marjoram essential oils (Baatour et al. 2010). However, during salt stress, various essential oil molecules such as a-bisabolol oxide B, a-bisabolonoxide A, chamazulene, a-bisabolol oxide A, a-bisabolol, and trans-b-farnesene were observed to be enhanced (Baghalian et al. 2008). In *Origanum vulgare* subjected to salt stress, Said-Al Ahl and Hussein (2010) found significant reductions in essential oil components such as carvacrol, p-cymene, and c-terpinene. Lemon balm, *Majorana hortensis*, *Matricaria chamomile*, *Salvia officinalis*, and basil all had significant salt-induced inhibitory effects (Said-Al Ahl and Omer 2011). During mild salt stress, essential oil compounds such as (E)-2-decenal, (E)-2-dodecenal, and dodecanal initially rose in coriander. The oil level, however, reduced dramatically at greater concentrations (Neffati and Marzouk 2008). Under regulated settings, the major components of essential oils in *Ocimum basilicum* were eugenol and linalool. The level of eugenol was reduced by salt stress, whereas the content of linalool was increased (Said-Al Ahl et al. 2010). Under control circumstances and 25 mM NaCl, the predominant essential oil molecule in *Salvia officinalis* was viridiflorol, but 1,8-cineole increased under 50 and 75 mM NaCl stress. Manool became the dominating component when the plants were subjected to 100 m NaCl stress (Ben Taarit et al. 2010). *Satureja hortensis* sage, thyme, and basil cultivated in salt-contaminated soils showed an increase in the proportion of essential oils (Baher et al. 2002;

Hendawy and Khalid 2005; Ezz El-Din et al. 2009). With increasing stress, essential oil elements such as carvacrol increased and c-terpene reduced dramatically in coriander roots (Neffati and Marzouk 2008). Under salt stress, the proportion of essential oils and oil output (ml per plant) in curly leafed parsley increased. Under stress, foliar scent components such as b-phellandrene, myristicin, b-myrcene, and apiole were also altered (Said-Al Ahl et al. 2016). So far, the differing impacts of salt stress on essential oil synthesis have been studied based on data for several medicinally significant plant species. Increased oil gland density, along with a larger absolute number of gland productions during stress, has been hypothesized as a possible cause of essential oil buildup in several plant species. Net assimilation or the partitioning of assimilates across growth and differentiation processes might be other considerations. Plants' main metabolism may slow down during times of stress, resulting in the buildup of intermediate products that are channeled into secondary metabolites like essential oil. The decreasing of essential oil levels in stressed plants might be related to the general anabolism being impeded by exposure to salty environments (Said-Al Ahl et al. 2016).

14.6.4 Steroid (Cardenolide) Metabolism and Salt Stress

Cardenolide is a steroid derivative found in *Digitalis purpurea* and other plants. This plant's extracts are used to treat heart failure. They are mostly found in the form of glycosides, which are sugar-derived structural groups. Morales et al. (1993) found that in moderate salinity conditions, the cardenolide level in *D. purpurea* leaves and roots was raised. When *D. purpurea* plants were exposed to 100 mM salt stress, their cardenolide level was greater than when they were exposed to 200 mM salt stress or when they were cultivated under control circumstances.

14.7 Conclusion and Future Prospects

Medicinal plants are grown for various plant parts, and their active ingredients are employed in a variety of applications, including pharmaceuticals. They have been regarded promising plants for marginal areas, freshly reclaimed soils, and semi-arid regions due to its high curative value and natural occurrence in varied habitats. Because a large number of literatures report on the sensitivity of medicinal and aromatic plants to salt stress, the high-yielding genotypes of these plants are highly encouraging. On medicinal plants, there is a scarcity of information. The overall goal of future research is to get a better knowledge of medicinal plants' responses to salt stress by evaluating the relative tolerance of different medicinal and aromatic plants, as well as their sensitivity at various plant stages; how varied environmental variables impact salt-stressed medicinal and aromatic plants; how salt-stressed medicinal and aromatic plants are affected by a variety of environmental factors; and the effects of feeding and other therapies on salt-stressed plants' growth, mineral absorption, photosynthesis, and active components. Medicinal plants' salt resistance systems should be alleviated.

Saline lands can be converted to more productive plant lands by a number of farm management practices:

- Drip or micro-jet irrigation: growth of deep-rooted perennial plants or trees are examples of better farm methods.
- Amelioration through fertilization: salinity causes nutritional imbalances in plants, mostly by lowering macro-element concentrations (N, P, K, and Ca). As a result, increasing nutrient concentrations in the root zone with larger fertilizer doses is the most straightforward strategy to restore normal nutrient concentrations inside the plant.
- Leaching: the most successful way for reclaiming saline soils is to leach them to remove soluble salts.
- Usage of salt stress tolerant plants: plant-breeding procedures incorporating the introgression of the genetic background from salt-tolerant wild species into cultivated plants are being used to develop salt-tolerant plant genotypes.

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Chapter 15
Role of Nano-Biotechnology in Medicinal Plant Production

Deepu Pandita

Abstract Nanotechnology tool has the capability and unprecedented boom to provide society with great benefits and manifold applications. Nanoparticulated systems are used as nano-fertilizers, nano-pesticides, nano-herbicides, biosensors, and quality stimulants, substantially contribute to superior plant yield, and produce greater concentrations of treasured secondary metabolites and nutritive significance of crops. Nanoparticles (NPs) on the basis of precise size, composition, concentration, and mode of delivery increased plant growth photosynthesis, biochemical characteristics, and overall plant performance in a range of medicinal plants. Medicinal plants possess healing properties and phytotherapeutic effects due to the presence of specific biologically active secondary metabolites and have been used by mankind since prehistoric times. Secondary metabolites act as principal compounds for designing effective modern-day drugs. NPs act as novel and effective elicitors and induce oxidative stress for enhancing secondary metabolite production in medicinal plants. Nanoparticles can alleviate both the biotic and abiotic stresses and improve morphological, physiological, and other biological parameters in medicinal plants and their production.

Keywords Nano-biotechnology · Medicinal plants · Secondary metabolites · Nanoparticles · Elicitors

15.1 Introduction of Nano-Biotechnology

Nanotechnology discipline deals with atoms or molecules (nanoparticles) in the size range of nanometer scale (1–100 nm) in a single dimension (Yu et al. 2020). Nanoparticles have uniqueness in terms of chemical composition, physical structure, interactions, and magnetic and optical effects. Size provides nanomaterial their unique property. Reduced size of nanomaterial has larger surface area. This leads to high chemical reactivity of nanoparticles (NPs). Nanostructures are more

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promising tools due to astounding catalytic reactivity, optical absorption, electrical conductivity, and mechanical characteristics (Pattan and Kaul 2014; Ali et al. 2021). According to the US National Nanotechnology Initiative (https://www.nano.gov), nanotechnology is "the understanding and control of matter at dimensions of roughly 1 to 100 nanometers, where unique phenomena enable novel applications." The European Commission defined nanotechnology as the "Key Enabling Technology" front-runners for sustainable competitively and development in quite a lot of industrial sectors (ec.europa.eu). Biotechnology is based on use of various technological tools and applications for the comprehensive understanding and development of biological molecules and organisms. The interdisciplinary approach of nano-biotechnology belongs to nanotechnology and uses techniques of both nanotechnology and biotechnology (i.e., nano-biotechnology). The NPs find use in the natural biological systems for the benefit of mankind. NPs offer an efficient mode to accomplish high yield of plant by mitigating the adverse effects of biotic and abiotic stresses (Jampilek and Kralova 2019, 2021). Nanoparticles evade undiscriminating and excessive usage of conservative pesticides and fertilizers in the plants. The majestic scenario of the nano-enabled agriculture which can withstand "farm to fork" chain can be viewed in a chapter written by Pandita (2020). The current chapter has highlighted the advancements in Nano-biotechnology and its applications towards medicinal plant production and applications.

15.2 Nanomaterials

Nanomaterials have dimensions ranging between 1 nm and 100 nm in one of the dimensions (Thakkar et al. 2010; Jeevanandam et al. 2018). NPs have varied characteristics (optical, electronic, and chemical), because of a large surface area besides quantum properties that are accessible on these scales (Khan et al. 2019). These novel characteristics have permitted us to find distinctive uses of NPs. Nanomaterials can be categorized on the basis of number of their dimensions in macro-scale: 0D consists of NPs with all dimensions on nano-scale, 1D includes nano-fibers and nano-wires with only one dimension in macro-scale, 2D includes nano-sheets and thin films, and 3D signifies materials in bulk (Singh 2016). Nanomaterials are categorized into carbon, polymeric, ceramic, and metal compounds on the basis of their chemical nature as well (Khan et al. 2019; Paramo et al. 2020). The nanomaterials of carbon nature comprise structures, for instance, fullerenes, grapheme, and CNTs, i.e., carbon nanotubes (Ealias and Saravanakumar 2017). Ceramics are inorganic solids comprised of ceramic compounds, for instance, titanium dioxide (TiO_2), iron dioxide (FeO_2), and zinc oxide (ZnO) (Thomas et al. 2015; Paramo et al. 2020); and metals contain nanomaterials of gold (Au), copper (Cu), silver (Ag), and nickel (Ni). Organic nanomaterials contain dendrimers obtained from organic nanoparticles symmetrical to the nucleus (Ealias and Saravanakumar 2017; Paramo et al. 2020).

15.3 Interaction of Plants with NPs

Plants can be exposed to the NPs through different methods, for example, direct injection of NPs into plant cells, spraying of NPs on plant leaves or any other plant part, contamination of soil with nanoparticles or irrigation of plants with nanoparticle suspensions, and infection of cellular pollen grains or seeds (Zhu et al. 2008; Corredor et al. 2009; Yin et al. 2012; Tarafdar et al. 2012; Poborilova et al. 2013; Speranza et al. 2013; Paramo et al. 2020). NPs invade naturally into plants through roots or aboveground plant parts. Cellulosic cell wall permits entry of small particles in a moderately easy mode and limits larger NPs. NPs of only 5 and 20 nm dimensions can pass the cell wall (Dietz and Herth 2011). Few NPs induce larger pore formation in cell wall facilitating access to large NPs (Navarro et al. 2008; Kurepa et al. 2010). Higher NP concentrations cause mutilation of the plant cell wall (first barrier) and plasma/cell membrane of plants. This facilitates penetration of NPs inside the cell which then interacts with various cellular processes (Mazumdar and Ahmed 2011; Mirzajani et al. 2013). Once inside the cell wall, NPs transport by process of endocytosis (Etxeberria et al. 2006). In different plant tissues, NPs show symplastic transport (Ma et al. 2010). Inside the cell, NPs affect electron transport chain (ETC) of powerhouse mitochondria and photosynthetic chloroplasts resulting in oxidative burst and increased ROS accumulation which in turn may induce apoptosis or necrosis and cell death (Dimkpa et al. 2013; Faisal et al. 2013; Jiang et al. 2013; Pakrashi et al. 2014; Cvjetko et al. 2017). In contrast, ROS positively has signaling function in a range of cellular processes as well as ecological stress tolerance (Sharma et al. 2012). To fight against stress, plants increase the concentration of antioxidants under the influence of nanoparticles (Rastogi and Pospíšil 2010; Sharma et al. 2012; Faisal et al. 2013; Jiang et al. 2013; Costa and Sharma 2016). This proves antioxidant system regulation in reaction to interaction of NPs with the plant. A range of hormonal pathways show up regulation or downregulation against various types of stresses influencing plant metabolism (O'Brien and Benková 2013). NPs affect concentration of the photosynthetic pigments as well as their activity in plants (Perreault et al. 2014; Tripathi et al. 2017).

15.4 Applications of Nano-Biotechnology

Nanometer-size enables easy penetration of NPs in cell walls, plasma membranes, cytoplasm, and cell organelles of plants and affects the plant metabolism efficiently (Nair et al. 2010). Nanotechnology through NPs has various benefits for plants, e.g., nano-pesticides for crop protection, nano-herbicides, nano-fungicides, nanoparticle-based gene target delivery for crop improvement, nano-fertilizers for crop improvement, and computerized control of nano-sensors for precision farming. Positive interactions of NPs at the cellular levels in plants may result into enhanced photosynthesis, nutrition and stress tolerance and soil nutrition, and seed nano-priming

(Iavicoli et al. 2017). Manifold characteristics of crop yield have been embarked by nanotechnology. The designed nanomaterials improve quality of soil (e.g., nano-fertilizers), increase plant growth (e.g., growth promoters, seed primers, photosynthetic enhancers), and provide protection to plants against disease-causing pathogens (e.g., nano-pesticides), among other applications (Fraceto et al. 2016). Nano-fertilizers stimulate development of crops through absorption of micronutrients for appropriate development of plant. Nano-fertilizers can be synthesized by zinc (Zn), silica (SiO_2), titanium dioxide (TiO_2), copper (Cu) nanoparticles (NPs), and polymeric nanoparticles (NPs) as dendrimers which act as nano-carriers ((López-Valdez et al. 2018; Kah et al. 2018; Vandevoort and Arai 2018). Nano-pesticides protect against abiotic stresses; pesticide encapsulation for controlled discharge improves selectivity and stability of pesticides. This reduces cost of pesticides and increases era of active chemical compounds (Pascoli et al. 2018). Nanomaterials can help in the remediation of contaminated environments (Yarima et al. 2020). These improvements may overcome forthcoming agricultural demands, snowballing quality and quantity (yield) of crops, reducing chemical pollution, or even defending crops against various ecological stresses (Liu and Lal 2015).

15.5 Role of Nano-Biotechnology in Medicinal Plant Production

Nano-biotechnology plays dynamic roles in the improvement of production of secondary metabolites due to their novel and exceptional characteristics (Giraldo et al. 2014). Plants produce different secondary metabolites after exposure to diverse inducer molecules or stress-inducing agents known as the elicitors (Zhao et al. 2005a, b). The elicitors can be categorized as biotic and abiotic elicitors. Several nanoparticles act as elicitors and defend the plant against them, and consequently, the plants produce stress-induced metabolites (Davey 2016). The elicitation in in vitro conditions induces stress to plants and activates plant defense system which then accumulates secondary metabolites in huge amounts. NPs are competent and novel elicitors used in biotechnology of plants for the enhancement of production of secondary metabolites (Fakruddin et al. 2012). Nanoparticles act as potential, novel, and effective nano-elicitors in a number of medicinal plants. *Momordica charantia* on elicitation with AgNPs at 5 mg/L concentration showed enhancement in the concentration of phenolics and flavonoids (Grover and Yadav 2004; Chung et al. 2018a). *Ocimum basilicum* showed elevated regeneration capacity through somatic embryogenesis by use of CuNPs (Ibrahim et al. 2019). Copper sulfate NPs ($CuSO_4$NPs) increased flavonoid content in it (Genady et al. 2017). CuONP elicitation in *Gymnema sylvestre*, *Stevia rebaudiana*, *Withania somnifera*, and *Cichorium intybus* increased TPC and TFC (Chung et al. 2019a; Laishram et al. 2018; Kurek and Krejpcio 2019). Nano-sized ZnO particles enhanced anthocyanin secondary metabolite in *Glycyrrhiza glabra* (Oloumi et al. 2015). Fe_3O_4NPs enhanced flavonoid content in *Dracocephalum kotschyi* which contains rosmarinic acid and methoxylated hydroxyflavones (Moradi et al. 2020; Nourozi et al. 2019). Chitosan-NPs increased phenolics and flavonoids in *Camellia sinensis* (Chandra et al. 2015). Nanoparticles show activity against various plant pathogens. TiO_2 shows antifungal activity against wheat rust (Irshad et al. 2020), CuO acts as a biosensor for the detection of *Aspergillus niger* (Etefagh et al. 2013), ZnO holds fungicidal activity against *Botryosphaeria dothidea*, *Alternaria mali*, and *Diplodia seriata* in apple orchards (Ahmad et al. 2020), CuO has also antifungal activity against *Colletotrichum gloeosporioides* (Oussou-Azo et al. 2020), Zn and Cu increase quantity and quality in the basil plant (Abbasifar et al. 2020), and graphene oxide-Fe_3O_4 is antifungal against *Plasmopara viticola* (Wang et al. 2017). Medicinal plants can also be used as sources of NPs. Ag-NPs (9–35 size) synthesized from leaf extract of basil and *Mangifera indica* improved fresh and dry weight and amount of photosynthetic green chlorophyll pigment and stimulated content of carbohydrates and proteins in wheat seedling at concentrations of 20 and 40 $\mu g\ mL^{-1}$ (Latif et al. 2017). The use of AgNPs produced from extracts of *Moringa oleifera* enhanced shoot and root lengths and leaves at trifoliate stage in wheat plant at a concentration of 100 $\mu g\ mL^{-1}$ (Iqbal et al. 2019). Various medicinal plant-based NPs and their applications are given in Table 15.1.

Spray application of nano-silver at a concentration of 20, 40, and 60 ppm on seeds of *Ocimum basilicum* enhanced height and dry weight of plant and improved yield of seeds (Nejatzadeh-Barandozi et al. 2014). Application of silver nanoparticles at concentrations of 100, 200, 500, 1000, 2000, and 4000 $mg\ L^{-1}$ after soaking of *Ricinus communis* seeds improved enzymatic activity and quantity of parahydroxy benzoic acid (Hojjat and Hojjat 2015). Silver nanoparticles at a concentration of 500, 1000, 1500, 2000, 2500, and 3000 ppm increased plant height, number, length, and weight of fruits in *Cucumis sativus* (Yin et al. 2011) and in *Foeniculum vulgare* at 0, 30, 60, 90, 120, 150, and 180 $mmol\ L^{-1}$ increased percentage of seed germination and improved fresh weight and length of roots (Thangavelu et al. 2018). Nano-silver at 0, 10, 20, 30, and 40 $g\ ml\ L^{-1}$ concentrations in *Trigonella foenum-graecum* enhanced germination of seeds, length of roots, and fresh and dry weight of plant roots (Lee et al. 2012) and in *Thymus kotschyanus* (0, 20, 40, 60, 80, and 100 ppm) improved diameter of canopy area, essential oil, yield, and α-terpinyl acetate amount and declined flowering time (Sharma et al. 2012). Silver NPs (20, 40, 60 ppm) increased number of seeds and inflorescences in *Carthamus tinctorius* (Zari et al. 2015) and root numbers, root lengths, and dry weight of leaves in *Crocus sativus* (0, 40, 80 or 120 ppm) (Mukhopadhyay 2014). CNTs, MWCNTs, and fullerols Ag NPs affected seed yield in *Ocimum basilicum*, growth and yield in wheat, and yield of fruits in bitter melon (Nejatzadeh-Barandozi et al. 2014; Kole et al. 2013; Razzaq et al. 2016). Au NPs at 10 ppm affected growth and seed yield in Indian mustard (Arora et al. 2012). Au NPs exhibited positive influence on germination of seeds (39.67%), biomass (by 2.40-fold), and fresh weight (by 5.18-fold) in *Gloriosa superba* (Gopinath et al. 2014). Cerium oxide nanoparticles (CeO_2 NPs) at 125 mg/kg concentration revealed significant rise in length of plant roots and shoots, biomass, and activities of shoot catalase and root ascorbate peroxidase in

Table 15.1 Medicinal plant-based NPs and their applications

Plant species	Parts used in extract	NPs	Characteristics of NPs		Biological applications of nanoparticles (NPs)	Reference(s)
			Shape	Size in nm		
Citrus × latifolia		Ag	Spherical	4 to 24	Destroys bacteria such as *Bacillus cereus*, *Bacillus subtilis*, and *Escherichia coli*	Linh et al. (2018)
Fortunella japonica (Thunb.) Swingle/ *Citrus japonica* Thunb.			Face centered cubic structure, almost spherical	11.6		Anh et al. (2019)
Combretum erythrophyllum (Burch.) Sond.	Leaves		Spherical	13.62	Destroys microbes like *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, *Proteus vulgaris*	Jemilugba et al. (2019)
Terminalia arjuna (Roxb.) Wight & Arn.	Leaves		Well dispersed	15–30	Antibacterial potential against *Staphylococcus aureus* (NCIM5021), *Pseudomonas aeruginosa* (NCIM5029), and *Salmonella typhimurium*	Dudhane et al. (2019)
Agrimonia pilosa Ledeb.	Aerial parts	Au	Hexagonal	20–25	Toxic to cancerous cell lines of gastric adenocarcinoma. Antioxidant and antifungal effect on oomycetes SR1 and BP1120	Patil et al. (2019)
Pongamia pinnata (L.) Panigrahi	Leaves		Small and unique	10–25	Antioxidant and antibacterial	Sukumar et al. (2020)
Citrus medica L.		Cu	Crystalline in nature	20	Antibacterial potential toward *Escherichia coli*, *Pseudomonas aeruginosa*, *Propionibacterium acnes*, *Klebsiella pneumoniae*, and *Salmonella typhi*. Antifungal properties against *Fusarium culmorum*, *F. oxysporum*, and *F. graminearum*	Shende et al. (2015)
Syzygium cumini L.	Leaves		Monoclinic and crystalline		Antimicrobial potential against *Bacillus* subtilis and *E. coli*	Aher et al. (2018)
Moringa oleifera Lam.			Spherical, solitary crystalline and face centered cubic phase	9.69	Toxic effect against HT-29 cancerous cells. Antibacterial against both the gram-positive and gram-negative bacterial strains	Ezhilarasi et al. (2016)
	Leaves	Ni		43.87–48.76	95% photocatalytic effect against CV dye	

Camellia sinensis (L.) Kuntze			Spherical and uniformly distributed			Rajan et al. (2015)
Rhamnus virgata Roxb.	Leaves		Spherical	25	Cytotoxic, antioxidant, and antibacterial against *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Bacillus subtilis*	Shankar et al. (2003)
Plantago major L.	Leaves	Fe	Spherical	4.6–30.6	83.33% decolorization of methyl orange	Dwivedi and Gopal (2010)
Eucalyptus robusta Sm.	Leaves		Spherical	0.8	Antimicrobial potential against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*	Li et al. (2014)
Allium saralicum R. M. Fritsch			Spherical	40–45	Wound healing properties, antioxidant, antifungal, and antibacterial	Rico et al. (2015)
Trianthema portulacastrum L.			Crystalline	25–90	Effective against *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger*, *A. flavus*, *A. fumigatus*. 91% photocatalytic toward Synozol Navy Blue-KBF dye	Hou et al. (2018)
Passiflora caerulea L.	Leaves	Zn	Spherical	70	Antibacterial potential against urinary tract infection (UTI)	Latowski et al. (2010)
Aloe socotrina	Leaves		Crystalline	15–50		Anjum et al. (2012a)
Allium eriophyllum Boiss.			Crystalline, uniform, spherical	22	Antibacterial, cytotoxic, antifungal, wound-curing potential	Anjum et al. (2012b)
Aloe barbadensis Mill.	Leaves	Ti	Spherical	20	Antibacterial against *Pseudomonas aeruginosa* PAO1	Noshad et al. (2019)
Nigella sativa L.	Seeds		Spherical	1–6	Antibacterial and anti-cancerous	Gunjan et al. (2014)
Azadirachta indica A. Juss.	Leaves	Se	Spherical, after 5 and 10 minutes of reduction time	153,278	Effective against positive and negative strains of bacteria	Mazumder et al. (2020)

Coriandrum sativum (Morales et al. 2013). Fullerol [C60 (OH) 20] upsurged levels of cucurbitacin-B (74%) and lycopene (82%) and antidiabetic compounds, including charantin (20%) and insulin (91%) in *Momordica charantia* (Kole et al. 2013). Nano-silver particles (Ag NPs) act as prospective elicitors and increased content of sesquiterpene lactone known as artemisinin in hairy root cultures of *Artemisia annua* (Zhang et al. 2013). *Aloe vera* nano-Ag and nano-TiO_2 effectively enhanced aloin production at 48 hours in cell suspension cultures of *Aloe vera* plant (Raei et al. 2014). Ag NPs increase overall phenols in *Bacopa monnieri* (Krishnaraj et al. 2012). An application of Fe and Zn nano-oxides induces production of hypericin and hyperforin in *Hypericum perforatum* (Sharafi et al. 2013). Table 15.2 shows numerous research investigations on the applications of NPs in medicinal plant production. NPs can have some negative effects as well in plants. TiO_2 increased root growth in *Arabidopsis thaliana* but declined total biomass and chlorophyll content at higher concentrations. Higher concentrations also cause lipid peroxidation, reduced growth, damage in germination of seeds, uptake of the minerals, ultrastructure of the cellular and subcellular organelles, oxidative stress and antioxidant response, and photosynthesis inhibition, hinder photosynthetic pathways by blockage of electron transport chain (ETC) and DNA damage, and change biosynthetic genes causing change in content of vitamin E (Szymańska et al. 2016; Kataria et al. 2019; Rajput et al. 2020; Paramo et al. 2020). The positive and negative consequences of NPs on the various medicinal plants are summarized in Fig. 15.1.

15.6 Conclusion

Nano-biotechnology plays a dynamic role to boost the production of secondary metabolites owing to their novel and exceptional characteristics. Plants produce secondary metabolites on exposure to diverse inducer molecules or elicitors. NPs trigger interference to electron transport chain (ETC) of chloroplasts and mitochondria inside the cell and generate ROS which will trigger apoptosis or necrosis and cell death. The complex networks of phytohormones up- or downregulate during stress. The medicinal plant-derived nanoparticles (NPs) possess various biological applications such as antimicrobial, antibacterial, cytotoxic, wound-healing potential, etc. NPs can give a new face to medicinal plant production in both positive and negative ways such as reactive oxygen species (ROS) production, damage to micronuclei and DNA with an upsurge in concentration, plant growth, enhancement in chlorophyll content and leaf area, upregulation of phenylalanine, metabolomics pathways related to defense and secondary metabolism, etc.

Table 15.2 NPs and their applications in medicinal plant production

Medicinal plant species	Nanoparticles (NPs)	Effects produced in medicinal plants	Reference(s)
Allium cepa L.	Root hairs of *Allium cepa* show interaction with Au NPs of various sizes (15 nm, 30 nm, and 40 nm)	Reactive oxygen species (ROS) production which enhances lipid peroxidation and chromosomal aberrations	Rajeshwari et al. (2016)
	Aluminum oxide	Increased concentration causes damage to DNA and micronuclei	De et al. (2016)
	Ag and Au (at 5.4 ppm)	Au improves seed germination, height of plant, length and diameter of leaves; Ag improved seed germination	Acharya et al. (2019)
	MWCNTs (0 $mg{\cdot}L^{-1}$ and 500 $mg{\cdot}L^{-1}$)	Enhanced height of plant, chlorophyll rate, and surface area of leaves	Abdul-Ameer and Almousawy (2019)
	ZnO/MWCNTs	Enhanced seedling growth	Kumar et al. (2018)
Arabidopsis thaliana (L.) Heynh.	TiO_2 (100–1000 $mg{\cdot}L^{-1}$)	Chlorophyll amount reduces modifications in biomass and antioxidant enzymes of plant	Szymańska et al. (2016)
	Carbon nano-horns (0, 0.01, 0.05, 0.1, 0.3, 0.5, 1, 5, 10, 50, and 100 $mg{\cdot}L^{-1}$)	Changed content of sugars and amino acids at 0.1 $mg{\cdot}L^{-1}$. Increase in nicotinamide, flavones, and purines	Sun et al. (2020) and Paramo et al. (2020)
Artemisia annua L.	Cobalt NPs of 10 nm diameter (5 $mg{\cdot}L^{-1}$)	Enhances content of artemisinin (medicinal compound)	Ghassemi et al. (2015)
	Ag–SiO_2	Increase in content of artemisinin and catalase (CAT) enzyme activity	Zhang et al. (2013)
Beta vulgaris subsp. vulgaris	TiO_2, ZnO $\leq$ 40 nm size (0.25 $ml{\cdot}L^{-1}$, 0.50 $ml{\cdot}L^{-1}$)	NPs increased chlorophyll amount, growth of beetroot plant, and terpene content	Siddiqui et al. (2019)
Brassica juncea (L.) Czern	Au	Increase in height of *Brassica* plant, diameter of stem, and yield and decrease in sugar content after foliar spray	Arora et al. (2012)
Brassica oleracea, *Solanum lycopersicum* L., *Lactuca sativa* L., *Amaranthus dubius* Mart. Ex. Thell	Graphene (500 mg L^{-1}–2000 mg L^{-1})	Inhibition of growth and biomass of plants, dose-dependent decrease in leaf number. Increase in reactive oxygen species and damage of cell; no notable toxicity in lettuce	Begum et al. (2011)

(continued)

Table 15.2 (continued)

Medicinal plant species	Nanoparticles (NPs)	Effects produced in medicinal plants	Reference(s)
Brassica rapa L.	CuO	Decrease in content of chlorophyll, carotenoid, and sugars; increase in content of proline and anthocyanin by CuO, production of ROS, malondialdehyde (MDA), glucosinolate, phenolic and flavonoid, and hydrogen peroxide	Chung et al. (2018b, 2019b) and Paramo et al. (2020)
Brassica rapa spp. pekinensis	CuO	Increase in glucosinolates and phenolic compounds	Chung et al. (2018b)
Calendula officinalis L.	AgNPs in blend with methyl jasmonate	Saponin content increased by 177%	Ghanati and Bakhtiarian (2014)
Capsicum annuum L.	ZnO	Increased germination of seeds, seed vigor up to 123.50%, 129.40%, and 94.17% (at 100 ppm, 200 ppm, and 500 ppm), phenolics and antioxidant activity	Garciá-López et al. (2018)
Capsicum annuum L., *Solanum melongena* L.	Graphene nanosheets (0.1 $g{\cdot}L^{-1}$, 0.2 $g{\cdot}L^{-1}$, and 0.3 $g{\cdot}L^{-1}$)	Increased yield and growth, sugars, and H_2O_2. Cell membrane not damaged; nanosheets located in chloroplast	Younes et al. (2019)
Catharanthus roseus (L.) G. Don	MWCNTs (0 $mg{\cdot}L^{-1}$, 50 $mg{\cdot}L^{-1}$, 100 $mg{\cdot}L^{-1}$, and 150 $mg{\cdot}L^{-1}$)	Increased growth, plant biomass, length of roots, chlorophyll, carotenoids, proteins, CAT, and POX enzymes	Ghasempour et al. (2019)
Chrysanthemum L., *Gerbera* L., *Streptocarpus* Lindl.	Ag and Au	Ag prevents rhizogenesis in *Chrysanthemum* and *Gerbera*. Gold NPs increased regeneration of roots in *Gerbera*. Ag and Au increased micro-propagation in cape primrose	Tymoszuk and Miler (2019) and Paramo et al. (2020)
Cicer arietinum L.	Molybdenum from small to huge concentrations ($1 \times 10^{-5} - 2\ mg{\cdot}L^{-1}$)	Declined plant growth at high concentrations. Iron (Fe) deficiency in young plant leaves. Declined number of leaves, flowers, and pods	Nautiyal and Chatterjee (2004)
	Water-soluble carbon nanotubes (wsCNTs)	Enhances growth rate of roots, shoots and branching.	Tripathi et al. (2011)

(continued)

Table 15.2 (continued)

Medicinal plant species	Nanoparticles (NPs)	Effects produced in medicinal plants	Reference(s)
	TiO_2 NPs at concentrations 0.5, 1.5, 3, 4, and 5 mg·L^{-1}	Increases contents of the phenolics and flavonoids	Mohammed AL-oubaidi and Kasid (2015)
Citrullus lanatus (Thunb.) Matsum. & Nakai	CuO (500 mg·L^{-1}–1000 mg·L^{-1})	Increase in biomass and fruit production	Elmer et al. (2018)
Citrus maxima Merr.	α-Fe_2O_3	α-Fe_2O_3 accumulates in roots. Decreased chlorophyll content	Hu et al. (2017)
Coriandrum sativum L.	Ni	Decreased water content, photosynthetic pigments, elongation of roots and shoots, antioxidant activity	Miri et al. (2017) and Paramo et al. (2020)
Cucumis melo L.	γ-Fe_3O_3; Fe_3O_4	Increased chlorophyll content and fruit weight at 200 mg·L^{-1} concentrations	Wang et al. (2019b)
Cucumis sativus L.	Foliar application of Ag of 20 nm size (4 and 40 mg/plant)	Antioxidant defense system activation, upregulation of phenolics, and changed membrane characteristics	Zhang et al. (2018) and Paramo et al. (2020)
Cucurbita maxima L.	Fe_3O_4 (100 mg L^{-1})	Presence in phloem sap revealed NP translocation; reduced levels of oil-related metabolites, such as 4-tetradecyl ester eicosane, methoxyacetic acid, and heneicosane	Tombuloglu et al. (2020) and Paramo et al. (2020)
Cucurbita pepo L.	MWCNTs	Reduced percentage of germination of seeds, shoot length, and biomass. Increase in oxidative damage	Hatami (2017)
Daucus carota L.	Ag	Reduced percentage of seed germination, growth, and protein content of seeds. Increased chlorophyll and H_2O_2 content	Park and Ahn (2016)
	Multi-walled CNTs	No alteration in germination. Seed protein and H_2O_2 content decreased, whereas chlorophyll content increased	Park et al. (2016)
Dracocephalum L.	TiO_2	Improved shoot length and quantity of essential oils	Mohammadi et al. (2016)

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Table 15.2 (continued)

Medicinal plant species	Nanoparticles (NPs)	Effects produced in medicinal plants	Reference(s)
Duckweed	CuO	Altered rate of plant growth and photosynthetic amount	Lalau et al. (2014)
Glycine max L.	Fe_2O_3, fulvic acid-coated $FeeO_3$, Fe-EDTA	Stress-related and plant growth disorders absent, improved content of chlorophyll, plant biomass, and development of roots	Yang et al. (2020a)
Hordeum vulgare L.	Cadmium oxide (CdO) (7–60 nm)	Altered primary metabolite content, saccharides, and amino acids (tryptophan and phenylalanine)	Večeřová et al. (2016)
Hyoscyamus niger L.	Bulk and nano-TiO_2	Increased superoxide dismutase (SOD) by both increased hyoscyamine and scopolamine amount in nano-TiO_2.	Ghorbanpour et al. (2015) and Paramo et al. (2020)
Hyoscyamus reticulatus L.	Fe_3O_4 (0 mg·L^{-1}, 450 mg·L^{-1}, 900 mg·L^{-1}, 1800 mg·L^{-1}, 3600 mg·L^{-1})	Increased antioxidant enzymatic activities, hyoscyamine and scopolamine	Moharrami et al. (2017) and Paramo et al. (2020)
Hypericum perforatum L.	ZnO, Fe_3O_4 (0 ppb, 50 ppb, 100 ppb, 150 ppb)	Higher hypericin and hyperforin production	Sharafi et al. (2013)
Ipomoea batatas L.	ZnO, CuO, and CeO_2	Yield of plants influenced at higher ZnO, CuO, and CeO_2 concentrations	Bradfield et al. (2017) and Paramo et al. (2020)
Lactuca sativa L.	Ag	No phytotoxic effect on foliar applications	Larue et al. (2014)
	Carbon dots	Increased yield and growth rate and decreased nitrate content	Zheng et al. (2017)
	CuO	Germination of seeds and root radicle growth stopped; S-nitrosothiols levels in radicles exhibited direct dose-dependent response	Pelegrino et al. (2020) and Paramo et al. (2020)
	Al_2O_3 (0.4, 1, and 2 mg·L^{-1})	Root absorption increased acquisition of macronutrients. Adsorption and aggregation of Al_2O_3 restricted translocation to roots	Hayes et al. (2020) and Paramo et al. (2020)
	Graphene oxide	Improved length, number of hair roots. Foliar application increased superiority of proteins, lettuce, sugars, and vitamin C (at 30 mg·L^{-1})	Gao et al. (2020) and Paramo et al. (2020)

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Table 15.2 (continued)

Medicinal plant species	Nanoparticles (NPs)	Effects produced in medicinal plants	Reference(s)
Lactuca sativa L. var. longifolia	CeO_2 (1000 and 2000 $mg \cdot kg^{-1}$)	Lesser content of photosynthetic chlorophyll and biomass	Zhang et al. (2017)
Lepidium draba L.	Fe_3O_4, CuO	Higher CAT and POD enzyme activities and concentration of sulforaphane	Riahi-Madvar et al. (2016)
Malus domestica Borkh.	GO	Inhibition of lateral roots, increased activity of CAT, POD, and SOD enzymes, auxin efflux carrier and auxin influx gene transcription at 0.1 $mg \cdot L^{-1}$	Li et al. (2018a) and Paramo et al. (2020)
Medicago sativa L., *Lactuca sativa* L.	Cu O (5, 10, and 20 $mg \cdot L^{-1}$)	Decline in length of roots and shoots, change in enzyme activity	Hong et al. (2015) and Paramo et al. (2020)
Mentha piperita L.	TiO_2 (150 $mg \cdot L^{-1}$)	Menthol contents increased by 105.1%	Ahmad et al. (2018)
Momordica charantia L.	Fullerol C60 (OH) 20	Increased biomass of plant and yield of fruits and content of charantin and insulin	Kole et al. (2013)
Nigella arvensis L.	Al_2O_4, NiO	Biomass of plant enhanced at 50 $mg \cdot L^{-1}$ and 100 $mg \cdot L^{-1}$ of Al_2O_3 and 50 mg L^{-1} of NiO. Biomass reduced at high NP concentration; increased antioxidant capacity, total saponins and total phenolics at 100–2500 $mg \cdot L^{-1}$ of Al_2O_3	Chahardoli et al. (2020) and Paramo et al. (2020)
Ocimum basilicum L.	Multi-walled CNTs	Induction of plant growth and content of the essential oil increased. Higher dosage (100 $mg \cdot L^{-1}$) causes toxic effects in plant	Gohari et al. (2020) and Paramo et al. (2020)
Oryza sativa L.	Zinc (1.5–8.5 mM)	Inhibits uptake of Fe, Cu, Mn, Ca, Mg	Shri and Pillay (2017)
	Ag	Increase in quantity of green photosynthetic chlorophyll a and carotenoids, activities of CAT, APX, and GR enzymes	Gupta et al. (2018) and Paramo et al. (2020)
	GO	Upregulation of phenylalanine, secondary metabolic pathways, and aquaporin inhibition	Zhou and Hu (2017) and Paramo et al. (2020)

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Table 15.2 (continued)

Medicinal plant species	Nanoparticles (NPs)	Effects produced in medicinal plants	Reference(s)
	Mesoporous carbon	Decrease in length of roots and shoots and increased phytohormones	Hao et al. (2019)
	CuO	Suppression in growth rate of seedlings; decreased quantity of green chlorophyll and carotenoid in plant leaves	Yang et al. (2020b)
	TiO_2	Improved biomass (>30%). Decreased rates of photosynthesis at 10 $mg \cdot L^{-1}$. Downregulation of energy consumption in metabolism	Zhang et al. (2020) and Paramo et al. (2020)
	Manganese (3 and 6 mM)	Growth inhibition, increased superoxide anion leads to oxidative stress	Srivastava and Dubey (2011)
Oryza sativa L., *Lolium* L., *Raphanus raphanistrum* subsp. sativus, *Cucurbita* L.	Au	Positively charged Au is absorbed by roots, whereas negatively charged Au is translocated into shoots	Zhu et al. (2012)
Phaseolus radiatus *Triticum aestivum* L.	CuNPs	Reduced growth rate in both species	Lee et al. (2008)
Phaseolus radiatus *Sorghum bicolor* L.	Ag	Seedling growth affected, growth rate not affected in soil media	Lee et al. (2012)
Phaseolus vulgaris L.	CeO_2 (500 $mg \cdot L^{-1}$)	Root exposure with NP suspensions increased soluble protein content by 204%	Majumdar et al. (2014)
	CeO_2 (25, 50, and 100 $mg \cdot L^{-1}$)	Accumulation into roots and translocation to aboveground plant parts, tissue-specific metabolic reprogramming	Salehi et al. (2020)
Pisum sativum L.	Cd, Co, and Pb at high concentrations	Catastrophe of germination and growth of seedlings	Majeed et al. (2019)
Raphanus sativus L.	TiO_2 (10 to 1500 $mg \cdot L^{-1}$)	Increased photosynthesis and phenols. Higher TiO_2 concentrations subscribe to rapid water-use efficiency	Tighe-Neira et al. (2020)

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Table 15.2 (continued)

Medicinal plant species	Nanoparticles (NPs)	Effects produced in medicinal plants	Reference(s)
Salvia officinalis L.	Titanium dioxide TiO_2	Content of phenolics and flavonoids was highest at 200 mg·L^{-1} and 100 mg·L^{-1}	Ghorbanpour (2015) and Paramo et al. (2020)
	TiO_2	Improved antioxidant activity. Maximum concentrations of phenols and flavonoids achieved at 200 and 100 mg·L^{-1}	Ghorbanpour (2015) and Paramo et al. (2020)
Salvia verticillata L.	Multi-walled CNTs	Increased oxidative stress in leaves and rosmarinic acid. Decreased photosynthetic pigments	Rahmani et al. (2020)
Satureja khuzestanica	MWCNTs	Increased flavonoid and phenols in callus culture	Ghorbanpour and Hadian (2015) and Paramo et al. (2020)
Silybum marianum L. Gaertn.	Monometallic and metallic alloys (Ag, Au, Cu, Au-Cu, Ag-Cu, and Ag-Au)	Increased frequency of seed germination, development of the shoots and roots. Ag-Cu and Ag-Au alloys increased phenolics. Others increased flavonoid content as well	Salman Khan et al. (2016) and Paramo et al. (2020)
Sinapis alba L.	CNTs	Seed germination energy and seed viability repressed at all concentrations but not at 0.01 g·t^{-1}	Polischchuk et al. (2020) and Paramo et al. (2020)
Solanum lycopersicum L.	CeO_2 (250–500 mg·kg^{-1})	Increased length of shoots and green chlorophyll amount	Barrios et al. (2016) and Paramo et al. (2020)
	$CoFe_2O_4$	No effect on seed germination but length of roots increased	López-Moreno et al. (2016)
	Multi-walled CNT-carboxylic acid functionalized single-wall CNTs (SWNT)	CNTs showed no response on growth and height of plant. SWNT increased content of salicylic acid	Jordan et al. (2020) and Paramo et al. (2020)
	Uncoated cerium oxide NPs and citric acid-coated cerium oxide NPs (500 mg·kg^{-1})	Increased length of shoots	Barrios et al. (2016) and Paramo et al. (2020)
Spinacia oleracea L.	CeO_2	Chlorophyll content, biomass, peroxidation of lipids, and plant biomass were unaffected. Downregulation of amino	Zhang et al. (2019)

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Table 15.2 (continued)

Medicinal plant species	Nanoparticles (NPs)	Effects produced in medicinal plants	Reference(s)
		acids and decrease in zinc and Ca in plant leaves	
Stevia rebaudiana (Bertoni) Bertoni	CuO	Oxidative stress triggers antioxidative molecule (phenols, flavonoids) production, increased production of rebaudioside A and stevioside	Javed et al. (2017a)
	ZnO-PEG, ZnO-PVP, CuO-PEG, CuO-PVP, CuO, ZnO.	Nanoparticles made of metal oxide capped with polymers increased content of steviol glycosides, phenolics, and flavonoids than uncapped metal oxide NPs	Javed et al. (2017b) and Paramo et al. (2020)
	ZnO NPs	Increased phenolics and flavonoids and antioxidant responses. ZnO NPs (1 mg·L^{-1}) increased nearly two times content of steviol glycosides	Javed et al. (2018), Kumar et al. (2018) and Paramo et al. (2020)
	CuO and ZnO	Act as abiotic elicitors to generate plants with elevated antioxidant levels	Javed et al. (2018)
Tanacetum parthenium L.	C60 and salicylic acid	Enhanced growth of plant at more concentrations. Extreme enhancement in flowers (1000 mg·L^{-1}), increased chlorophyll amount at lower C60 levels	Ahmadi et al. (2020)
Thymus daenensis	MWCNTs	Increase in biomass and height of *Thymus* seedling, highest content of total phenolics, flavonoids, and antioxidant activity at 250 μg·mL^{-1}	Samadi et al. (2020)
Trigonella foenum-graecum L.	Ag produced by silver nitrate reduction	Enhanced shoot length, number of the leaves, chlorophyll, carotenoids, phenolics, flavonoids, and tannins	Sadak (2019)
Triticum aestivum L.	TiO_2	Early growth parameters badly influenced	Zahra et al. (2019)
	NiO2	Decreased growth of plant, increased antioxidant quantity and inhibition of photosynthesis	Saleh et al. (2019) and Paramo et al. (2020)
	ZnO	Increased length of plant roots and shoots	Singh et al. (2019) and

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Table 15.2 (continued)

Medicinal plant species	Nanoparticles (NPs)	Effects produced in medicinal plants	Reference(s)
			Paramo et al. (2020)
	Fe_2O_3 (500 mg·kg^{-1})	Improved length of roots, height, biomass, green chlorophyll amount. NPs translocate into leaves and cause damage to root tip	Al-Amri et al. (2020)
	GO, GO quantum dots, and reduced GO	Decrease in mineral elements, upregulation of amount of sugars. rGO downregulates proteins and decreases amylopectin, prolamin, amylose, and globulin.	Li et al. (2018b) and Paramo et al. (2020)
	TiO_2	Enhanced length of the roots and shoots, nutrient quantity in shoots, and crude protein quantity at 50 mg·L^{-1}	Ullah et al. (2020) and Paramo et al. (2020)
	TiO_2, Fe_2O_3, CuO (50 and 500 mg·kg^{-1})	CuO application decreased contents of Zn, Fe, and essential amino acids. TiO_2 improved accumulation of amino acids. Fe_2O_3 increased contents of cysteine and threonine	Wang et al. (2019a) and Paramo et al. (2020)
	TiO_2	Roots upregulated monosaccharides and azelaic acid, triggering of tyrosine metabolism; leaves upregulated reserve sugars and pathways of tocopherol, tryptophan, phenylalanine	Silva et al. (2020) and Paramo et al. (2020)
	ZnO NPs size of 35 nm (15, 62, 125, 250, and 500 mg·L^{-1})	Length of roots and shoots increased. ZnO provides Zn micronutrient for development of the plant	Singh et al. (2019)
	Fe_2O_3 and ZnO	Enhanced height, area of leaves and shoot dry weight of plant. Zn and Fe increased in leaves	Fathi et al. (2017)
Triticum vulgare L.	Fe, Cu, Ni	Fe stimulates growth. Ni and Cu produced toxicity on growth as metal content increased. Ni and Cu at low	Mikhailovna Korotkova et al. (2017)

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Table 15.2 (continued)

Medicinal plant species	Nanoparticles (NPs)	Effects produced in medicinal plants	Reference(s)
		concentrations reduced growth of roots	
Vicia faba L.	Single-bilayer GO	Decreased growth, activity of catalase and ascorbate peroxidase, increased leakage of electrolytes	Anjum et al. (2014)
Vigna radiata L.	TiO_2	Alterations in length of shoots and roots, quantity of chlorophyll, and total soluble leaf proteins	Raliya et al. (2015)
Zea mays L.	Phytochemical capped Au NPs	Increased germination of aged seeds	Mahakham et al. (2016)
	Fe_2O_3 (50 and 100 mg L^{-1})	Decrease in root length	Li et al. (2016)
	Al2O3 (2000 mg·L^{-1})	Somewhat poisonous to elongation of roots	Yang et al. (2015)
	Y_2O_3 (10, 30, 50, 100, and 500 mg·L^{-1})	Rates of germination of seeds was unaffected; increased peroxidase, catalase, polar metabolites exhibited dose-dependent rise in NPs	Gong et al. (2019)
	Fe_3O_4	No effect on biomass of plant and photosynthetic process. Fe accretion increased in underground roots. Metabolomic pathways connected to defense machinery were inactivated	Yan et al. (2020)
	TiO_2, SiO_2 (1000 mg L^{-1})	SiO_2 decreased length and fresh weight of shoots; TiO_2 reduced chlorophyll content	Ghoto et al. (2020)
Zea mays L. *Oryza sativa* L.	γ-Fe_2O_3	γ-Fe_2O_3 increased percentage of germination of seeds and seedling vigor index at 500 ppm in both plants	Kasivelu et al. (2020) and Paramo et al. (2020)
	ZnO (2000 mg·L^{-1})	Decrease in elongation of roots	Yang et al. (2015) and Paramo et al. (2020)
Zea mays L. *Glycine max* (L.) Merr.	MWCNTs	MWCNTs accumulated in vascular tissues. Growth stimulation in *Zea mays* and growth inhibition in *Glycine max*. Dry biomass of *Zea mays* increased	Zhai et al. (2015) and Paramo et al. (2020)

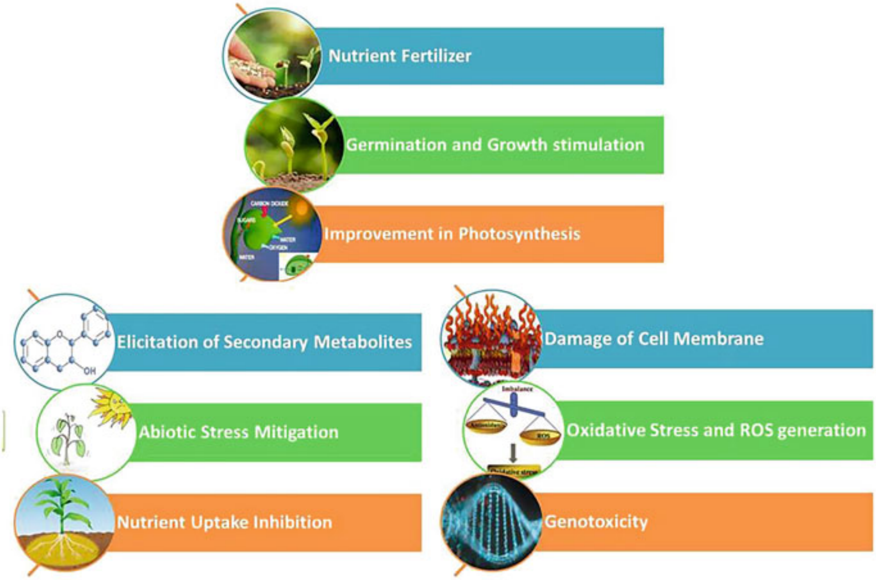

Fig. 15.1 Positive and negative influences of NPs on medicinal plants

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Chapter 16

An Insight into Plant Nanobionics and Its Application to Overcome the Impact of Environmental Stress on Medicinal and Aromatic Plants

Arian Amirifar, Arash Hemati, Behnam Asgari Lajayer, Janhvi Pandey, and Tess Astatkie

Abstract Nanomaterials can significantly boost plant growth and crop yield. They exhibit exceptional traits compared to other materials such as high surface area, tunability, adjustable pore size, and prominent reactivity. They can be utilized in the production of various herbicides, pesticides, fertilizers, etc. Recent developments in the nanomaterial's framework and their application have improved photosynthetic properties of various plant species and reduced destructive impacts of reactive oxygen species (ROS) and oxidative stress. Utilization of medicinal plants has been embedded in different cultures since ancient times all around the world, mainly due to numerous benefits and applications of secondary metabolites extracted from them. It has been suggested that nanomaterial application can enhance the secondary metabolite production of medicinal plants. Several utilizations and benefits of various nanoparticles of silver, copper, carbon, titanium, silicon, zinc, magnesium, and molybdenum are discussed in this chapter. Nanotechnology is a science that is performed at the nanometer measures, which ranges from 1 to 100 nanometers. "Plant nanobionics" can be characterized as a new area of research incorporated between nanotechnology and botanic biology. Thus, it is a field in plant science correlating with the utilization of nanoparticles and their cooperation with the plants that arises with an innovative function.

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16.1 Introduction

The word “nanobionics” is made up of two words: nano and bionics. Both words have Greek origins. “Nano” is derived from Greek word νᾶνος meaning dwarf, and “bionics” originates from the Greek word *bios* meaning life; also bionics is a portmanteau word consisting of biology and electronics. Nanomaterials are categorized and recognized by their nanoscale size, in at least one of the three dimensions. Nanomaterials have various utilizations, and recently their use in agricultural sector is being broadly researched as a propitious approach to enhance the development of plant species and their productivity (Gogos et al. 2012; Giraldo et al. 2019). Significant progress in the field of nanotechnology amalgamated with biotechnology has elevated the applicability and relevance of nanotechnology in various research fields (Ashkavand et al. 2015). The exceptional characteristics of nanomaterials provide them with several properties such as catalytic, magnetic, thermal, electrical, and fluorescence that are required for medical, electronics, energy, and environmental remediation applications (Sharma and Madou 2012; Prasad et al. 2014). Nanomaterials have various herbicidal, pesticidal, bactericidal, and fertilizing traits that target specific plant parts (Prasad et al. 2014). There are several types of nanomaterials including fullerene (C60), graphene, carbon dots, polymeric nanoparticles, and metal-based nanoparticles such as iron, aluminum, copper, silica, zinc, gold, and silver. Some other oxidized nanoparticles like zinc oxide, titanium dioxide, and cerium oxide have been synthesized for various applications like bioremediation, energy storage, catalytic reactions, photocatalytic utilizations, fuel cells, sensors, agriculture and biomedical operations involving nanomedicine, drug transport/delivery, and wound-healing properties (Joshi et al. 2018). Recently, nanomaterials are being extensively used in agricultural fields to enhance the productivity of crops and their resilience to pathogen infection (Chen et al. 2015; Ismail et al. 2017; Gupta et al. 2018). Nano-enabled agriculture can promote more efficient plant protection and growth, as well as introduces new functions to modify plants through specific interactions shown in Fig. 16.1.

Diminished size of arable land, water resources scarcity, ramifications of global warming, and low effectiveness of the commonly used chemicals aggravate the abiotic and biotic stress among species and subsequently lower the yield potential. For example, salinity and drought stress provoke enormous financial crux due to crop losses each year. Efficient utilization of nanoparticles can help the germination of seeds and their treatment, species development, pathogen identification and analysis, and detection of harmful chemicals present in the agricultural products (Nuruzzaman et al. 2016). Consequently, plant nano-biotechnology might stimulate viable agricultural production through mechanisms distinct from those of chemical and genetic engineering (Kah et al. 2019; Giraldo et al. 2019; White and Gardea-Torresdey 2018).

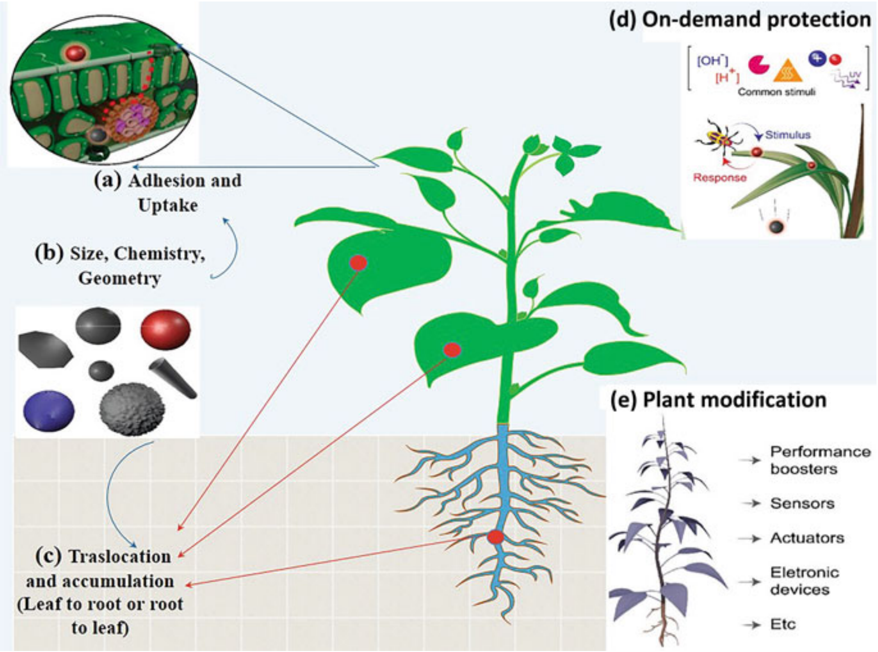

Fig. 16.1 Benefits of nano-enabled agriculture: (**a**) Promotes more efficient plant protection and growth, as well as introduces new functions to modify plants through specific interactions, (**b**) adhesion and uptake of particles is tethered to the particulate properties (size, chemistry, geometry, etc.), (**c**) impacts heavily the translocation and accumulation of particulate inside plants, (**d**) targets smart delivery systems that can be designed to respond to specific stimuli, such as pH, light, enzymes, ionic strength, and temperature, and (**e**) modifies plants toward several end-goals to achieve through targeting particles designed to accumulate at specific sites of plant tissues

16.2 Nanobionics for the Improvement of Photosynthetic System

Team Strano at Massachusetts Institute of Technology did a novel research using nanobionics in which they extracted the chloroplasts of *Arabidopsis thaliana*, put them into a buffer solution, and then added polymer-coated nanoparticles containing cerium oxide (ceria). Ceria nanoparticles reduce the destructive effects of reactive oxygen species (ROS), such as hydrogen peroxide and super oxides. Radicals can have an adverse effect on the molecules involved in photosynthesis, consequently detaining its ability to function. Nano-cerium oxide particles were labeled with fluorescent dyes and monitored with confocal fluorescence as well as transmission electron microscopes. It was observed that cerium oxide nanoparticles spontaneously penetrate the chloroplast's outer envelope and accumulate there, which exhibits sudden significant drop in the ROS levels in the chloroplasts. Nanoparticles enhance light energy conversion into electron flow. Subsequently, carbon nanotubes are introduced into the chloroplasts, and their uptake is monitored via near-infrared fluorescence microscope. Spontaneous movement of carbon nanotubes inside chloroplasts is captured at the single particle level suggesting that the nanoparticles enhance the conversion of light energy into electron flow (Kah et al. 2019). Once infiltrated by carbon nanotubes, they are tested as chemical sensors. By adding nitric oxide on the leaf surface, the fluorescence level inside leaves decreases drastically. Single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) are the two major types of nanotubes. Tensile strength of these nanotubes is about 200 Gigapascals; hence, they are ideal for reinforcing various composites and for applications in nanoelectromechanical complexes. Furthermore, their semiconducting and metallic traits offer astonishing opportunities to fabricate electronic circuits or even fully functional nanodevices. Nanotube systems involve graphite layers wrapped into cylindrical forms. Lately, fluorescent nanoparticles and quantum dots have been advanced for labeling and detecting plant proteins (Pyrzynska 2011).

The mechanical, electronic, and thermal properties and relevancy of carbon nanotubes are evaluated through their geometrical dimensions, specifically thickness. Taking advantage of these properties of nanotubes, new possibilities arise for producing various types of nanodevices that confer specific conductive, optical, and thermal properties for sustainable agriculture (Raliya and Tarafdar 2013). It has been reported that carbon nanotube-based delivery system can be used to mark chemical agricultural compounds that reduce tissue damage and control chemical release to the environment (Raliya and Tarafdar 2013; Hajirostamlo et al. 2015).

Numerous organelles that accumulate and utilize nanomaterials have been recognized. Vacuolar uptake of SWCNTs has been reported by Serag et al. (2015) by labeling the same with fluorescein isothiocyanate. In their experiment, the levels of fluorescence signals were monitored in the cell vacuoles after subsequent incubation of plant tissues with fluorescein isothiocyanate tagged SWCNTs. Vacuolar accumulation was maintained by diffusion coefficient (Deff) measurement, which was quantified utilizing fluorescence recovery in a photobleached area (FRAP). FRAP assisted the study of molecules that are capable of recovering in the photobleached area fraction and consequently approved the aggregation of SW-F in the vacuoles (Serag et al. 2015).

Crop yield can be boosted by bioengineering plants for tolerating various harsh stress conditions and enhancing yield quality, particularly the efficiency of photosynthesis. Recently, nanomaterials with unique functional characteristics like enhanced solar energy harvest have been developed. Such nanomaterials and nanobionic plants were developed by confining the nanomaterials within organelles with photosynthetic activity. Inclusion of nanomaterials or nanotubes enhanced the capability of chloroplasts to capture carbon.

Furthermore, nanotubes like polyacrylic acid-nanoceria (PAA-NC) and single-walled nanotube-nanoceria (SWNT-NC) diminish the proportion of ROS inside chloroplasts and impact sensing process in plants, which is crucial for a broad range of physiological procedures (Giraldo et al. 2014).

Enzyme-imitating nanoparticles (Ceria, C60) have been validated to increase and boost the development of various plant species and alleviate their tolerance under extreme conditions by methods that are different from ROS scavenging. For example, Borišev et al. (2016) demonstrated that fullerol nanoparticles reduce the stress incited by oxidative damage in beet during dry and arid conditions by acting as an additional intracellular source of water (Rossi et al. 2016). It has also been reported that under salt stress conditions, nanoparticles boost the amount of biomass, chlorophyll content, and photosynthetic efficacy of the plants. Later, Rossi et al. (2017) demonstrated that ceria nanoparticles reduce the amount of apoplastic root barriers, consequently raising Na^+ movement to shoots and less convergence in roots of *Brassica* plants. Additional research is required to investigate the intracellular transportation of Na^+ from root to aerial parts, because overaccumulation of the same can lead to photosynthetic reduction in the leaves of plants.

Photosynthesis is a process that utilizes light energy to transform atmospheric carbon dioxide to chemical energy source such as glucose. As the organelle behind photosynthesis, the highest glucose concentration in plants belongs to chloroplasts. Nonetheless, because of their micron-sized dimensionality, direct glucose gathering from chloroplasts will need standalone machines that can operate inside a single plant cell or an organelle. So far, the possibility of chloroplast to take advantage of the energy harvesting can be estimated from a biofuel study that was based on confined chloroplasts. Boghossian et al. (2013) measured 40 and 110 μW cm^2 with and without 14 W m^2 illumination when isolated chloroplasts were placed between two electrodes with glucose oxidase and catalase on the cathode and laccase on the anode. Several nanoparticle antioxidants were studied as possible ROS confiner to conserve the activity of chloroplasts. The chloroplasts inserted with dextran-wrapped nanoceria exhibited maximum capability of light harvesting.

ROS scavenging is mainly performed by antioxidant enzymes, for instance, superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, glutathione peroxidase, and peroxidase, as well as by some non-enzymatic metabolites with a relatively low molecular mass (ascorbic acid, alpha tocopherol, polyphenols). In extreme circumstances, metabolic pathways that are related to scavenging of ROS will also get activated, for instance, synthesis of shikimate-phenylpropanoid, ascorbic acid, and aldaric acid metabolism (Zhang et al. 2018). Abiotic stresses, even if they are presented in a form of drought, extreme radiation and heat, high salinity, frigid temperatures, scarcity of vital nutrients, toxic chemicals like heavy metals, or stress induced by oxidation, are the prime reasons for crop damage and loss around the globe (Atkinson and Urwin 2012) and lead to a decline in the production of major crop plants by more than 50%. Abiotic stress gives rise to many morphological, physiological, biochemical, and molecular variations in plants that have a negative impact on their development, progress, yield, and productivity (Atkinson and Urwin 2012). Fundamental approaches used by plants to overcome stress involves upregulation of protectants, like antioxidants and osmolytes (Wang et al. 2003). Studies have shown that ROS are produced organically by plant species as a consequence of biochemical interactions (Tripathy and Oelmüller 2012) in chloroplasts, mitochondria, peroxisomes, and other organelles via metabolic processes like photosynthesis and respiration (Tripathy and Oelmüller 2012). In small amounts, ROS operate as signaling molecules involved in the development and defense; on the other hand, convergence of the same in plants during stress conditions is followed by the damage to the cell membranes, DNA, proteins, enzymes, and other cell components leading to the suppression of plant development (Tripathy and Oelmüller 2012).

16.3 Non-nanoenzymes (Fe, TiO_2, SiO_2, ZnO)

Some nanoparticles without enzyme-imitating properties enhance the tolerance of plants under stress conditions. Kim et al. (2015) discovered that nano-zerovalent iron activates high H^+-ATPase activities which diminishes apoplastic pH and increases the size of leaves and the width of stomatal aperture. However, the drought sensitivity of species treated with nano-zerovalent iron did not exhibit a noticeable change compared to the control displaying rise in the rate of CO_2 absorption and assimilation. At present, fundamental methods that enhance tolerance in plants against abiotic stress via iron-based nanoparticles are unclear. Nanoparticles that do not sweep ROS at first hand but instead increase the encoding of genes engaged in antioxidant defense mechanism and boost stress resistance have been demonstrated. For example, Abdel Latef et al. (2018) reported better stress resistance in plants treated with 0.01% titanium oxide nanoparticles due to enhancements in chlorophyll amount and biomass.

Shallan et al. (2016) suggested that the spray of titanium oxide nanoparticles or silica nanoparticles boosts the tolerance of cotton toward drought stress. The benefits of silicon nanoparticles for plants include an improved flexibility to various environmental stresses. Siddiqui and Al-Whaibi (2014) reported that silica nanoparticles (ranging from 1.5 to 7.5 grams per liter) enhanced the expression of catalase, peroxidase, superoxide dismutase, ascorbate peroxidase, and glutathione reductase genes and raised chlorophyll amount as well as enhanced both the efficiency of photosynthesis and biomass in tomato (*Lycopersicum esculentum*) plants Mill.).

Alharby et al. (2016) reported that salt stress reduced the expression of superoxide dismutase and glutathione peroxidase genes in tomato (*Solanum lycopersicum*). The impact was inverted by introducing zinc oxide nanoparticles in plants, representing a positive feedback of metabolism to zinc oxide nanoparticles in salt-induced stress. The procedure for zinc oxide nanoparticles that raise salt stress tolerance depends on proteins or enzymes that are linked with resistance regulation. Comparable results were reported by Haripriya et al. (2018), suggesting that a foliar spray of zinc oxide nanoparticles lessened salt-induced stress in finger millet (*Eleusine coracana*).

16.4 Medicinal Plants' Responses to Synthetic Nanoparticles

The utilization of medicinal plants has been the part and parcel of human civilizations since time immemorial. It is mainly because of their therapeutic properties imparted to them due to the presence of specific secondary metabolites and alkaloids. Their identification acted as a backbone for the advancement of novel potent synthetic medications. One of the recent ways to boost the yield of secondary metabolites is to utilize nanoparticles that have characteristics of an elicitor. Nonetheless, depending on the particle size, arrangement, concentration, and utilization paths, nanoparticles might impart some beneficial traits to medicinal plant species (for instance, plant development and overall growth enhancement and boost in the photosynthetic rate). But the utilization of nanoparticles must be done wisely as it has been reported that their high concentrations might damage plants mechanically, negatively affect biochemical and morphological characteristics of plants, and exhibit cytotoxic and genotoxic results.

Basically, there are two approaches for the development of nanoparticles: Physical (top to bottom) and chemical (bottom to top). Physical method mainly constitutes of grinding and crushing using stabilizing agents and was the initial approach for nanoparticle production (Masarovicova et al. 2014). By using this method, nanoparticles with size greater than 10 nm are produced, which have rather large size variation that makes them less efficient. The second approach, i.e., chemical method, is much unique, and, nowadays, it is considered as a more feasible and efficient way to produce nanoparticles, which is a better way to control the grain size of the nanoparticles (Jampilek and Kralova 2019, 2020). A broad variety of various stabilizers such as donor ligands, polymers, and detergents are used to control the size of nanoparticle grains and to inhibit their coalesce and convergence. Electrochemical reduction of metal salts and organized breakdown of organometallic compounds is done to produce metal nanoparticles.

In the nucleation's initial state, the metal salt is reduced to atoms which coalesces with various ions or clusters and forms a permanent nucleus (Ma et al. 2020; Singh et al. 2019). The diameter of the core is usually lower than 1 nm that relies upon the strength of the bonds, metal salt's redox potential, and reducing agents that are utilized in the process (Linh et al. 2020, Adrees et al. 2020).

Numerous plants or microbial derivatives are associated with the green synthesis of nanoparticles that involve several active biological complexes for the reduction of metal ions and stabilization (Oliveira et al. 2015; Abbasi Khalaki et al. 2021). Biologically active compounds that are adsorbed on the nanoparticles considerably boost the interaction speed of nanoparticles with cells and improve their effectiveness within the organisms (Makhlouf and Barhoum 2018).

Different nanoparticles affect plant species distinctively, and this impact relies upon the chemical structure and concentration of nanoparticles along with environmental factors like pH (Jampilek and Kralova 2019; Kralova et al. 2019). Nanoparticles applied as "enrichers" induce plant development and productivity as well as impart beneficial impact on the crop's nutritional value (Jampilek and Kralova 2017, 2019; Masarovicova et al. 2014), while nano-sized herbicides inhibit the growth of undesirable weeds (Jampilek and Kralova 2015, 2017, 2018; Masarovicova et al. 2014; Oliveira et al. 2015). It has been reported that the introduction of certain nanoparticles in plants imparts their better development and repels pathogens and insects (Jampilek and Kralova 2015, 2017, 2018, 2019, 2020).

It has also been reported that in various plants, introduction of nanoparticles induces oxidative stress, which further enhances the alkaloid and metabolite production (Jampilek and Kralova 2021; Patel et al. 2020; Ma et al. 2020; Singh et al. 2019; Kralova et al. 2021; Moharrami et al. 2017; Anjum et al. 2019). Thus, the utilization of nanoparticles has been suggested to reduce the negative impacts of abiotic stress on plants, which ultimately enhances crop yield (Jampilek and Kralova 2019, 2021; Linh et al. 2020; Adrees et al. 2020; Morales-Espinoza et al. 2019; Xiao et al. 2019).

16.5 Nanoparticle Functionality Against Biotic Stresses (Nanopesticides)

"Nanopesticides can be explained as any pesticide formulation or product consisting of engineered nanomaterials as active ingredient and including biocidal traits, as a section of the engineered structure" (Adisa et al. 2019). Several nanoparticles, such as silver copper and aluminum nanoparticles, display antibacterial and pest control properties (Gogos et al. 2012). Some nanoparticles with pesticidal characteristics are discussed below (Table 16.1).

Table 16.1 Research on nanoparticles with pesticidal characteristics

Nanoparticle	Major finding	Reference
Silver	Silver nanoparticles in the range of 30 to 150 mg killed 99% of the nematodes	Cromwell et al. (2014)
Copper	Copper nanoparticles possess microbicidal property against the pathogens *Escherichia coli* and *Bacillus subtilis*	Yoon et al. (2007)
Titanium	Titanium dioxide nanoparticles have photochemical antibacterial property	Paret et al. (2013)
Cerium	250 mg per liter cerium oxide nanoparticles reduce the progression of fungal disease by 53%	Adisa et al. (2018)
Magnesium	Magnesium hydroxide nanoparticles inhibited the growth of *Pseudomonas syringae* and *E. coli* within 4 hours	Huang et al. (2018)
Silicon	Silicon nanoparticles initiated the phenolic compound production, delivering better tolerability to plants against *Aspergillus* spp.	Suriyaprabha et al. (2012)

16.5.1 Silver-Based Nanoparticles

The expanding population of harmful fungi and pests that are resistant to routine chemical pesticides has stirred the need to develop new ways or products that help in sustenance of plants. Owing to their broad range of antibacterial properties, silver nanoparticles are considered as a potent nanopesticide in agriculture. Ocsoy et al. (2013) synthesized DNA-directed silver nanoparticles on graphene oxide and reported that these composites at 16 mg/L concentration significantly reduced the activity of cultured *Xanthomonas perforans* (pathogen causing bacterial leaf spot in tomato and pepper worldwide). This pathogenic bacterium infection in tomato leads to 10–50% reduction in yields. Similar results were reported in a glasshouse experiment using Ag@dsDNA@GO (DNA-directed silver Ag nanoparticles developed on graphene oxide [GO]) at 100 milligrams per liter. Additionally, silver nanoparticles have exhibited their potential against nematodes, a common soil-borne organism (Vicente et al. 2006). Cromwell et al. (2014) reported that silver nanoparticles in the concentration range of 30–150 milligrams per milliliter can eradicate 99% of the population of nematodes (*Meloidogyne* spp.) within 6 days of application. In another field experiment, silver nanoparticles in the range of 150 milligrams per milliliter reduced the number of nematodes by 82% and 92% at day 2 and day 4, respectively.

Compared to chemical pesticides, synthesized green silver nanoparticles are environmentally safe to produce. Reducing agents derived from plant and bacterial extracts play a role in the fabrication of silver nanoparticles. Mishra et al. (2014) utilized *Serratia* sp., a plant growth-promoting rhizobacterium (PGPR), to synthesize silver nanoparticles biologically. It has been reported that bio-synthesized silver nanoparticles exhibit powerful antifungal characteristics against *Bipolaris sorokiniana*, which causes spot blotch and root rot disease of wheat, in greenhouse conditions. Utilizing leaf extract of turnip, Narayanan and Park (2014) synthesized green silver nanoparticles, approximately 16 nm in size, and demonstrated their broad spectrum antifungal activity against wood debasing fungi, e.g., *Gloeophyllum abietinum*, *G. trabeum*, *Chaetomium globosum*, and *Phanerochaete sordida*. Ali et al. (2015) utilized an extract derived from *Artemisia absinthium* for green-synthesizing silver nanoparticles, which subdued *Phytophthora* contagion on plant species and improved plant overall health when applied at a concentration of 10 mg/ml. Despite these positive results in the horticultural utilization of silver nanoparticles, their potency to induce toxicity in plants when used in larger concentration has increased concern. Zhang et al. (2018) investigated the toxicity of commercially available silver nanoparticles at the molecular levels and discovered that the utilization of silver nanoparticles (0.4 milligrams/plant) on leaves incited oxidative stress in cucumber leaves. More studies on the response of plants to silver nanoparticles dosage and the effect of such particles are required to permit their environmentally safe usage. Also, green synthesis procedure of nanoparticles which is quickly evolving in nanotechnology must be promoted so that it can take over the utilization of toxic chemicals and time expenditure.

16.5.2 Copper-Based Nanoparticles

The bactericidal and fungicidal impacts of copper ions are already evident. Copper hydroxide nanoparticles are the active compounds in the commercially available pesticide called “Kocide 3000.” Copper nanoparticles possess microbicidal properties against pathogens *Escherichia coli* and *Bacillus subtilis* (Yoon et al. 2007) together with the plant fungal pathogens *F. oxysporum*, *C. lunata*, *A. alternate*, and *P. destructiva* (Kanhed et al. 2014). A research comparing the antibacterial effectiveness of copper nanoparticles against the Bavistin fungicide (Devistin and carbendazim 50% WP) demonstrated better performance of copper nanoparticles. More recently, Borgatta et al. (2018) compared the ability of copper oxide nanoparticles and $Cu_3(PO_4)_2{\cdot}3H_2O$ nanosheets to treat ailment incited by *Fusarium oxysporum* f. sp. niveum in watermelon (*Citrullus lanatus*). In a glasshouse field study, $Cu_3\ (PO_4)_2{\cdot}3H_2O$ nanosheets (10 milligrams per liter) significantly reduced fungal infection by 58% and enhanced crop yield. The functionality of $Cu_3(PO_4)_2{\cdot}3H_2O$ nanosheets was better than copper oxide nanoparticles that had significant impacts on disease only at extreme doses (1000 milligrams per liter). The researchers accredited the dissimilar performance of nanoparticles to traits of the nanoparticle’s dissolution. The potential toxicity incited by the massive levels (1000 milligrams per liter) requires examination. Lately, Cumplido-Najera et al. (2019) demonstrated the effects of copper nanoparticles and K_2SiO_3 nanoparticles on tomatoes cultivated in hydroponic culture and infected with *Clavibacter michiganensis* pathogen. It was reported that the copper nanoparticles reduced the *C. michiganensis* infection in tomato plants. The researchers deduced that the combined application of both particles enhanced the levels of both enzymatic and non-enzymatic metabolites essential for protection of plants and increased their tolerability against *C. michiganensis* infection accordingly. The pesticidal actions of copper nanoparticles are evident. Le Van et al. (2016) analyzed the impacts of copper oxide nanoparticles on Bt-transgenic cotton. Nanoparticles boosted the exogenous genes expression encoding Bt toxin in cotton plant at a dose of 10 mg/liter, consequently raising their resistance. Ayoub et al. (2018) synthesized copper oxide and calcium oxide nanoparticles and reported their entomotoxic effects against *S. littoralis*, recommending the application of these nanoparticles as advantageous pesticides. Copper nanoparticles exhibited more immediate entomotoxic impacts compared with calcium oxide nanoparticles, with a lethal dose of 232 milligram per liter after 3 days, whereas for calcium oxide nanoparticles, the lethal dose of 129 milligram per liter was attained after 11 days, posttreatment.

Native medicinal plant of Ethiopia was utilized for the first time to scrutinize the synergistic impact of phyto-constituents in green nanoparticles of copper (g-Cu nanoparticles) regarding the improvement of antimicrobial activities of nanoparticles. Murthy et al. (2020) reported the green synthesis of Cu nanoparticles utilizing *Hagenia abyssinica (Brace) JF. Gmel.* leaf extract.

Wang et al. (2014) examined the fungicidal characteristics of six carbon-based materials, namely, SWCNTs, multi-walled carbon nanotubes, graphene oxide, reduced graphene oxide, fullerene, and activated carbon (at 500 mg/liter concentration each), against *Fusarium graminearum* and *Fusarium poae*. These nanomaterials were incubated along with *F. graminearum* and *F. poae* for 5 and 12 hours in the dark. SWCNTs exhibited maximum fungicidal activity, followed by MWCNTs, graphene oxide, and reduced graphene oxide (500 milligrams per liter), contemplating that the activated carbon at these concentrations exhibited no fungicidal impacts. At a concentration of 500 milligrams per liter, fullerene suppressed the initiation of *F. graminearum* spore's germination but wasn't able to suppress the *F. poae* spore germination. Researchers suggest that the side effects of these nanomaterials' application include inhibition of water uptake and plasmolysis.

16.5.3 Titanium-Based Nanoparticles

Titanium dioxide and zinc oxide are photo-chemically operative. When exposed to light and in the presence of oxygen, excited electrons are generated resulting in the superoxide radical's synthesis by direct electron transfer. Photo-chemically functional titanium dioxide nanoparticles possess bactericidal effects, hence can be used as nanopesticides. Paret et al. (2013) suggested the high photo-catalytic activity and antibacterial potency of titanium dioxide nanoparticles against *X. perforans*, the pathogen that causes spot disease in tomatoes. In glasshouse surroundings, titanium dioxide and zinc nanoparticles, at 500 to 800 milligrams per liter, significantly diminished severity of bacterial spots compared to both untreated and copper-treated controls.

16.5.4 Cerium-Based Nanoparticles

How cerium-based nanoparticles boost plant protection is still unclear. Recently, Adisa et al. (2018) set up a greenhouse trial to test the activity of cerium oxide nanoparticle against *Fusarium* wilt infection in tomatoes. Cerium oxide nanoparticles (50 and 250 milligrams per liter) were introduced in a 3-week-old tomato (*Solanum lycopersicum*) seedlings grown in soil media through root and shoot pathways. Results indicated that 250 milligrams per liter of cerium oxide nanoparticles applied to the soil and foliage notably reduced disease progression by 53 and 57%, respectively. The science behind fungicidal properties of cerium oxide nanoparticles is still not very clear, and more research is required in this area.

16.5.5 Magnesium-Based Nanoparticles

Imada et al. (2016) examined the effects of magnesium oxide nanoparticles on tolerability of tomatoes cultivated in 50% vermiculite and 50% perlite against *Ralstonia solanacearum* pathogen. The pretreatment of roots with magnesium oxide nanoparticle suspension noticeably suppressed the infection. This method involves the improvement of jasmonic acid inducible LoxA, salicylic acid inducible PR1, and expression of GluA genes, which are related to resistance and defense and are ethylene inducible. Cai et al. (2018) reported that magnesium oxide nanoparticles at 200 or 250 milligrams per liter efficiently inhibit *R. solanacearum* infection responsible for the wilt of *Nicotiana tabacum* plants. Suggested method involves damaging of cell membranes and ROS convergence in different parts. In an additional research conducted by Huang et al. (2018), antibacterial activities of magnesium hydroxide nanoparticles were compared with the properties of Kocide 3000 in which copper hydroxide is the active compound. Magnesium hydroxide nanoparticles inhibited the growth of *Pseudomonas syringae* and *E. coli* within 4 hours. Pesticidal activities of magnesium hydroxide nanoparticles were same as Kocide 3000 activity, additionally suggesting their potency as a copper alternate.

16.5.6 Silicon-Based Nanoparticles

Suriyaprabha et al. (2012) examined the resistance of maize plants treated with silicon nanoparticles (20 to 40 nanometer in size, 15 kg/ha) compared to plants treated with bulk silicon, against pathogens. Silicon nanoparticles initiated the production of phenolic compounds in plants, resulting in better tolerability of the plants against *Aspergillus* spp. More recently, Buchman et al. (2019) determined the ability of mesoporous silica nanoparticles (36 nm in size, 500 milligrams per liter) with chitosan coating to suppress wilt caused by *Fusarium* in watermelons cultivated in soil. Both mesoporous silica nanoparticles and chitosan-coated mesoporous silica nanoparticles diminished disease infection, by 40 and 27%, respectively. Hence, silicon nanoparticles could be utilized as a possible alternate fungicidal agent in agriculture.

16.6 Utilization of Nanoparticles as Nanofertilizers

Micronutrients play an important role in plants' defense system against several disease and pathogen infections (Servin et al. 2015). To make plants disease resistant, a recent approach is to enhance the nutritional status of plants by nanoparticle introduction in the same. For instance, Elmer and White (2016) fabricated sprays with micronutrients consisting of metallic oxide nanoparticles (alumina, copper oxide, ferric oxide, manganese oxide, nickel oxide, and zinc oxide) then applied them on pathogen-infected tomato and eggplants in a field experiment. They reported that the nanoparticles containing copper oxide, manganese oxide, and zinc oxide suppressed the *Fusarium* wilt infection in both plants and enhanced their yield. It has been reported that such nanoparticles can perform as fertilizers and pesticides. Furthermore, Kah et al. (2019) revealed that the average improvement in effectiveness of nanofertilizers corresponding to commercial produces is about 20–30%.

In subsequent sections, recent studies conducted on the utilization of nanoparticles involving nutrients for amplifying development and productivity are reviewed. Some nanoparticles have been reported to be functional in promoting plant growth under normal circumstances and improving stress resistance in plant species.

16.6.1 Iron-Based Nanoparticles

Iron has a vital role in numerous physiological procedures in plant cells, as well as photosynthetic pigment biosynthesis, photosynthesis, and respiration (Clarkson and Marschner 1996). Various researches have demonstrated that iron-based nanoparticles boost plant growth under standard surroundings. In a research done by Liu et al. (2016), lettuce (*Lactuca sativa*) seedlings grown in aquatic media were spiked with various levels of FeOX nanoparticles. At a concentration of 5 to 20 milligrams per liter, FeOX nanoparticles remarkably improved the size of lettuce shoot, by 12–26%. Ghafariyan et al. (2013) also reported that iron (III) oxide nanoparticles at 30 to 60 milligrams per liter remarkably enhanced the chlorophyll content of soybean (*Glycine max* L., Oxley), grown in hydroponic culture. Growth rate and root length of maize seedlings cultivated in hydroponic culture containing 20 milligrams per liter γ-Iron (III) oxide was significantly increased by 27.2 and 11.5%, respectively (Li et al. 2016). Application of iron oxide nanoparticles in small doses encourages better plant development. Palchoudhury et al. (2018) conducted a study on the impact of α-Iron (III) oxide nanoparticle application on the growth of legume roots and uncovered that soaking the plants with nanoparticles at a minimum dose of 5.54×10^{-3} milligrams per liter notably boosted root length, by 88 to 366%. Rui et al. (2016) also reported the efficacy of iron (III) oxide nanoparticles as an alternate of iron-based fertilizers. Alidoust and Isoda (2013) assessed the effectiveness of nano-iron (III) oxide on seedlings of soy at two exposure areas (root and leaf). They reported that a spray of vitamin C-coated nano-iron (III) oxide applied on the leaves notably boosted root length and photosynthesis rate. In another experiment, peanut plants (*Arachis hypogaea*) were spiked with various levels of γ-Iron (III) of 20 nm size, for more than a month. Plants that were exposed to 2 mg/kg of γ-Iron (III) oxide exhibited significant boost in their photosynthetic rate and biomass yield. It has been reported that γ-Iron (III) oxide nanoparticles fight with oxidative stress in *Brassica napus* cultivated under drought conditions in soil by lowering the levels of hydrogen peroxide (by applying 2 milligrams per liter γ-Iron (III) oxide) and malondialdehyde (by applying 1 milligram per liter γ-Iron (III) oxide) (Palmqvist et al. 2017).

Likewise, chitosan-coated ferrosoferric oxide nanoparticles with diameters ranging from 3 to 22 nm applied at dosages around 200 and 400 milligrams per kilogram exhibited significant effects on seed germination and development of *Capsicum annuum* seedlings (Bahrami et al. 2018). In another experiment, the yield of chilies and marigold plants almost doubled when their 28-day-old saplings were dipped in iron sulfide nanoparticle solution (100 micro-grams per milliliter concentration) for 3 hours just before transplantation (Jangir et al. 2019).

16.6.2 Zinc-Based Nanoparticles

Zinc has a regulatory effect on the processes, organization, and activity of various enzymes (Brown et al. 1993). There are significant evidences that ZnO nanoparticles improve development and boosts biomass yield in certain plants. For example, Dhoke et al. (2013) examined the impact of zinc oxide nanoparticles on the development of mung bean (*Vigna radiata*) seedlings cultivated in hydroponic culture. Application of nanoparticles on the shoots boosted the biomass yield of root and aerial parts of the seedlings. Dimkpa et al. (2017) examined the impact of application of zinc oxide nanoparticles or zinc salt on the growth of sorghum plants. The potency of vulnerability paths (soil of shoot application) was examined with respect to the yield, efficiency of macronutrients, and enrichment of grain by zinc. Application of both zinc oxide and zinc salt to the shoots considerably increased the yield of sorghum plants and nutrient quality of grain under low and high NPK input. The researchers recommended an approach on the nanoscale for increasing crop productivity, nutrition rate of grains, and efficiency of nitrogen assimilation. Maize development can also be fortified by the application of zinc oxide nanoparticles (Subbaiah et al., 2016). In the experiment, maize plants were subjected to zinc oxide nanoparticles (25 nm size) of 50–2000 ppm concentrations. At 1500 ppm, zinc oxide nanoparticles notably raised the percentage of germination by 80% and improved the seedling overall health, whereas at 2000 ppm, zinc oxide nanoparticles did not suppress seed germination; likewise the same dosage of zinc sulfate leads to inadequate inhibition of plant growth. Maximum grain yield (3298 kg/ha) was attained with 400 ppm application of zinc oxide. Aside from its advantageous role in species growth under normal circumstances, it has been reported that zinc oxide nanoparticles additionally enhance performance of plants facing salinity stress (Alharby et al. 2016), drought stress (Dimkpa et al. 2019), and stress incited by cadmium (Rizwan et al. 2019). Zinc nanoparticles can also be synthesized using plant leaves as reported in a recent research done by Naseer et al. (2020). In the experiment, zinc oxide nanoparticles were synthesized utilizing leaf extracts of *Cassia fistula* and *Melia azedarach* medicinal plants. For it, 0.01 M zinc acetate dihydrate solution was applied as a precursor in extracts of respective plant leaves for nanoparticle synthesis.

16.6.3 Copper- and Magnesium-Based Nanoparticles

Copper, manganese, and zinc improve disease defiance by triggering the defense enzymes of plant species like phenylalanine ammonia-lyase and polyphenol oxidases (Servin et al. 2015). Saharan et al. (2016) conducted a study on the impact of copper chitosan nanoparticles on the growth and development of maize seedlings, particularly on physiological and biochemical alterations. Copper chitosan boosted the development of maize seedlings by encouraging α-amylase activity and starch content.

Magnesium is a micronutrient that is necessary for the development of plant species and is involved in many major physiological processes (Li et al. 2001). It is constantly engaged in photosynthetic processes and is an essential component of chlorophyll (Kashem and Kawai 2007). Delfani et al. (2014) treated black-eyed pea with magnesium nanoparticles by spraying it to the leaves and suggested that 0.5 gram per liter of these nanoparticles remarkably boosts the photosynthetic rate and the amount of biomass. Likewise, Rathore and Tarafdar (2015) reported that the spray of bio-synthesized magnesium nanoparticles (<5.9 nm, 20 mg/L) to the leaves of wheat enhanced length, number of newly developed tips, and biomass of roots. Moreover, magnesium oxide nanoparticles (50, 150, 250 milligrams per liter) enhanced superoxide dismutase and peroxidase activity in tobacco plants grown in matrix medium (Cai et al. 2018). Magnesium is a crucial micronutrient and has the ability to improve activities of antioxidant enzymes. Magnesium nanoparticles can be applied to plants suffering from magnesium inadequacy symptoms and can enhance plant stress tolerability. For instance, compared to bulk magnesium oxide, usage of magnesium oxide nanoparticles (150 and 250 mg/L) notably suppressed *Ralstonia solanacearum* infection in *Nicotiana tabacum* plants (Cai et al. 2018).

16.6.4 Manganese-Based Nanoparticles

Manganese acts as a cofactor for various redox enzymatic reactions and is engaged in many metabolic processes (Najeeb et al. 2009). Superoxide dismutase is a key factor of antioxidant defense system in plants (Wang et al. 2005). In a research that analyzed the action of nanoparticles on plant species at cellular and vegetative growth levels, Pradhan et al. (2013) examined the impacts of application of manganese nanoparticles at a concentration of 0.05 milligrams per liter compared with manganese sulfate (a widely available salt of manganese) on the growth of mung bean plants. Hoagland's nutrient solution with manganese nanoparticles and $MnSO_4$ was utilized to cultivate mung bean plants for 15 days. The plants receiving Mn nanoparticles (0.05 mg/liter) exhibited enhancement in their biomass within the range of 39–53.6%. The researchers additionally isolated chloroplasts from healthy leaves and investigated the impact of manganese nanoparticles and manganese sulfate on the photosynthetic activities of chloroplasts. The researchers reported more significant photophosphorylation and evolution of oxygen in chloroplasts of plants treated with manganese nanoparticles than those in the plants treated with manganese sulfate and control. Hence it is suggested that manganese nanoparticles might act as a unique nano-modulator with photochemical characteristics and can be utilized in agricultural fields for enhancing crop production. Scrutinizing the impact of nanoparticles on the chloroplasts will enhance our insight into the system of improved photosynthesis rate at the entire-plant level. Same research group additionally scrutinized the impact of manganese nanoparticles and manganese sulfate salt on the uptake of nitrate, assimilation, and metabolism in mung bean plants. They further reported that manganese nanoparticles activate the assimilatory characteristics in plants without nodules by boosting the net flux of nitrogen assimilation via various pathways (Pradhan et al., 2014). Furthermore, the application of manganese oxide nanoparticles (40 nm in size, 1 milligram per liter concentration) lowered disease severity by 28% in *Solanum lycopersicum* plants infected with *Fusarium oxysporum* f. sp. lycopersici, as compared to untreated control groups (Elmer and White 2016). Additionally, it has been suggested that trimanganese tetraoxide nanoparticles that act as nanoenzyme can effectively scavenge ROS in various plant species (Yao et al. 2018).

16.6.5 Molybdenum-Based Nanoparticles

Molybdenum is a cofactor of nitrate reductase and nitrogenase enzymes that are essential in fixation of nitrogen, reduction of nitrate, and nitrogen translocation in plants (Alam et al. 2015). Molybdenum disulfide nanoparticles are utilized as semiconducting material with extraordinary electronic, catalytic, and optical traits (Parzinger et al. 2015). Li et al. (2018) conducted an experiment to investigate the impact of application of molybdenum disulfide nanoparticles (1.5 micrometer in size, application on shoot, 32–500 micro-grams per milliliter concentration) on the development, amount of chlorophyll, peroxidation of lipids, and activity of antioxidant enzymes in rice plants. Molybdenum disulfide nanoparticle (125 mg/L) exposure did not have any significant impact on the rate of seed germination, malonaldehyde content, or activity of antioxidant enzymes but significantly boosted the biomass and chlorophyll levels. Nanoparticles also enhanced the expression of aquaporin gene in rice. Recently, Chen et al. (2018) synthesized MoS_2 nanoparticles that imitate the activity of superoxide dismutase, catalase, and peroxidase and can be recommended for boosting the stress tolerance of various species.

Nanotechnology is a rapidly evolving field; and molybdenum-based nanoparticles are being used extensively in different places, but their uncontrolled usage and their consequential effects on the environment have been a serious concern. In a study on nano-toxic impacts and methods of nano-molybdenum treatment on the soybean-rhizobia symbiotic association in decontaminated blend of sand and vermiculite growing medium, it was reported that exposure to various concentrations and types of molybdenum-based nanomaterials causes physiological and biochemical toxicity to soybean plants. The application of nano-molybdenum in the sand-vermiculite media also enhanced the concentration of molybdenum in plant tissues. The molybdenum-based nanoparticles introduced in the plant tissues were reported to alter not only the development of plants but also the structure of the roots and the action of rhizobium in the symbiotic association diminishing the nitrogen fixation capability (Yang et al. 2020).

16.6.6 Silicon-Based Nanoparticles

The beneficial impacts of silicon are well understood; nevertheless, the benefits of silicon nanoparticles over its bulk matter are areas that were not studied enough. Nanoparticles of silicon have noticeable physiological traits that let their entry into the plants' systems and affect their metabolic activities. Mesoporous characteristics of silicon nanoparticles make them better nominees for suitable nano-carriers of various molecules that aid in agriculture. Various studies have demonstrated the significance of silicon nanomaterials in agriculture (Rastogi et al. 2019).

Silicon improves plants' resistance to both abiotic and biotic stresses, but until now, not much research has been conducted to understand the mechanism. Guntzer et al. (2012) analyzed the impact of silica nanoparticle application (12 nm in size, 8 grams per liter) on the germination of *Solanum lycopersicum* seeds (*L. esculentum* Mill. cv. B strain). Silica nanoparticles notably improved seed germination, mean germination time, index of seed germination, and fresh weight of the seedlings. In a different research, Sun et al. (2016) scrutinized the results of mesoporous silica nanoparticle application (0 to 2000 milligrams per liter) on wheat and lupine plants' development, grown in nutrient solution. They reported that the mesoporous silica nanoparticles absorbed by the roots were relocated to chloroplasts, and they substantially raised photosynthetic activity. Mesoporous silica nanoparticles at dosages of 500 and 100 milligrams per liter also boosted germination of seeds and enhanced total biomass, content of total protein, and the chlorophyll amount.

16.6.7 Impact of Other Nanoparticles on Plant Species

In crop plants, biotic and abiotic stresses diminish photosynthetic rate in leaves and subsequently the crop yield. Nanoparticles on their own will not aid the nutrients that are essential and crucial for development of plant species; they can enhance rate of photosynthesis.

In the research conducted by Giraldo et al. (2014), application of single-walled carbon nanotubes (5 milligrams per liter) boosted photosynthesis rate in spinach leaves by 31%, which might be attributed to the improvement in the transfer rate of electrons. Chandra et al. (2014) reported that carbon quantum dots in concentrations of 50, 54.2, and 75 milligrams per liter enhanced non-cyclic photo-phosphorylation, synthesis of ATP, and release of oxygen in isolated chloroplasts of mung bean plants. This result might be attributed to a raised transfer rate of electrons in thylakoid membranes. Moreover, nanoparticles boost photosynthetic rate by light harvesting, transfer of electrons, and ROS scavenging, as reported by Swift et al. (2019) and Liu et al. (2019). Their research enhanced the knowledge in the utilization of nanofertilizers with agricultural applications to boost plant development and yield.

16.7 Conclusion

Plant nanobionics is a rapidly evolving research area, due to its tremendous amount of applications in different fields. It involves the utilization of special nanoparticles to augment different processes like photosynthesis and yield production. Several nanopesticidal and nanofertilizer applications of various nanoparticles such as silver, copper, carbon, titanium, cerium, magnesium, iron, zinc, manganese, molybdenum, and silicon have been discussed in this chapter. Nanobionics can boost plants' endurance and reduce stress effects under harsh environments and, thus, boost their yields. It is a newly developed field, and therefore, more detailed and extensive research is needed to obtain greater benefits for society.

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Chapter 17
Phytoremediation Capacity of Medicinal Plants in Soils Contaminated with Heavy Metals

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Abstract Phytoremediation is a set of technologies that reduce, in situ or ex situ, the concentration of various compounds through biochemical processes carried out by plants. Phytoremediation uses plants to remove, reduce, transform, mineralize, degrade, volatilize, or stabilize contaminants present in soil, water, and air. Plants to be used for phytoremediation are selected primarily for their physiological characteristics, such as presence of specific enzymes, tolerance and assimilation of toxic substances, plant growth rates, root depth, and ability to bioaccumulate and/or degrade contaminants. A wide diversity of species has been utilized in

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phytoremediation. Some of these are known as hyperaccumulators, due to their high capacity to accumulate heavy metals. Phytoremediation offers several advantages, among which are the following: (i) it constitutes a sustainable technology, (ii) it is carried out without the need to transport the contaminated substrate (thus decreasing the dissemination of contaminants through air or water), (iii) it is a set of technologies that are efficiently applied to both organic and inorganic contaminants, and (iv) its cost-effectiveness is driven by conventional agronomic practices, i.e., specialized personnel and energy are not required. The benefits of the practice are also associated with the following: (v) it is minimally harmful to the environment, (vi) it improves the physical and chemical properties of the soil due to the formation of vegetation cover, (vii) it has a high probability of being accepted by the public as the plants involved are often pleasing to the eye, (viii) it does not involve excavation work and heavy traffic, and (ix) it can be employed in water, soil, air, and sediments and allows for the recycling of resources (e.g., water, biomass, metals). Therefore, this chapter aims to (a) select the main medicinal plants with the potential to phytoremediate soils contaminated by heavy metals (e.g., uranium, copper, nickel, cobalt, mercury, cadmium, lead, chromium, zinc, selenium, aluminum, iron, and manganese), (b) report on the tolerance mechanisms of phytoremediation, and (c) indicate the concentration and accumulation levels of toxic heavy metals in medicinal plants.

Keywords Bioconcentration · Heavy metals · Hyperaccumulator plants · Phytoremediation · Phytotechnologies

17.1 Introduction

Phytoremediation is the process that seeks to decontaminate different environments using plants, extracting contaminants from the environment. Depending on the species, these plants have the capacity to remove different contaminants such as pesticides and heavy metals, thus preventing their spread through the soil and surface and groundwater (United States Environmental Protection Agency-A 2001). The use of plants to remove pollutants from the environment is based on taking advantage of their natural processes. These processes include absorption by roots, transpiration by leaves, and enzymatic activity, which in some cases have the capacity to transform contaminants into useful elements for the plant, thus removing them from the environment (Pilon-Smits 2005). Heavy metals in the environment originate due to naturally occurring geological processes and human activities (Bradl 2005). Their high toxicity and the constant increase of these activities have raised great concern for assessing and remediating the environmental contamination generated by these elements. Phytoremediation has been widely accepted as an emerging technology, thanks to its qualities that characterize it as an environmentally sustainable technology. In addition, its low implementation cost makes it cost-effective in the long term (Gerth et al. 2000). Even so, there are many questions about how phytoremediation works. Research and study of this technology still have a long way to go to accurately understand all the processes involved in phytoremediation. Important tools in the investigation of phytoremediation processes are genetics and molecular biology, which can be key in taking advantage of all the potential that this technology has to offer (Pilon-Smits 2005). Even so, phytoremediation has limitations such as the lack of knowledge of all the physiology involved in the phytoremediation process or the identification of the appropriate plants to use depending on the contaminated medium or contaminant. Therefore, this chapter aims to (a) select the main medicinal plants with the potential to phytoremediate soils contaminated by heavy metals (e.g., uranium, copper, nickel, cobalt, mercury, cadmium, lead, chromium, zinc, selenium, aluminum, iron, and manganese), (b) report on the tolerance mechanisms of phytoremediation, and (c) indicate the concentration and accumulation levels of toxic heavy metals in medicinal plants.

17.2 Advantages of Phytoremediation

Phytoremediation offers several advantages. Its main attraction lies in the fact that decontamination processes are carried out with a relatively low investment. Likewise, its implementation improves the environmental quality of the area where it is used, reducing the erosion caused to the soil by the effect of water and wind, preventing pollutants from dispersing, and improving soil quality at the same time. This same effect is generated when applied in water bodies, making technology generate additional processes of improvement of the environmental characteristics, proving to be a process that can be implemented in a sustainable development framework (Pilon-Smits 2005). Another characteristic that makes this technology so attractive is its cost-effectiveness. Conventional processes such as the use of activated carbon, physicochemical separation of contaminants, filtration, soil washing, or aerobic and/or anaerobic processes represent a high cost and even more when implemented in large areas (Susarla et al. 2002).

Energy requirements are the main aspect that raises the costs of a decontamination process, and this is where phytoremediation presents a great advantage. The fact that plant activity is based on the use of sunlight means that operating costs can be up to 80% less than conventional processes (Moriwaka and Erkin 2003). This characteristic makes phytoremediation a technology with a great future in developing countries such as ours. Considering that the application of phytoremediation processes can be carried out in situ, it usually improves the environmental conditions of the area to be decontaminated, and added to the fact that phytoremediation processes are carried out naturally, this technology does not require a large investment. In fact, the simplest phytoremediation mechanism does not require human interference, only monitoring. This process, called natural attenuation, generates a lower level of decontamination than one where there is constant intervention, but it is nevertheless considerable (Pilon-Smits 2005). Around the world, phytoremediation has been widely accepted because it has been called a “green” technology. It is considered environmentally sustainable, mainly because it does not generate greenhouse gases and helps to conserve natural resources, so much that the European Union and the United States allocate considerable resources for research in this area (Lasat 2000; Barceló and Poschenrieder 2003).

17.3 Important Factors in Phytoremediation Processes

In recent years, soil and water contamination has taken on great relevance in our society. The emergence of new technologies of high efficiency and low cost generates viable alternatives for the management of environmental contamination, particularly in developing countries. Phytoremediation is one of these alternatives. There are several characteristics that the plants of a phytoremediation system must have to achieve good performance (Godwin and Thorpe 2000) (Pulford and Watson 2003).

Some of these characteristics are listed below:

1. Tolerance to the contaminant: it is of utmost importance that the plant can tolerate the contaminant and carry out its development in its presence.
2. Remediation capacity: remediation must be carried out since this is the fundamental objective of the implementation of phytoremediation.
3. Reproduction and growth: the plant must have a rapid growth that allows good pollutant removal rates and optimizes the phytoremediation processes. It is also important that they maintain their reproductive capacity in the presence of disturbances through succession.
4. Biomass production: with a greater biomass, the plant can carry out a greater removal of pollutants.
5. Resistance to stress: it is important that the plant can resist stress situations generated by chemical, physical, biological, or climatic conditions.
6. Native plants: if possible, it is advisable to use native plants to alter the local ecosystem as little as possible.

17.4 Plant Strategies

Plants present different strategies to adapt to the presence of pollutants in the environment where they develop.

17.4.1 Accumulator Plants

These plants have the capacity to accumulate pollutants in their tissues. These are found in much higher concentrations than those present in the environment (Ghosh and Singh 2005). Table 17.1 shows the main metal hyperaccumulator plants.

Table 17.1 Main families and genera of metal-hyperaccumulating species

Element	Number of taxes	Main families	Main genus
Ni	317	Violaceae	*Hybanthus*
		Flacourtiaceae	*Homalium*
		Euphorbiaceae	*Phynthus*
		Brassicaceae	*Alyssum* L.
Zn	18	Brassicaceae	*Thlaspi* and *Cardaminopsis*
Pb	14	Poaceae	*Arrhenatherum*
		Brassicaceae	*Thlaspi*, *Brassica*
		Fabaceae	*Sesbania*
		Apocynaceae	*Hemidesmus*
		Plantaginaceae	*Plantago*
Cu	37	Lamiaceae	*Aeollanthus*
		Scrophulariaceae	
		Convolvulaceae	*Ipomoea*
		Crassulaceae	*Crassula*
		Commelinaceae	*Commelina*
Co	28	Lamiaceae	*Haumaniastrum*
		Scrophulariaceae	*Crotalaria*
		Asteraceae	
		Fabaceae	
Mn	9	Myrtaceae	*Austromyrtus* and *Gossia*
		Phytolaccaceae	*Phytolacca*
		Proteaceae	*Virotia*
		Celastraceae	*Maytenus*
cd	4	Brassicaceae	*Thlaspi* and *Arabidopsis*
		Asteraceae	*Bidens*

17.4.2 Indicator Plants

These plants also accumulate contaminants; however, their concentrations are usually a reflection of the concentration present in the medium, increasing or decreasing as the medium fluctuates (Ghosh and Singh 2005).

17.4.3 Exclusionary Plants

These plants prevent the entry of contaminants into their aerial tissues. They usually retain them in their roots, immobilizing them and preventing them from being transported in the medium (Ghosh and Singh 2005). In this way, they do not allow pollutants to be leached.

17.5 Bioavailability Associated with pH

For the phytoremediation process to take place, it is necessary that contaminants are available for the removal processes carried out by plants. The conditions of the medium can determine how efficient the process can be. In the case of heavy metals, plants generally need to find the chemical elements in their soluble-water form to incorporate them into their metabolic processes. In this aspect, the pH plays a fundamental role because it is usually found in an acidic environment form. As pH increases, the availability of heavy metals in their dilute form decreases, and they tend to form other compounds that the plant can use (Sandrin and Hoffman 2007). It is important to consider the valence of the metal, which will define whether it will be bioavailable or not under the present conditions.

Plants possess highly specialized mechanisms to stimulate the availability of metals in the rhizosphere and thus increase the ability of roots to remove metals from the medium. It has been found that some plants can exude mugineic and avenic acid and can even release H^+ ions directly from the roots, acidifying the medium to increase the availability of metals in diluted form (Lasat 2002).

17.6 Phytoremediation Processes

Plants tend to take up pollutants from the medium in which they are found, whether in soil or water (Lasat 2002). For example, some metals are essential nutrients such as copper, zinc, and nickel, while others do not appear to have any physiological activity such as mercury and cadmium (Lasat 2002). Adequate levels of required micronutrients in plant tissue are diverse, for example, the concentration in weights for zinc is 20 ppm, for copper 6 ppm, and for nickel 0.1 ppm (Taiz and Zeiger 2006).

Although plants can accumulate different metals, they can become toxic in high concentrations. However, there are plants that have the capacity to accumulate these metals in high concentrations without being affected in their growth or physiological activities. These plants are called hyperaccumulators (Salt et al. 1998). There are different ranges of heavy metal concentrations and their phytotoxicity in tolerant plants, for example, for cadmium (normal, 0.05–2 mg/kg; toxic, 5–700 mg/kg), for copper (normal, 3.5–30 mg/kg; toxic, 20–100 mg/kg), for lead (normal: 0.5–10 mg/kg), and for zinc (normal, 10–150 mg/kg; toxic, (100 mg/kg) (Padmavathiamma and Li 2007). Examples of hyperaccumulator plant species are as follows: *Thlaspi calaminare* accumulates 3960 ppm zinc, *Aeollanthus biformifolius* accumulates 1370 ppm copper, *Phyllanthus serpentinus* accumulates 3810 ppm nickel, and *Thlaspi caerulescens* accumulates 1800 ppm cadmium (Bradl 2005).

Phytoremediation can be applied under three models. The first is in situ phytoremediation. This method consists of the implementation of phytoremediation plants in the area where the contaminated soil or water is located. It is important to consider that the plant can have access to the contaminants in order to carry out an efficient decontamination (Susarla et al. 2002). The second model is ex situ phytoremediation, which occurs when the plant does not have access to the pollutants being phytoremediated. Generally, treatment areas are designated where the contaminants are moved for the plants to carry out the phytoremediation process. Once the contaminants are removed from the environment, the remediated soil or water is returned to the area from which it was extracted. Moving the contaminants generates a higher cost than the in situ method (Susarla et al. 2002).

Finally, there is in vitro phytoremediation. In this method the phytoremediation process is achieved not directly with the plant but by isolating and using the elements that the plant uses to carry out phytoremediation, such as its enzymes. For example, these components can be used by adding plant extracts to the contaminated water volume. This method also requires the designation of a treatment area. If to this we add the need of understanding the behavior of each enzyme, we can conclude that this is the most expensive method (Susarla et al. 2002). There are several phytoremediation processes, depending on the systems and strategies that the plant uses to carry out the decontamination. In some cases, more than one may be present in the process of contaminant removal.

17.7 Phytoextraction or Phytoaccumulation

Also called phytoaccumulation, phytoextraction consists of the capture and transport of pollutants present in the environment, which are then transferred and stored in different plant tissues. In these tissues, contaminants are accumulated in very high concentrations. Phytoextraction consists in the absorption of contaminants by roots; it is the capacity of some plants to accumulate contaminants in their roots, stems, or foliage. This mechanism has been extensively studied in plants that accumulate metals and recently radioactive materials.

This process is commonly known as phytomining. It has the advantage that after harvesting the plant material and depending on the type of treatment for its disposal, the contaminants can be recycled. In the case of soil application, the material for disposal is almost 10% of what it would be to directly dispose contaminated soil (Moriwaka and Erkin 2003; Brooks et al. 1998).

17.8 Phytovolatilization

This process consists in the capture of contaminants by the plant roots and then, through enzymatic reactions, converted into volatile compounds and released into the atmosphere through the stomata in the leaves. This occurs as growing plants absorb water along with soluble organic contaminants. Some of the contaminants may reach the leaves and evaporate or volatilize into the atmosphere. (Núñez et al.

2004). This type of process works for highly volatile metals such as mercury and arsenic. This process is influenced by environmental conditions such as humidity, temperature, and wind speed (Moriwaka and Erkin 2003).

17.9 Phytodegradation

Also known as phytotransformation, phytodegradation consists in the transformation of organic pollutants into simpler molecules. In some cases, the degradation products are used by the plant to accelerate its growth, and in other cases the pollutants are biotransformed; this is a process where the plant takes contaminants from the environment and through physiological processes, including enzymes and their cofactors, degraded to be incorporated into plant tissues and used as nutrients. Generally, the final compounds are less toxic (Gerth et al. 2000; Susarla et al. 2002).

17.10 Rhizodegradation

In this process, pollutants are degraded in the rhizosphere. These processes are mainly carried out by microbial activity associated with the rhizosphere. Also, some exudates produced by the plant can induce bacterial enzymes or serve as food for microorganisms and help enhance the degradation of pollutants, and the plant can maintain optimal conditions for microorganisms, for example, by releasing oxygen to promote aerobic conditions.

17.11 Filtration and Rhizofiltration

It is defined as the use of plants with the capacity to absorb, concentrate, and, in some cases, precipitate contaminants present in aqueous solutions such as lead, cadmium, copper, chromium, zinc, and nickel. For their efficiency, it’s essential that plants have roots with a large surface area. Very fibrous roots have a high removal capacity (Subroto et al. 2007). This type of process is particularly used to treat industrial discharges and runoff from agricultural areas. It can be implemented both ex situ and in situ. Once the plants reach a saturation level, they are harvested and transported for disposal. The combination of rhizofiltration and harvesting activity generates a process equivalent to phytoextraction. Similarly, the hydraulic force generated by the absorption of water from the trees prevents the leaching of pollutants (Pilon-Smits 2005). This process is based on the utilization of plants grown in hydroponic cultures; roots of terrestrial plants with high growth rate and surface area are preferred to absorb, concentrate, and precipitate contaminants.

17.12 Phytostimulation or Rhizodegradation

Plants generate root exudates that stimulate the growth of native microorganisms capable of degrading xenobiotic organic compounds.

17.13 Phytostabilization

A mechanism that uses the plant to develop a dense root system that allows it to reduce the unavailability and mobility of pollutants by preventing their transport to underground layers or to the atmosphere.

17.14 Biological Models of Phytoremediation

Calow (1993) points out the potential of using onion (*Allium cepa* L., Liliaceae), beet (*Beta vulgaris* L., Chenopodiaceae), rice (*Oryza sativa* L., Poaceae), and radish (*Raphanus sativus* L., Brassicaceae) to assess the toxicity and risk of hazardous chemicals in the environment. However, the effect of heavy metals on vascular plants is not well known (De Jong and De Haes 2001). Other metals, such as cadmium (Cd) and zinc (Zn), can be absorbed to a greater extent in plants such as radishes and carrots. Radish leaves accumulate a higher content of the metal, causing wilting and a decrease in root length and biomass, while carrots report the same degree of root shortening and a greater accumulation of the metal (Intawongse and Dean 2006).

Manganese (Mn) available in some soils can substitute essential elements for plants such as calcium (Ca) and magnesium (Mg) (Kabata-Pendias 2000). In studies conducted on radish and spinach plants, it was observed that Mn tends to accumulate in radish leaves and a high content in spinach leaves and lower concentrations in roots of both plants (Intawongse and Dean 2006). As can be seen, the tendency of this metal, although it may be a metal absorbed by soils to a lesser degree, is more easily absorbed by plants and has greater mobility to reach the different parts or organs of the plant, causing damage to them. Metal-producing plants: both on land and in water, some species that you may be familiar with have a great potential to accumulate heavy metals – A. sunflower (*Helianthus annuus* L.), B. red mangrove (*Rhizophora mangle* L.), C. mustard (*Sinapis alba* L.), D. castor bean (*Ricinus communis* L.), E. duckweed (*Lemna minor* L.), F. water lily (*Eichhornia crassipes* (Mart.) Solms.), G. salvinia (*Salvinia natans* L.), and H. white water lily (*Nymphaea ampla* L.).

Actually, the use of medicinal plants has increased worldwide. However, to guarantee the safe use of medicinal plants, it is necessary to quantify their heavy metal content. Recently, Carranza-Álvarez et al. (2016) quantified the concentration of two toxic elements, cadmium and lead (Cd and Pb), and of a micronutrient, iron (Fe), in three medicinal plants (*Justicia spicigera*, *Arnica Montana*, and *Hamelia patens*) collected from three different sites in two municipalities of the Huasteca Potosina, Mexico. Cd and Pb are two toxic elements with no known biological function, which can cause severe damage when introduced into the food chain, while iron is an essential element. However, iron is a major element that can form oxides and trap trace elements such as Pb and Cd and favor bioaccumulation of these elements in plants. It is important to mention that in Huasteca Potosina the three plants mentioned above are used for empirically treatment of iron deficiency. Therefore, it was important to study the concentration of the three elements in both medicinal plants. The results of this analysis showed that the medicinal plant preparations contain higher values than those considered safe for Cd and Pb, respectively. Furthermore, it is confirmed that these three medicinal plants could be an important source of Fe. This suggests that the collection of medicinal plants should be carried out in sites free of potentially hazardous toxic elements.

Some phytoremediating species of lead (Pb) are *Melastoma malabathricum* (Selamat et al. 2014), *Cynodon dactylon* L. (Prabha and Li 2007), *Adiantum capillus-veneris* L. (Yanqun et al. 2005), *Helianthus annuus* (Arias et al. 2015), *Paspalum notatum* (Yoon et al. 2006), *Bidens alba*, *Cyperus esculentus*, *Rubus fruticosus*, and *Plantago major* L. Some phytoremediating species of lead (Pb) and cadmium (Cd) are *Amaranthus hybridus* (Ortiz-Cano et al. 2009; Boonyapookana et al. 2005), *Catharanthus roseus* (Subhashini and Swamy 2017), *Matricaria chamomilla* (Voyslavov et al. 2013), *Artemisia princeps* (Morishita and Boratynski 1992), *Artemisia vulgaris* (Jara-Peña et al. 2014), *Solanum nitidum*, *Brassica rapa*, *Fuertesimalva echinata*, *Urtica urens* L, and *Lupinus ballianus*. Some lead (Pb), cadmium (Cd), and mercury (Hg) phytoremediation species are *Lupinus albus* (Fumagalli et al. 2014), *Artemisia absinthium* (Zornoza et al. 2010), and *Artemisia campestris* (Radanović and Antić-Mladenović 2012). The species *Lupinus uncinatus* is a cadmium (Cd) phytoremediation plant (Ehsan et al. 2009).

17.15 Contaminants

Phytoremediation is a technology applicable to the remediation of several organic and inorganic pollutants; however, the characteristics of each pollutant determine the type of phytoremediation process to be carried out as well as the type of plant to be used. Organic pollutants originate mainly from anthropogenic activities such as the use of herbicides and pesticides in agriculture and the use of solvents, fuels, or industrial wastes. For the phytoremediation of these pollutants, their bioavailability, mobility, solubility, and the presence of other compounds must be considered.

Likewise, the time it takes to implement phytoremediation for their treatment must be considered, since the longer the delay in its application, the lower the remediation efficiency (Cunningham and Ow 1996). Inorganic contaminants, such as heavy metals, can be found naturally. They become toxic in high concentrations, and their presence is mainly altered by anthropogenic activities such as agriculture, mining, and industrial activities. The removal of these pollutants is influenced in the same way by the previously mentioned aspects (Bradl 2005).

17.16 Origin and Effects

There are several natural sources of heavy metals, including igneous or magmatic rocks as well as sedimentary rocks. The composition and content of metals vary according to the type of rock (Bradl 2005). Some rocks of this type are biotite, apatite, albite, muscovite, and magnetite (Mitchell 1964). Hydrothermal sources contribute important quantities of metals, as well as volcanic activity that brings material to the surface of the earth's crust. Even winds can play an important role by transporting ash and dust and depositing it over long distances. High weathering of minerals and metal ions in rocks, as well as erosion, play an important role in the dynamics and mobility of metals (Bradl 2005). There are also many anthropogenic sources. Agricultural activities generate a considerable number of heavy metals, mainly associated with agrochemicals. The use of phosphorus-based fertilizers contains significant amounts of zinc and cadmium. Similarly, the pesticides used generate a considerable amount of arsenic, lead, and mercury (Bradl 2005). In addition, untreated industrial sewage sludge contains considerable loads of cadmium, zinc, copper, lead, selenium, molybdenum, chromium, arsenic, and nickel (Adriano 1992). Mining generates an important contribution due to the oxidizing environment that acts on the extracted rocks, generating acidic conditions that drain the material, mobilizing metals such as cadmium, arsenic, copper, mercury, and lead (Bradl 2005). Emissions from fuel burning contribute significantly to atmospheric levels.

17.17 Heavy Metals

The term heavy metal is defined as metallic elements that have a relatively high density and is toxic or poisonous at even very low concentrations. Examples of heavy metals, or some metalloids, include mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), and lead (Pb), among others (Lucho et al. 2005).

Heavy metals are generally found as natural components of the earth's crust, in the form of minerals, salts, or other compounds. They cannot be easily degraded or destroyed naturally or biologically because they do not have specific metabolic functions for living organisms (Abollino et al. 2002).

Heavy metals are hazardous because they tend to bioaccumulate in different crops. Bioaccumulation means an increase in the concentration of a chemical in a living organism over a certain period compared to the concentration of that chemical in the environment (Angelova et al. 2004). The uptake of heavy metals by plants is generally the first step for their entry into the food chain. Uptake and subsequent accumulation depend primarily on the movement (species mobility) of metals from solution in the soil to the plant root. In plants, the concept of bioaccumulation refers to the aggregation of contaminants; some of them are susceptible of being more phytoavailable than others (Kabata-Pendias 2000).

17.18 Phytoremediation Mechanism for Heavy Metals

The first pollutants subject to phytoremediation processes were heavy metals due to the identification of several hyperaccumulator plant species. Likewise, the wide use of heavy metals in industry and everyday items, such as batteries, generated a particular interest in their removal from the environment. The fact that traditional removal methods were so expensive somehow promoted the use of phytoremediation as an efficient and economical method for their removal (Lasat 2000; United States Department of Agriculture 2000). A particular characteristic of plants is that they tend to absorb metals that are used in various metabolic processes such as copper, nickel, and zinc. On the other hand, other metals have no function in the plant but are important pollutants such as cadmium, mercury, or lead. Several of these metals are toxic to different organisms, including humans, affecting populations present in contaminated sites (Pilon-Smits 2005; Li et al. 2000).

Virtually all phytoremediation processes can be carried out in the removal of heavy metals. In the case of rhizofiltration, it uses plants with high tolerance to these pollutants by immobilizing them in the rhizosphere. Here, metals are accumulated or precipitated avoiding their integration into the trophic chain. In this process, the implementation of water cress (*Eichhornia crassipes*) and some grasses such as *Festuca rubra* and *Agrostis capillaris* for the phytoremediation of zinc, nickel, arsenic, chromium, and lead has been successful (Ingole and Bhole 2003; United States Department of Agriculture 2000; Li et al. 2000; Wing et al.2003).

Eichhornia crassipes has been reported as a plant with a high potential to accumulate metal ions (Larcher 2003). This capacity varies depending on the type of metal present in the water, as well as the environmental conditions. *Eichhornia crassipes* works very efficiently for low concentrations of heavy metals, between 5 and 10 mg/L (Skinner et al. 2007; Hu et al. 2007); in some cases removal can reach 94% for zinc and 84% for chromium (Tripathi and Mishra 2009). At higher concentrations, a reduction in the removal efficiency of arsenic, chromium, mercury, and lead has been noted, where the process is maintained, but the higher concentration in the plant seems to reach a limit of accumulation eventually preventing the macrophyte from removing more metals from the water, although this effect is not as marked for nickel and zinc (Ingole and Bhole 2003).

In the case of chromium, the association of the cudweed with chromium-reducing bacteria improves the removal of chromium from the water body. This microbial activity concentrated in the rhizosphere reduces chromium from Cr (VI) to Cr (III), which is less toxic. Although the removal by the crawfish decreases, the total removal increases (Faisal and Hasnain 2005). When using chromium-tolerant bacteria, the highest concentration of this metal is found in the roots, probably associated with the high activity in the rhizosphere (Abou-Shanab et al. 2007). For cadmium, the cudweed has been reported as a plant with high removal capacity, with high transfer from the root to other parts of the plant. In this case, in addition to immobilization in cell walls, phenomena such as adsorption and chelation may be of relative importance (Toppi et al. 2007). Another process used for metal removal is phytovolatilization. This has been carried out using plants such as *Arabidopsis thaliana* or commercial plants such as broccoli. In the first case, it has the capacity to reduce the mercuric ion Hg^{2+} to Hg^0 which is phytovolatilized by the stomata of the plant. Broccoli, on the other hand, performs a similar process by volatilizing selenium (Rugh et al. 1996; Zayed and Terry 1994).

The most used process in phytoremediation of heavy metals is phytoextraction. Hyperaccumulator plants which can accumulate heavy metals in concentrations of up to 1000 mg/kg dry weight in their aerial tissues are used. It is also believed that such concentrations serve as a defense against pathogens and predators such as fungi and insects. This phytoextraction is most efficient when copper, nickel, or zinc is involved since they are elements that are incorporated into metabolic pathways. An example of this is the removal of zinc by *Thlaspi caerulescens* where it has been found to be able to accumulate up to 26,000 ppm of zinc without any apparent damage. This is not the case for some metals such as lead (Pulford and Watson 2003; Lasat 2002; United States Department of Agriculture 2000; Li et al. 2000). For phytoremediation processes of heavy metals to be efficient, several aspects must be considered. In the first place is the degree of tolerance of the plant to the metal since this will define its accumulation capacity. Likewise, optimum plant growth must be guaranteed. The use of plant hormones such as cytokinins has been considered to increase growth and biomass (Salt and Kramer 2000; Khan 2005). The bioavailability of the metal is another fundamental factor since only the available fraction can be removed by the plant. The use of chelating agents can increase the metal removal capacity by increasing metal availability. Metal uptake depends on the ability to pass through the root cell walls either passively or actively. Finally, root transport to aerial tissues is very important for good accumulation (Salt and Kramer 2000). It is of utmost importance to know the life cycle of the plant in the different environments where it is found. Once the plant completes its cycle, its decomposition can lead to the release of heavy metals previously removed from the environment. This type of information is important to develop a biomass removal or harvesting plan that ensures the highest possible removal efficiency.

In the case of arsenic, it was found that *Eichhornia crassipes* presented removal processes of about 21 days (Alvarado et al. 2008). Heavy metals are more dangerous because they are neither chemically nor biologically degradable. Once released, they can remain in the environment for hundreds of years. Moreover, their concentration in living beings increases as they are ingested by others, so the ingestion of contaminated plants or animals can cause poisoning symptoms.

17.19 Presence of Heavy Metals in Soil

Heavy metals are present in soils as natural soil components or as a consequence of anthropogenic activities. Different metals can be found in soils, forming part of their own minerals, such as silicon (Si), aluminum (Al), iron (Fe), calcium (Ca), sodium (Na), potassium (K), and magnesium (Mg). Manganese (Mn) can also be found, which is generally present in the soil as an oxide and/or hydroxide, forming concretions together with other metallic elements. Some of these metals are essential in plant nutrition, so some of them are required, such as Mn, essential in the photosystem and activation of some enzymes for plant metabolism (Mahler 2003). Figure 17.1 shows responses of tolerant plants to the presence of heavy metals in soil.

Heavy metals include elements such as lead, cadmium, chromium, mercury, zinc, copper, and silver, among others, which constitute a group of great importance, since some of them are essential for cells, but in high concentrations they can be toxic for living beings, soil organisms, plants, and animals (Spain 2003), including humans. In the Earth's crust, there is a similarity of distribution between nickel (Ni), cobalt (Co), and iron (Fe). In the surface soil horizons (arable layer), Ni is bound to organic

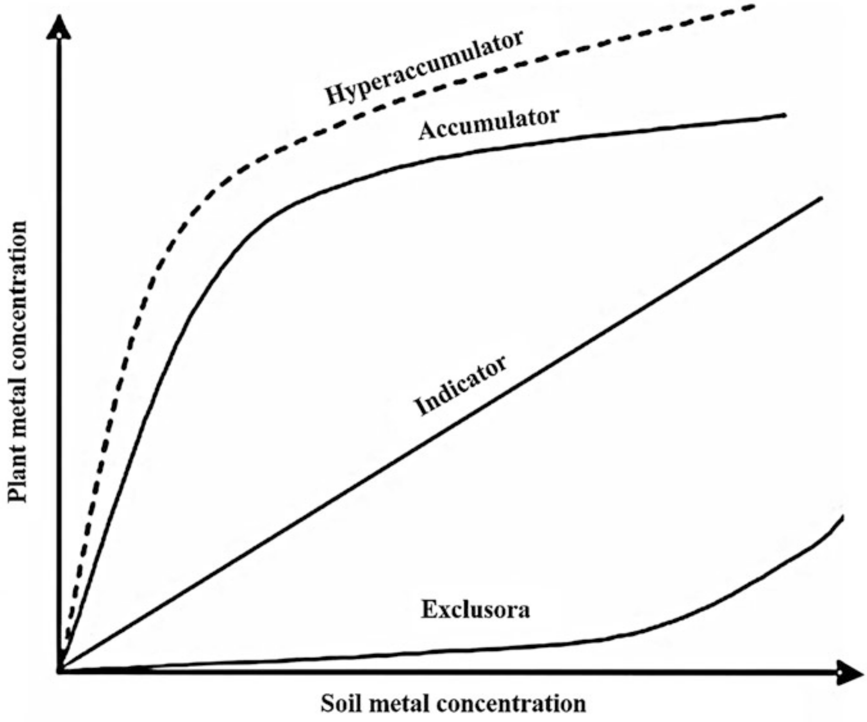

Fig. 17.1 Responses of tolerant plants to the presence of heavy metals in soil

forms (Corinne et al. 2006), part of which can be found as easily soluble chelates. Nickel (Ni) is also an essential element for plant metabolism, even though plants require less than 0.001 mg kg^{-1} dry weight (Mahler 2003). Zinc (Zn) can also be found naturally in soils and is a nutrient required by plants for their development (Mahler 2003).

Natural geological activities, such as the erosion of hills and volcanoes, are an important source of heavy metal inputs into the soil. Anthropogenic activities such as the mining industry, which is listed as one of the industrial activities that generates most of the heavy metals, are also a source of heavy metals. In the soil, heavy metals can be present as free or available ions, compounds of soluble metal salts, or insoluble or partially soluble compounds such as oxides, carbonates, and hydroxides (Pineda 2004).

The relative mobility of trace elements in soils is of utmost importance in terms of their availability and their potential to leach from soil profiles into groundwater and differs depending on whether their origin is natural or anthropogenic and, within the latter, on the type of anthropogenic source (Burt et al. 2003).

Among the heavy metals are the so-called trace elements, which can serve as micronutrients for crops, since they are required in small quantities and are necessary for organisms to complete their life cycle. After a certain threshold, they become toxic, such as B, Co, Cr, Cu, Mo, Mn, Ni, Fe, Se, and Zn and the metalloid As. There are also heavy metals with no known biological function, whose presence in certain quantities in living beings leads to dysfunctions in the functioning of their organisms. They are highly toxic and have the property of accumulating in living organisms, elements such as Cd, Hg, Pb, Sb, Bi, Sn, and Tl (García and Dorronsoro 2005). When the content of heavy metals in the soil reaches levels that exceed the maximum permissible limits, they cause immediate effects such as inhibition of normal plant growth and development and a functional disturbance in other components of the environment as well as a decrease in soil microbial populations; the term used or employed is soil pollution (Martin 2000).

In the soil, heavy metals as free ions can have a direct action on living beings through the blocking of biological activities, i.e., enzymatic inactivation by the formation of bonds between the metal and the -SH (sulfhydryl) groups of proteins, causing irreversible damage to the different organisms. Soil contamination by heavy metals occurs when soils are irrigated with water from mine wastes, contaminated wastewater from industrial and municipal parks, and seepage from tailings dams (Wang and Chao 1992). Once in the soil, heavy metals can be retained in the soil, but they can also be mobilized into the soil solution by different biological and chemical mechanisms (Pagnanelli et al. 2004).

Heavy metals added to soils are slowly redistributed and distributed among the components of the soil solid phase. Such redistribution is characterized by rapid initial retention and subsequent slow reactions, depending on the metal species, soil properties, level of introduction, and time (Han et al. 2003).

The factors that influence the mobilization of heavy metals in soil are soil characteristics: pH, redox potential, ionic composition of the soil solution, exchange capacity (cationic and/or anionic), presence of carbonates, organic matter, and texture, among others. The nature of the contamination and the origin of the metals and forms of deposition and environmental conditions produce acidification and changes in redox conditions, temperature, and humidity variations in soils (Sauquillo et al. 2003).

In general, heavy metals incorporated into the soil can follow four different pathways: first, they are retained in the soil, either dissolved in the soil aqueous phase or occupying exchange sites; second, they are specifically absorbed on inorganic soil constituents; third, they are associated with soil organic matter; and fourth, they are precipitated as pure or mixed solids. On the other hand, they can be absorbed by plants and thus incorporated into trophic chains; they can pass into the atmosphere by volatilization and can be mobilized to surface or groundwater (García and Dorronsoro 2005).

To elucidate the behavior of heavy metals in soils and prevent potential toxic risks requires the evaluation of their availability and mobility (Banat et al. 2005). Metal toxicity depends not only on their concentration but also on their mobility and reactivity with other components of the ecosystem (Abollino et al. 2002).

Heavy metals contribute strongly to environmental pollution; the number of metals available in the soil is a function of pH, clay content, organic matter content, cation exchange capacity, and other properties that make them unique in terms of pollution management (Sauve et al. 2000).

Lead (Pb), for example, is a highly toxic environmental contaminant; its presence in the environment is mainly due to anthropogenic activities such as industry, mining, and smelting. In soils contaminated with lead (Pb), cadmium (Cd), and zinc (Zn) (Hettiarchchi and Pierzynski 2002) are also often found (Hettiarchchi and Pierzynski 2002) due to the analogy between their metallic properties and characteristics, something similar to what occurs for the Fe-Ni-Co triad.

In these cases, the soil-plant barrier limits the translocation of Pb into the food chain, either by chemical immobilization processes in the soil as reported by Laperche et al. (1997) or by limiting plant growth before the absorbed Pb reaches values that can be harmful to humans. Pb present in contaminated soils can be inhibited by the application of phosphorus and magnesium oxides; however, these treatments can affect the bioavailability of other essential metals such as Zn (Hettiarchchi and Pierzynski 2002).

On the other hand, in places where wastewater has been used for agricultural irrigation, an increasing trend in the concentrations of metals in soils has been reported, because of the time (years) of use of this water, where the quantities of metals extracted and measured in these soils have been positively associated with the time of use of wastewater; Ni and Pb show a higher annual accumulation rate. In soils studied with different pH and contents of clay and organic matter, and where concentrations of Pb and Zn have been intentionally added, the capacity of absorption of these metals in each type of soil has been determined. Lettuce was planted, and after harvesting, the soils were re-evaluated, and it was observed that the concentration of these metals in the soils decreased (Stevens et al. 2003), which shows that these contaminated soils are a health risk because plants can absorb these metals.

Also, in studies conducted on European soils, which varied in their physicochemical characteristics and with the objective of quantifying copper (Cu) uptake in plants, they were amended using $CuCl_2$ to obtain a range of seven concentrations including an unamended control. For these studies, barley and tomatoes were planted in these soils, and root elongation (for barley) and overall growth (for tomato) were evaluated in the plants. The Cu concentrations caused inhibition of root elongation and overall growth of the tomato plant; on the other hand, the most calcareous soils showed the highest Cu retention, which demonstrates once again that soil characteristics have a direct and determining influence on the concentrations of metals in the soil and on the levels of availability to plants (Rooney et al. 2006; Zhao et al. 2006).

Phytoremediation is not a simple remedy or recipe that is applicable to all contaminated soils; before this technology can become technically efficient and economically viable, there are some limitations that need to be overcome. For example, their molecular, biochemical, and physiological mechanisms are poorly known and insufficiently understood; however, despite this, a large number of plants defined as hyperaccumulators can still be made known and identified (Freitas et al. 2004). Phytoremediation of contaminated soils is a technique with great potential. The use of plant species tolerant to high levels of metals in soils and water allows restoration activities with less environmental impact on the land than other traditional techniques, which are more invasive and have adverse side effects (Robinson et al. 1997). Organic amendments can also be used for the remediation of contaminated soils. The bonding between organic matter and metals (formation of complex molecules of high stability) can decrease the phytoextraction capacity, thus decreasing phytotoxicity and allowing the reestablishment of vegetation on contaminated sites (Robinson et al. 1997).

Since 1991, the Chinese government has developed and reported guidelines for monitoring and evaluating heavy metal levels in contaminated sites (Chen et al. 1996; Wang et al. 1994). These guidelines are mainly based on soil properties and the effect of heavy metals on water quality, on the activity of microorganisms in soils, on human health, and on crop yields and quality. Three values have been formulated to assess soil quality: the A Values (defined as the upper limit of frequent concentration of heavy metals found in soils), B Values (defined as the acceptable level of heavy metals in soils), and C Values (excessive levels in very high concentrations of metals that indicate the need for intervention for solutions, i.e., pollution control becomes necessary and mandatory).

17.20 Bioaccumulation

Of all the traced elements present in the soil, there are 17 that are considered to be both highly toxic and readily available in concentrations that exceed toxicity levels. These are Ag, As, Bi, Cd, Co, Cu, Hg, Ni, Pd, Pt, Sb, Sn, Te, Tl, and Zn; of these, ten are easily mobilized by human activity in proportions far exceeding that of geological processes (Novotny 1995). What makes heavy metals toxic is not generally their essential characteristics but the concentrations at which they can occur, as they tend to bioaccumulate.

Bioaccumulation is defined as an increase in the concentration of a chemical in all living organism over time, compared to the concentration of the chemical in the environment, and almost more importantly, the type of species they form in a given environment.

17.21 Soil Contamination

The way to remediate soil contamination has been sought and solutions have been found, but they are costly and limiting. In the last decades of the twentieth century, technologies based on the use of living organisms emerged to decontaminate contaminated soils or sites and recover the affected ecosystems. When these technologies are based on the use of plants, they are globally known as phytoremediation (phytorecuperation, phytocorrection, phytorestoration). It is defined as the use of green plants to eliminate pollutants from the environment or to reduce their hazardousness (Salt et al. 1998). Phytoremediation mechanisms include the following.

17.22 Mechanisms of Phytoremediation

The absorption of pollutants takes place through the roots and leaves by means of the stomata and the cuticle of the epidermis. This absorption occurs in the rhizodermis of young roots, which absorb compounds by osmosis depending on external factors such as soil temperature and pH. Other important factors that influence contaminant penetration are their molecular weight and hydrophobicity, which determine that these molecules cross the plant cell membranes. After crossing the membrane, contaminants are distributed throughout the plant (Harvey et al. 2002). Contaminants that are taken up by the roots are excreted via the leaves (phytovolatilization). When contaminant concentrations are high, only small fractions (less than 5%) are excreted without changes in their chemical structure. Detoxification of organic compounds takes place via mineralization to carbon dioxide.

17.23 Conclusion

Phytoremediation is an emerging technology based on higher plants and microorganisms associated with the rhizosphere, which constitutes an option for in situ or ex situ removal of pollutants. In natural ecosystems, plants act as filters to remove, reduce, transform, mineralize, degrade, volatilize, concentrate, or stabilize pollutants (organic and inorganic) in soil, sludge, water, and sediments. The implementation of phytoremediation techniques is advisable because it generally does not alter ecosystems; it is carried out without harming them; it is a passive, friendly technique; and it is not harmful to the environment.

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Chapter 18
Stress-Tolerant Species of Medicinal Plants and Phytoremediation Potential

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Abstract Presently, there seems to be an increase in interest in cultivating medicinal plants across the globe. Medicinal plants offer huge potential to be grown on contaminated sites to recover soil health, in addition to oil production and eco-tourism, to address the rising demand for pharmaceuticals, essential oils, and bioenergy. In the present chapter, efforts have been made to collect and analyze available information regarding stress tolerance capabilities and the phytoremediation potential of medicinal plants, which will provide valuable insight into understanding the putative mechanisms involved in stress tolerance and pollution alleviation. The medicinal plants that can withstand stress and be used for the phytoremediation of environmental contaminants have also been explored.

Keywords Abiotic stressors · Heavy metals · Medicinal plants · Phytoremediation · Salinity · Stress tolerance

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18.1 Introduction

Humans have a strong bond with mother nature, which commenced with their birth on the earth. Humans do not live devoid of nature. Humans used to collect their food from nature and gradually learned the pharmacological value of the plants. Since the dawn of human civilization, medicinal plants have been the primary source for curing, healing, and alleviating a wide range of illnesses. These epiphanies resulted in the emergence of folk medicinal practices. Folk medicinal practices can be split into preventive (traditional foods, clothing, and hygiene systems) and therapeutic medicine (curative for common ailments). Despite the advancements of the contemporary medical and pharmaceutical industries, medicinal plants have become a vital component of daily life throughout the years. They are increasingly being utilized in cosmetology, the food industry, herbal tea, and alternative remedies (Rasool Hassan 2012). For a variety of factors, demand for medicinal plants is growing in both developed and developing countries. The rising interest in medicinal plants and their capacity to remediate environmental pollutants provide dual economic benefits as part of the greener economic growth and bioenergy production movement. Enriched (contaminated) biomass for bioenergy is an old technique that provides a low-cost alternative and renewable energy source (McKendry 2002).

Medicinal plants, as compared to conventional hyperaccumulator plants and crops, may be used to remediate contaminated sites due to their ease of provision, low cost, higher economic threshold level, and low chance of metal transfer from soil to essential oil, as well as alterations in its composition (Zheljazkov et al. 2006), which is predominantly due to the process (such as steam distillation) used for oil extraction (Bernstein et al. 2009; Pandey and Singh 2015; Scora and Chang 1997), thereby have limited or no risk of tropic chain contamination. Medicinal plants have a promising future as there are approximately 500,000 plants globally. The majority of their pharmacological, stress tolerance and phytoremediation potential has yet to be explored, necessitating existing and future studies. In the present chapter, efforts have been made to collect and analyze available information regarding stress tolerance capabilities and the phytoremediation potential of medicinal plants, which will provide valuable insight into understanding the putative mechanisms involved in stress tolerance and pollution alleviation. The medicinal plants that can withstand stress and be used for the phytoremediation of environmental contaminants have also been explored.

18.2 Multi-aspects of Medicinal Plants

Cultivation of medicinal plants for direct (i.e., therapeutic) as well as indirect (mitigation of environmental issues) purposes is an ancient practice (Gupta et al. 2013; Tamari et al. 2014). There are several medicinal plants worldwide with multipurpose benefits that protect animals, humans, and the environment

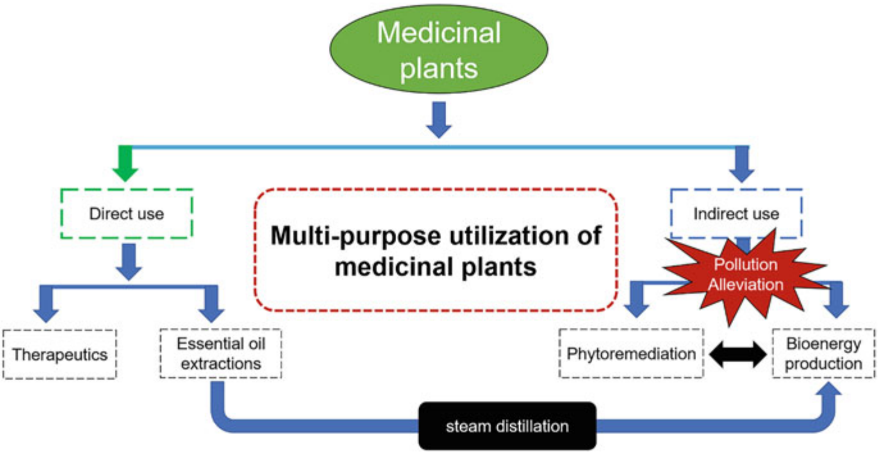

Fig. 18.1 Multipurpose uses of medicinal plants

(Fig. 18.1). Additionally, numerous medicinal plants exhibit biotic and abiotic stress tolerance and play a vital role in the accumulation/phytoextraction of environmental contaminants (both organic and inorganic) from polluted sites (Gupta et al. 2013).

18.3 Stress Tolerance Capability of Medicinal Plants

Stress tolerance refers to a plant's (particularly tolerant) capacity to withstand stress (both biotic and abiotic) episodes traumatic enough to cause severe physiological and cellular anomalies in susceptible plants. Abiotic stresses such as drought (water-deficit condition), salinity, temperature, light, and heavy metals impede plant survival and inhibit their growth, development, and production globally. Rapid urbanization puts extra burden on arable land and agricultural sustainability. Furthermore, the scarcity of freshwater resources, climate change, and overexploitation of agrochemicals (insecticides and pesticides) exacerbate these stressors, negatively influencing crop growth and production (Suzuki et al. 2014; Zhao et al. 2020). As earlier stated, each of the stressors has a negative influence on the physiology, morphology, and metabolism of the plant. Plants are prone to oxidative stress as a result of increased production of reactive oxygen species (ROS), including the superoxide anion (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH) (Lajayer et al. 2017). These ROS are highly toxic and can cause macromolecule damage, eventually leading to programmed cell death. Plants develop a plethora of resistance mechanisms to cope with the detrimental effect of adverse environmental conditions. The main tolerance mechanisms are modifications in membrane structure, escape from unfavorable conditions, modulation of the antioxidant defense system, production of compatible solutes (proline, soluble protein, soluble sugar) for cell osmotic adjustment, and alterations in biochemical and molecular attributes. Various plants utilize different coping strategies based on the degree/severity of stress, the plant species, and the growth stage (Mohammadi et al. 2020). When subjected to a specific stress, the tolerant plant led to stress adaptation in a time-dependent manner (Verma et al. 2013). To combat adverse biotic and abiotic stressors, medicinal plants employ a variety of phytochemicals (such as tannins, glycosides, and flavonoids). Secondary metabolites are responsible for plant adaptation to harsh environmental conditions (Maplestone et al. 1992). Furthermore, at molecular and physiological levels, plants trigger diverse metabolic processes or biosynthetic pathways in response to environmental stimuli (drought, salinity, light, temperature, and heavy metal stress) (Dangl and Jones 2001; Holopainen and Gershenzon 2010; Loreto and Schnitzler 2010).

Drought, a major environmental stressor, limits plant growth and productivity around the globe (Seleiman et al. 2021). Around 70% of global water is often used in the agricultural sector, and this figure is expected to increase to 83% by 2050 to meet the growing population's food demand (Facts 2012). Crop losses cost billions of dollars owing to drought and salinity stresses (Shabala et al. 2014). According to Selmar et al. (2017), water scarcity promotes the biosynthesis of key metabolites in spice and medicinal plants. Medicinal plants respond to drought stress by enhancing inherent bioactive components (Khan et al. 2011). Drought stress causes oxidative stress in plants, which reduces the photosynthetic rate and produces phenols. These phenolic compounds assist plants' defensive mechanisms (Jaafar et al. 2012). Enzymatic activity of phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS) has been proven to affect polyphenols in *Origanum vulgare* L (Lattanzio et al. 2009). The performance of plant's active and herbal constituents is altered by drought stress. Alavi-Samani et al. (2015) stated that different irrigation levels significantly influence the essential oil contents of *Thymus vulgaris* and *Thymus daenesis* such as carvacrol, γ-terpinene, and p-cymene in drought-stressed treated plants as compared to untreated plants. Mahdavi et al. (2020) concluded that the determinant compounds in drought stress adaptation were α-phellandrene, O-cymene, γ-terpinene, and β-caryophyllene in their study of *T. vulgaris* var. Wagner and *T. vulgaris* var. Varico3. Nowak et al. (2010) reported that drought stress substantially improved the content of terpenes in *Salvia officinalis*. García-Calderón et al. (2015) reported that drought stress caused an increase in kaempferol and quercetin contents in *N. tabacum*. Jujube (*Ziziphus jujuba* Mill.), a well-known plant for stress tolerance, has a dual capacity of stress avoidance and tolerance under drought stress conditions (Maraghni et al. 2014). Temperature is a major determinant in influencing the rate of plant growth and development, and each plant species has a varying temperature range. In recent years, the recurrence of heat waves or extreme temperature events has become predictable (Bhatla and Tripathi 2014). *S. rebaudiana* (Bertoni) is a species exhibiting natural sweeteners with zero calories. It has antibacterial, antifungal, anti-inflammatory, antimicrobial, cardiotonic, diuretic, hypoglycemia, diabetic benefit, hypotensive tonic, and vasodilator properties. Due to their remarkable medicinal properties, *S. rebaudiana* was subjected to water stress conditions, and their morpho-physiological and biochemical characteristics were evaluated (Kanta et al. 2016). Medicinal plants have evolved biochemical mechanisms to protect themselves from the impacts of light-induced damages (Ghassemi et al. 2021). These mechanisms provide an effective means of safely absorbing light (Ghassemi et al. 2021). Cold stress is one of the detrimental abiotic stressors which limits the geographical distribution as well as production of medicinal plants. Cold acclimation is a process that causes medicinal plants to develop resistance against cold stress (Ghassemi et al. 2021).

Salinity is a global agricultural problem that reduces plant growth and crop production in saline parts of the globe (Gull et al. 2019). It is widely acknowledged as a serious issue affecting medicinal plant growth and herbal yield (Mondal and Kaur 2017). Anthropogenic activities, salt composition, and land topography are all factors that contribute to soil salinity (Kotagiri and Kolluru 2017). Previous studies demonstrated that salinity induces water loss and ion toxicity, resulting in nutritional deficiency, reduced growth, and ultimately plant death, particularly in salt-sensitive genotypes (Mohammadi et al. 2020). Salt stress lowers chlorophyll levels, preventing chlorophyll production and triggering chlorophyll degradation. These physiochemical alterations in plants cause an imbalance in chlorophyll metabolism, impaired photosynthetic activity, and, in severe cases, necrosis. Plants have developed a unique system to combat salt stress, which regulates salt levels in various tissues. This coping mechanism controls the distribution of toxic ions (Na^+ and Cl^-) across the cells, tissues, and organs; besides, osmolytes are also involved in maintaining the photosynthetic process. Nonetheless, tolerant genotypes have a better coping mechanism than susceptible genotypes.

Previous studies also reported that salt stress reduced essential oil levels in mint and *Trachyspermum ammi* (Ashraf and Orooj 2006; Aziz et al. 2008), whereas Baghalian et al. (2008) reported a spike in the amount of essential oil in *Matricaria recutita* under salinity stress. Furthermore, they stated that *Matricaria recutita* could be the ideal choice to be grown in saline areas in order to boost agronomic yields with adequate therapeutic value where other crops cannot thrive. Several researchers have discovered that saline conditions improve the percentage of essential oil in different plants (*Satureja hortensis*, *Salvia officinalis*, *Thymus vulgaris*) under saline conditions (Baher et al. 2002; El-Din et al. 2009; Hendawy and Khalid 2005). According to Neffati and Marzouk (2008), salinity stress augmented carvacrol in the root but inhibited the amount of γ-trepine in the shoot. Kotagiri and Kolluru (2017) studied five distinct *Coleus* species under salinity stress. Of them, two species, *Coleus aromaticus* and *Coleus amboinicus*, showed better performance by exhibiting increased carbohydrate contents and water absorption potential. They further concluded that increased carbohydrate content under salinity indicates a metabolic substrate or osmolytes, conferring salt tolerance.

18.4 Phytoremediation Potential of Medicinal Plants

Various metal species have been used in wide-ranging industrial products; however, the unauthorized disposal of metal-enriched waste has contaminated soil, sediments, and groundwaters. Phytoremediation is the use of plants and plant products to remediate contaminants from the environment (Hayat et al. 2020; Menhas et al. 2021). Since this phytotechnology first evolved, considerable focus has been placed on identifying plants that accumulate contaminants, including hazardous metals. Recently, the use of plant-mediated phytoremediation approaches (notably the phytoextraction technique) to clean up polluted areas or water bodies has been regarded as a viable and ecologically sustainable strategy (Augustynowicz et al. 2014; Malar et al. 2014; Sasmaz et al. 2015; Syukor et al. 2016). So far, several plant species have been investigated as phytoremediators, including medicinal plants. However, several of them were classified as hyperaccumulators (plants that can accumulate higher levels of metals in roots and readily transfer them into above-ground parts, i.e., stems and leaves). Hyperaccumulators, on the other hand, are generally confined to metal-enriched soils, such as those occurring on serpentine outcrops and metalliferous rocks (Reeves 2006). The selection of plants for pollutant removal is an important step in phytoremediation. Meanwhile, researchers seek plants that could accumulate contaminants while simultaneously provide certain value-added products (at the same time or the postharvest stage of the crop). It is worth noting that in resource-constrained nations, the complications in reclaiming contaminated arable lands arise from governments' inability to give incentives to farmers or through the area being constantly under cultivation (Jisha et al. 2017; Zheljazkov et al. 2008). Thus, employing high biomass plant species such as maize, oats, and rapeseed (edible plants); tree and shrub species; and medicinal and aromatic plants is indeed an alternative option (Szczygłowska et al. 2011); however, utilizing primary food crops increases the risk of adding heavy metals into the food chain. Therefore, medicinal plants come into the picture; nevertheless, medicinal plants used for the phytoremediation process may not be particularly herbaceous and must have a high growth rate or phytoremediation capability compared to crop plants (Brown et al. 1995; Cosio et al. 2004; Lone et al. 2008). Recently, several medicinal plants have been found to be resilient to biotic and abiotic stressors that might lead contaminants to accumulate, suggesting that they could be utilized as phytoremediator plants (Mafakheri and Kordrostami 2021). *Senecio coronatus* (Thunb.) Harv. (Asteraceae), a medicinal plant, is one of the nine nickel (Ni) hyperaccumulating plants in Africa (Przybyłowicz et al. 1995). Similarly, two medicinally important African *Datura* species (Solanaceae), *Datura metal* L. and *Datura sativa* L., are termed as metallophytes (i.e., a cobalt and nickel accumulator), while also been regarded as phytomonitors (Bhattacharjee et al. 2004), whereas *Datura innoxia* has been regarded as a metal-tolerant species (Kelly et al. 2002). Likewise, *Helichrysum candolleanum* (Asteraceae) and *Blepharis diversispina* (Acanthaceae) exhibit high metal tolerance capabilities (Nkoane et al. 2005). *Chrysopogon zizanioides*, often known as vetiver grass, has been reported to be effective in removing both organic (e.g., 2,4,6-trinitrotoluene, phenol, and petroleum hydrocarbon) and inorganic (particularly hazardous metals such as lead, cadmium, copper, zinc, and arsenic) pollutants (Balasankar et al. 2013; Brandt et al. 2006; Chen et al. 2004; Datta et al. 2011; Ho et al. 2013; Makris et al. 2007; Singh et al. 2008; Singhakant et al. 2009). In a study done by Datta et al. (2011), vetiver grass was found to be a promising phytoremediator of As when grown in various soil types. When cultivated in 45 mg As kg^{-1} soil, the plant has been shown to possess a remarkable As removal efficiency of 10.6%. Hamzah et al. (2016) investigated the cadmium accumulation efficiency of various indigenous plants. They revealed that *Euphorbia hirta*, an industrially important plant (especially in the healthcare and petrochemical industries), had a high Cd bioaccumulation efficacy. *Euphorbia hirta* has also been proven useful for decontaminating radioactive waste (Hu et al. 2014). *Hypericum perforatum* L. is a valuable medicinal plant that could accumulate a considerable amount of Cd and is often used as an antidepressant (Kim et al. 1999; Malko 2002; Müller 1999; Schneider and Marquard 1995; Verotta 2003). Researchers indicated that *Hypericum* sp. may accumulate a high Cd concentration in their aerial parts without noticeably affecting growth or dry biomass. When grown on a Cr-contaminated substrate, hypericin, a key essential oil component, was not affected (Tirillini et al. 2006). *Ricinus communis*, also known as castor, has been used as a medicinal plant since very ancient times for anti-implantation activity, anti-inflammatory activity, antitumor activity, anti-asthmatic activity, and so on and has recently gained popularity as a potent phytoremediator for the cleanup of numerous pollutants, both organic and inorganic (Abreu et al. 2012; Adhikari and Kumar 2012; Bauddh et al. 2016a, 2015, 2016b; Bauddh and Singh 2012a,b, 2015a,b). Castor extracts have acaricidal and insecticidal properties (Zahir et al. 2010). The plant may readily grow up to 150 mg/kg Cd and accumulate a significant amount of the metal in its roots and shoots (Bauddh and Singh 2012a). The plant has a high level of tolerance to both biotic and abiotic stressors. Furthermore, *R. communis* is an annual plant that produces high biomass, which may be an alternative source of bioenergy. For instance, it has a high concentration of nutrients, while the husk and cake obtained after oil extraction can also be used as manure (Zahir et al. 2010). Heavy metals such as Cd, Ni, Pb, Cu, As, Cr, Zn, Ba, and others have been shown to accumulate at higher concentrations in the plant (Abreu et al. 2012; Adhikari and Kumar 2012; Bauddh et al. 2015, 2016a, b; Bauddh and Singh 2012a,b, 2015a,b; Mahmud et al. 2008; Romeiro et al. 2006). When grown on wasteland areas, *R. communis* planting has also enhanced soil physicochemical characteristics (Wu et al. 2012). *Hypericum perforatum* L., a medicinally important plant used to treat depression (Verotta 2003), is effective for soil Cd removal (Malko 2002; Schneider and Marquard 1995). The plant showed no visible phytotoxic effects on growth or dry biomass production. Bishehkolaei et al. (2011) uncovered that *Ocimum basilicum* L. accumulates chromium (Cr) effectively in its tissues. By limiting the metal in its roots, the plant evolved a defensive mechanism. In a recent study, *Ocimum basilicum* demonstrated its potential for phytoremediation of Cd-contaminated soil, which was enhanced when the plants were treated with various fertilizers (Zahedifar et al. 2016). According to Rai et al. (2004), *Ocimum* *tenuiflorum* L. could tolerate Cr phytotoxicity by altering several metabolic pathways. MM et al. (2013) evaluated the phytoremediation capability of six wild plants, namely, *Citrullus colocynthis*, *Datura stramonium*, *Lycium shawii*, *Malva parviflora*, *Phragmites australis*, and *Rhazya stricta*, for Cd, Zn, Cu, Ni, and Pb. Of these, *Datura stramonium* is a medicinal plant that can be used to phytostabilize soil polluted with Ni and Cu. Lal et al. (2008) assessed the phytoremediation potential of *Cymbopogon martinii*, *Cymbopogon flexuosus*, and *Vetiveria zizanioides* for Cd 2008. Cd tolerance was also identified in *Vetiveria zizanioides*. Many publications have reported the metal (e.g., Ni, Cr, Cd, Al, etc.) tolerance and bioaccumulation potential of several *Mentha* species (Manikandan et al. 2015; Zurayk et al. 2001, 2002). Zurayk et al. (2001) cultivated 12 different hydrophyte species as well as 4 *Mentha* species: *Mentha longifolia*, *Mentha aquatica*, *Mentha pulegium*, and *Mentha sylvestris*, in 1.0 ppm Cr, Ni, and Cd contamination. They discovered that all *Mentha* sp. accumulated a significant amount of all metals tested. *M. longifolia* accumulated Cr (1076.8 g Cr plant^{-1}), whereas *M. sylvestris* accumulated Ni (1822 g Cr plant^{-1}), the greatest amount of metal accumulation among all (12) plants studied. *Mentha aquatica* L. and *Mentha sylvestris* L. were grown for 14 days in solutions containing 1.0, 2.0, 4.0, and 8.0 mg Ni L^{-1} (Zurayk et al. 2002). *M. aquatica* and *M. sylvestris* both collected a significant quantity of Ni in their roots (8327 mg Ni kg^{-1} dry weight in *M. aquatica* and 6762 mg kg^{-1} dry weight in *M. sylvestris*), indicating that this plant is an efficient phytoremediator. Two medicinally important plants (*Centella asiatica* and *Orthosiphon stamineus*) were analyzed for the phytoremediation potential of different heavy metals (Abd Manan et al. 2015; Arifin et al. 2011; Mohd Salim et al. 2013). Abd Manan et al. (2015) compared the Zn, Cu, and Pb accumulation and translocation of two medicinal plants (*C. asiatica* and *O. stamineus*). They concluded that *C. asiatica* had more than one translocation factor for all studied metals (Zn = 1.34, Cu = 2.77, and Pb = 1.42). A translocation factor greater than one implies that *C. asiatica* has the potential to be utilized in phytoextraction. However, *O. stamineus* has less than one translocation factor and may be ideal for phytostabilization. *Allium sativum* (garlic) was reported to have a high cadmium accumulation capacity in its roots, 1826 times more than the control (Jiang et al. 2001). The plant had adequate tolerance to Cd and did not exhibit toxic effects at lower doses. *Cannabis sativa* var. sativa L. (hemp) is an industrial (for fiber) and important medicinal plant grown in Ni-, Pb-, and Cd-contaminated soil to assess its phytoremediation capacity and the impact of these metals on fiber quality (Linger et al. 2002). They advocated that the plant's leaves exhibited the highest concentration of all tested metals. Furthermore, they prophesied that none of the studied metals have an impact on fiber quality. Citterio et al. (2003) cultivated *Cannabis sativa* in Cd-, Ni-, and Cr-contaminated soil and discovered no significant differences in plant development (morphology) after 2 months of seed sowing under contaminated conditions. Metals stimulated the synthesis of phytochelatins in *C. sativa*, indicating a robust coping mechanism against metal toxicity. *Cymbopogon martinii*, also known as palmarosa, was discovered to be a viable candidate for metal phytostabilization when grown in tannery sludge mixed soil (Pandey et al. 2015).

Table 18.1 Phytoremediation potential of medicinal plants

S. No	Medicinal plants	Heavy metals	Major findings	References
1.	*Centella asiatica* and *Orthosiphon stamineus*	Zn, Cu, and Pb	*Centella asiatica* is a tolerant species and suitable for zinc, copper, and lead phytoextraction. By contrast, *Orthosiphon stamineus* is a moderate accumulator of the tested metal elements	Abd Manan et al. (2015)
2.	*Cunila galioides* Benth.	Al	The André da Rocha population is the most tolerant to aluminum, while Bom Jardim da Serra is the most sensitive. Higher flavonoid concentrations in tolerant counterparts	Mossi et al. (2011)
3.	*Pfaffia glomerata* (Spreng.)	Pb	Higher SOD and CAT antioxidant activity in the root and shoot	Gupta et al. (2011)
4.	*Mentha piperita*	As, Cd, Ni, Pb	Higher concentrations of Cd, Ni, and Pb were accumulated in different parts of the plant, except for As. Mentha piperita is suitable for metal phytostabilization	Dinu et al. (2021)
5.	*Salvia sclarea* L.	Zn	*S. sclarea* is an economically attractive plant for the phytoextraction and/or phytostabilization of Zn-contaminated soils	Dobrikova et al. (2021)

Acalypha indica is a Pb accumulator plant, and antioxidants might play a key role in detoxifying Pb-induced negative impacts. Various plant species, including *A. indica* L., have recently been demonstrated to accumulate heavy metal ions such as Cd, Pb, Ni, Cr, Zn, Fe, and Cu from contaminated environments (Olowu et al. 2015). This plant has numerous medicinal properties and therapeutic applications, including antibacterial, anti-inflammatory, antifungal, and wound-healing activities, cardiac disorders, biliousness, rheumatism, hemorrhages, ulcers, amenorrhea, and treatment of various skin diseases (Jagatheeswari et al. 2013). The Cd/Pb combination had negligible phytotoxic effects on *Ligusticum chuanxiong* Hort. which were associated with enhanced photosynthetic processes, subcellular distribution, the chemical forms of Cd and Pb, and secondary metabolite production (Zeng et al. 2020). These findings have implications for plant production strategies in heavy metal-contaminated soils. *Artemisia annua* (Asteraceae) is a dicotyledonous medicinal plant native to China, which is also extensively distributed in temperate and subtropical zones, particularly in Asia. *A. annua* has key features such as a fast growth rate and high biomass production, ease of culture, and a peculiar weedy habit, all of which are crucial for plants used for phytoextraction of metals and metalloids from contaminated soils (Rai et al. 2014). A study found that *Centella asiatica* was resistant to zinc, copper, and lead based on bioaccumulation, translocation, and enrichment factors, making it appropriate for phytoextraction. *Orthosiphon stamineus*, on the other hand, was a moderate metal accumulator (Abd Manan et al. 2015) (Table 18.1).

18.5 The Fate of Enriched Plant Biomass

In general, steam distillation is employed to extract essential oils from medicinal plants since it has the slightest possibility of allowing pollutants to transfer into the oil. After collecting the oil, the remaining biomass of medicinal plants can be used as a renewable bioenergy resource. This bioenergy can be generated either by direct burning of biomass or by producing biogas through biomass gasification. The use of plant biomass for bioenergy is an old technique that provides a low-cost, renewable energy source. This integrated strategy will decrease the cost of petroleum oil while also contributing to the development of a sustainable model that will mitigate multiple environmental issues such as the reduction of greenhouse gases and the alleviation of pollution (Fig. 18.2).

18.6 Conclusions and Future Prospects

Since ancient times, medicinal plants have been used for wide-ranging medicinal and therapeutic purposes. Medicinal plants offer exceptional stress tolerance and phytoremediation capabilities, in addition to numerous medicinal and pharmacological characteristics. As a result, it is seen as a viable alternative for the restoration of contaminated areas that would not involve the contamination of essential oils. Further research is needed to understand stress tolerance responses in medicinal

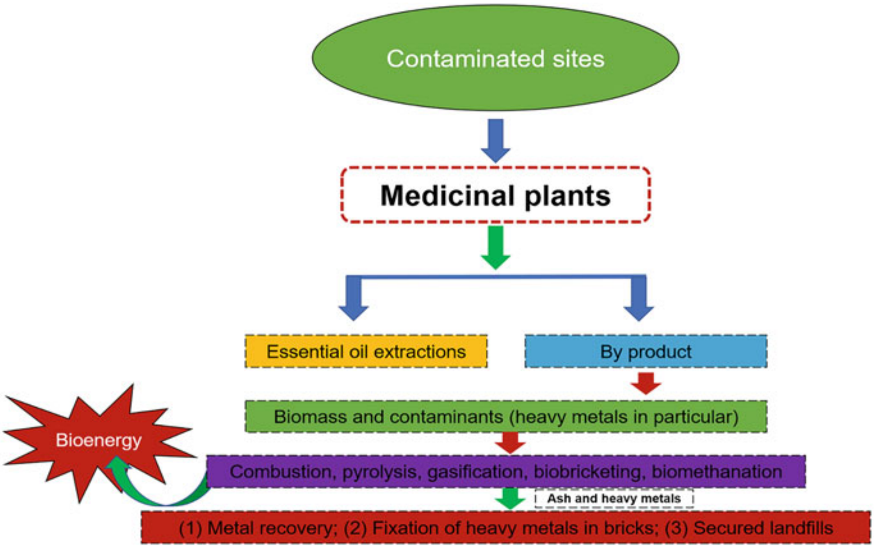

Fig. 18.2 Employing medicinal plants for decontamination/phytoremediation of polluted environments

plants since chemical compounds play an important role in defensive mechanisms and concomitant phytoremediation processes.

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Chapter 19
Breeding Advancements in Fenugreek for Environmental Stresses

Muhammad Azhar Nadeem, Muhammad Tanveer Altaf,
Muhammad Abu Bakar Zia, Tolga Karaköy, Faheem Shehzad Baloch,
and Muhammad Aasim

Abstract The world is witnessing a simultaneous problem of climate change and rapid increase in population. Climate change is becoming a big threat to agriculture production system, and it is believed that it will become more and more adverse in coming years. Under these circumstances, plants are combating various environmental stresses they never faced before. Plants are developing various mechanisms in order to deal with these stresses. Human being is directly dependent on the plants to fulfil its basic needs like food, shelter, and medicine. Medicinal plants are important resource for the human being in order to treat various diseases and remained a significant part of civilizations and cultures. Among these, fenugreek is one of the most important medicinal plants belonging to Leguminosae family. It is much known to the world for its aromatic, condimental, and medicinal properties. Its seeds are utilized as dietary proteins having antipyretic, antidiabetic, digestive, lactogogue, hypolipidemic, and cholesterol-reducing properties. Most of the cited literature provided deep insight about its medicinal properties. However, there is less available information that documented how fenugreek responds to various environmental stresses like drought, salt, and cold stress. This book chapter is aimed to provide information how plants especially fenugreek respond to environmental stress and how these stress influence the growth and development of this plant. We believe that provided information will motivate the scientific community to conduct more studies in order to develop climate-resilient fenugreek cultivars.

Keywords *Trigonella foenum-graecum* · Medicinal plant · Drought · Salinity · Cold stress

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19.1 Introduction

There is no uncertainty that food security and agricultural sustainability are threatened by environmental changes. Plants have evolved a variety of methods to deal with environmental stresses. The steady variation in the environment can lead to plant adaptability, whereas short variation in the environment can cause a cumulative reaction. The reaction of individuals to environmental stresses depends on the various factors, for instance, plant species and type and, duration of stress. Plants are exposed to harsh climatic circumstances like intense heat or cold, UV and water scarcity, heavy metals, nutrient insufficiency, light stress, salt stress, and air pollution; as a result, reactive oxygen species and the hydroxyl radical, hydrogen peroxide, and superoxide are produced (Bahuguna and Jagadish 2015). Plants have evolved a variety of survival ways to deal with harsh environmental conditions (Fig. 19.1). Plants that survive in severe conditions cause incredible changes at the molecular and cellular levels. Temperature and water are two of the most important environmental factors that influence consequently agricultural sustainability and plant distribution in various parts of the globe (Chinnusamy et al. 2007). Plants reacted to unfavorable ecological stresses (i.e., drought, flooding, heat, salinity, heavy metal, and UV) by numerous physiological, molecular, and biochemical variations. As an example, various kinds of phytochemical compounds are utilized to control biotic and abiotic stresses. Nonetheless, secondary metabolites play a vital role in plant's adaptation into their habitat. Plants displayed several biochemical responses to salt, heavy metals, temperature, drought, and light stress (Holopainen and Gershenzon 2010). These responses take place at physiological and molecular levels that eventually affect phytochemical compounds (Loreto and Schnitzler

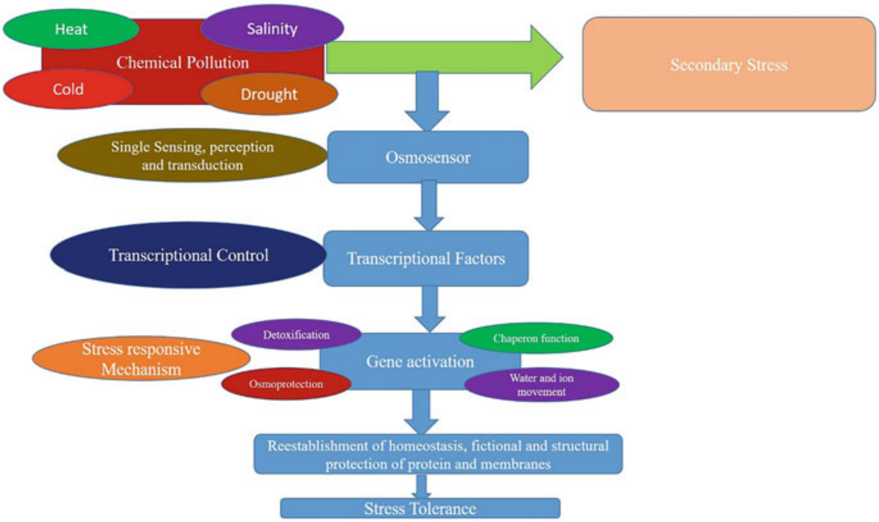

Fig. 19.1 Plant response to abiotic stress

2010). To survive under severe conditions, medicinal plants limit secondary metabolite synthesis to a particular metabolite. The current findings revealed that plants utilize varied ways to deal with stressful circumstances that depend on plant species, stressors, and time of occurrence. Additionally, findings revealed that the biochemical, molecular, and physiological changes in relations occur during environmental stresses.

Throughout life, humans have relied on nature to fulfil their basic needs such as scents, tastes, shelters, food, medicines, modes of transportation, clothing, and fertilizers (Srivastava et al. 2020). Medicinal plants have long been playing an important part in the growth of human society (Aasim et al. 2018; Nadeem et al. 2018). These plants have always been at the forefront of practically all cultures of civilizations as a source of medicine. Medicinal plants are considered abundant sources of traditional medicines, and many modern medicines are produced from them (Dar et al., 2017). Medicinal plants have been used to treat illnesses, flavor and preserve food, and stop disease epidemics for thousands of years (Sofowora et al., 2013). In 2008, the World Health Organization (WHO) published a report confirming the importance of these plants in daily life and reported that 80 percent of the world's population relied on them as traditional medicine, either directly or indirectly. Europe uses around 1300 medicinal herbs, 90 percent of which are obtained from the wild and natural resources. One hundred eighteen of the 150 most often used medicines are based on natural resources (Balunas and Kinghorn, 2005). According to a survey by the International Union for Conservation of Nature and the Globe Wildlife Fund, the world uses between 50,000 and 80,000 flowering plant species for medical purposes (Chen et al., 2016). Natural products and medicines derived from those products treat 87 percent of all human diseases, including cancer, bacterial infection, and immunological problems (Cragg and Newman 2013).

Around the globe, there is a large genetic diversity of medicinal plants, and this pool of genetic variants serves as the base for both selection and plant improvement (Karık et al. 2019). Hence, characterization, documentation, and identification of the gene pool of medicinal plants are necessary for this goal (Gantait et al., 2014). The sequences of the genome contain crucial details about the origin of plants, epigenomic regulation, inheritable traits, development, evolution, and physiology, all of which are necessary for deciphering genome diversity and chemo diversity at a molecular basis (Hao and Xiao, 2015). High-throughput sequencing of traditional medicinal plants could help with molecular breeding of high-yielding medicinal cultivars as well as shed light on the biosynthesis paths of medicinal compounds, particularly secondary metabolites, and their regulatory mechanisms (Boutanaev et al., 2015; Hao and Xiao, 2015).

Fenugreek (*Trigonella foenum-graecum* L.) is an ancient medicinal plant with an extraordinarily therapeutic and nutritional profile (Flammang et al. 2004; Aasim et al. 2018). Fenugreek belongs to the family *Fabaceae* that originated in central Asia approximately 4000 BC (Altuntaş et al. 2005). Fenugreek is being commercially farmed in Pakistan, Turkey, Iran, Spain, India, Nepal, North Africa Egypt, Morocco, France, Middle East, Argentina, and Afghanistan (Flammang et al. 2004; Altuntaş et al. 2005).

19.2 Drought Stress

Increasing austerity and occurrence of drought conditions due to abnormality in fluctuating rainfall designs are predicted as climatic altering situations (Klein et al., 2014). As compared to the current yield, water scarcity is expected to decrease the world's crop production by 30% by the year 2025 (Grafton et al., 2015). There is a vast range of phenotypic variability and stress injuries in response to water scarcity, allowing for a wide range of drought-resistant varieties not only within species but also within genotypes of the same species (Grafton et al., 2015; Jaleel et al., 2009). These kinds of variations are mandatory constituents for the breeding program of drought resistance (Ashraf, 2010). These variations can help to improve the understandings of the exact mechanism and their responses for recovery. Furthermore, effective and consistent biochemical as well as physiological indicators are the basic requirements for selecting landraces that are drought tolerant. Many studies in direction, however, have extensively focused on drought tolerance, and consequently, recovery phase studies have been abandoned by the scientists. Drought stress tolerance is typical of simple as well as compound traits being controlled by numerous hereditary, biochemical, and physiological methods (Vassileva et al., 2011). Plant response to drought stress is elaborated in Fig. 19.2. Due to an imbalance between excitation of electrons and application during photosynthesis, water stress causes cellular accumulation of reactive oxygen species (ROS).

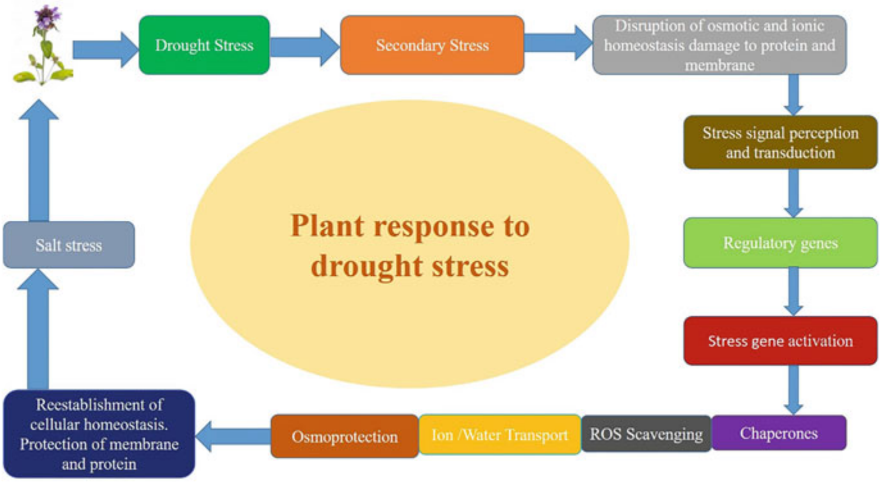

Fig. 19.2 Plant response to drought stress

Oxidative stress caused by ROS consequence can lead to harmful effects on proteins, cellular molecules, nucleic acids, pigments, cell membranes, and other critical procedures (Saed-Moucheshi et al., 2014). When the cell membrane integrity is damaged, a series of cellular and biochemical events will occur, which can be measured through the intercellular concentration of malondialdehyde (MDA) (Hasanuzzaman et al. 2012). In water scarcity conditions, the plant implements several internal mechanisms to combat oxidative harm. The antioxidant defense system, which includes both antioxidants (non-enzymatic and enzymatic antioxidants) is a stress-protective mechanism for reducing and controlling the harmful repercussions of oxidative injuries (Abid et al., 2018; Hasanuzzaman et al. 2012). For example, enzymatic antioxidants (i.e., ascorbate peroxidase (APX), glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), and others) play an important character to maintain cellular redox and in the ascorbate-glutathione route (Hasanuzzaman et al., 2019). By scavenging free radicals, phenolic chemicals serve a substantial protective role in the non-enzymatic antioxidant protection mechanism (Aziz et al. 2019). The carotenoids are non-enzymatic antioxidants that dissipate surplus energy and scavenge reactive oxygen species (ROS). Havaux (1998) and Pompelli et al. (2010) stated that carotenoids provide and maintain photosynthetic membrane and decrease SOD activity. Varying water associations by producing and accruing well-matched solutes like carbohydrates and free amino acids that are water-soluble is another way for maintaining plant cellular utilities suffering under water deficiency circumstances (Abid et al., 2018; Mafakheri et al., 2011). It's worth noting that osmotic adjustment would be the retrieval of metabolic events after rewatering (Abid et al., 2018). Fenugreek is a medicinal annual plant which is from the family Fabaceae, used in different ways like vegetables, medicinal plants, as well as spices. Fenugreek use includes an extended range of health profit cases, as well as antidiabetic, anticancer, antipyretic, anti-inflammatory, antitumor, and antioxidant effects encouraging things like rising breast milk and minimizing blood pressure and cholesterol (Amiriyan et al., 2019). For the cause of the fenugreek's great adaptableness to diverse environments, it is extensively grown all over the world (Talib et al., 2014). Amiriyan et al. (2019) revealed that fenugreek has a lot of genetic variation in Iran. Furthermore, just a few studies on fenugreek's drought tolerance have been carried out (Saxena et al., 2017; Sharghi et al., 2018; Zamani et al. 2020).

Maleki et al. (2021) conducted a study in which 16 different landraces of fenugreek were collected all over Iran and 2 varieties from India and Egypt were also involved. They concluded that increased sensitivity toward water scarcity does not essentially result in low retrieval capacity in landraces. Moreover, immense genotypic diversity in physiological and biochemical characters among the varieties specifies the significance of such traits in picking for drought stress tolerance, particularly in the environments like arid and semi-arid. Varietal's comparisons were undertaken by various authors: Acharya et al. (2008) and Chhibba et al. (2000). Choosing the varieties that are tolerant or improving tolerance deliberated the improved methods to effective farming of crops in arid and semi-arid regions (Painawadee et al., 2009; Basu et al., 2009). The practice of using drought-resistant cultivars is a significant approach to contest the drought-related problem. Such cultivars should be able to deliver greater yield under water scarcity. Genetic diversity for drought resistance has also been stated in peanuts (Painawadee et al., 2009).

19.3 Salt Stress

Since many of the herbal plants have no side effects, therefore, the attention in consuming herbs to treat different diseases has significantly amplified around the world. Figure 19.3 explains how plant responds to salinity stress. Valdiani et al. (2012) revealed that medicinal plants are one of the most significant crop groups that have traditionally been utilized for illness inhibition and treatment. In areas like arid and semi-arid, salinity is one of the main aspects restricting the growth and development of plants and seed germination, additionally affecting the quality and quantity of plants. The sprouting of many species is restricted and hindered with the rise of salinity level, and responses of the plant may differ significantly liable on species (Läuchli and Grattan, 2007). According to estimation around 50% of agriculture and 20% of arable land on the planet are salt-affected (Shrivastava and Kumar 2015).

Surface soils have a higher salt concentration than subsoil soils; therefore seeds may experience a harsher environment than mature plants (Läuchli and Grattan, 2007). Khan and Ungar (1997) revealed that one of the key causes for the sprouting stage's vulnerability to salinity is the high salt buildup rate caused by rapid cell

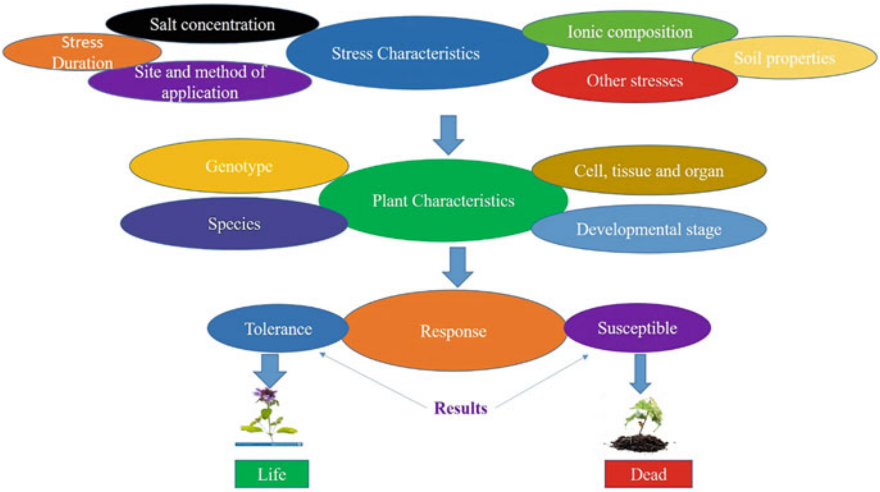

Fig. 19.3 Plant response to salt stress as a function of the interactions between stress and plant characteristics

development. Consequently, propagation and seedling formation are the important phases in plants' life span (Fenner and Thompson 2005). Munns and Tester (2008) and Bueno et al. (2017) stated that the method through which a plant bears salinity is complicated and species to species fluctuate. Salt has an osmotic influence on seed sprouting (Khan and Ungar 1997), toxicity's ion, or with the mixture of both of these (Khan and Ungar 1997; Munns and Tester, 2008). The osmotic pressure can prevent water intake, which is crucial for activation of enzyme, breakdown, and seed reserves translocation (Ashraf and Foolad, 2007; Munns and Tester, 2008). Moreover, the ionic imbalance can disrupt critical metabolic processes such as cell dividing and expand, and it can be poisonous in large amounts (Munns and Tester, 2008). Surpluses of sodium (Na+) and chlorine (Cl−) can impact physiological processes in plant cells (Munns and Tester, 2008; Morais et al., 2012). The proportion of salt tolerance fluctuates between plant species and even between stages of development within a species (Ahmad et al., 2013; Bueno et al., 2017). Salt-tolerant plants, also known as halophytes, have adapted to flourish in high-salinity environments due to the presence of several mechanisms in them for salinity tolerance. Ahmad et al. (2013) defined that salt-tolerant plants, also known as halophytes, have modified to flourish in high-salinity environments due to the existence of several systems in them for salinity tolerance. However, the vast range of plant species (glycophytes) that grow in non-saline conditions is salt-tolerant. Munns and Tester (2008) stated that the resistance of halophytic and glycophytic species to salt stress varies substantially.

Ratnakar and Raib (2013) and Zehtab-Salmasi (2008) stated that different medicinal plant species have varying salinity resistance levels. Ehtesham-Neya (2007) investigated the influence of various salt phases (i.e., 0, 2, 4, 6, and 8 bar) on medicinal plant growth, including bitter apple (*Citrullus colocynthis*), basil (*Ocimum basilicum*), flax (*Linum usitatissimum*), milk thistles (*Silybum marianum*), fennel (*Foeniculum vulgare*), black cumin (*Nigella sativa*), and safflower (*Carthamus tinctorius*). During the germination stage, as compared to other species, they discovered that milk thistles and safflower showed strong resilience to salinity. Fenugreek and dill seed germination were unaffected through 40 mM concentrations of NaCl (Ratnakar and Rai 2013; Zehtab-Salmasi, 2008). The species of plants used in recent research are famous due to medicinal characteristics, which include immunomodulatory (dragonhead and fenugreek), antipyretic (fenugreek), anti-ulcerogenic (fenugreek), antidiabetic (fenugreek), anti-inflammatory (dragonhead, savory, fenugreek, and dill), antihypertensive (dill, dragonhead), antifungal and antibacterial (dill, fenugreek, and savory), anticancer (fenugreek, savory, and dill), hypocholesterolemic (dill, fenugreek), antineuralgic (dragonhead), and antinociceptive (savory, dragonhead) effects (Satheeshkumar et al. 2010; Valady et al. 2010; Rafii and Shahverdi 2007; Haouala et al. 2008; Amanlou et al. 2005; Güllüce et al. 2003). These plants have long been utilized in traditional medicine and are commonly grown in semiarid and arid areas where salt is an issue (Sukhdev et al. 2006). Plants that can resist salt offer an alternative, highly profitable income crop that can be grown in dry and subtropical environments where salt-sensitive varieties are prohibited. Evidence regarding the extent of salinity assist plant is required as a support for choosing species most probably to prosper agriculture in salt-contrived regions.

19.4 Cold Stress

Plant growth is a complex physiological process that is influenced by external elements such as light, temperature, and water. These elements influence the germination individually or combined (Shaban, 2013). Appropriate temperature is crucial for the start of enzyme activation and, as a result, for quick imbibition and sprouting (Baskin and Baskin 1998). The seed's membrane permeability and cytosolic acid production are affected by temperature (Biligetu et al., 2011). The rate of germination increases as the temperature rises until it reaches the optimum temperature. For cool-season species, the optimal temperature for germination varies between 10 and 20 degree Celsius (Baskin and Baskin, 1998). The germination rate is greatest in this range of temperature; however most cool-season species can start germination at lower temperatures. However, under drought conditions, low temperatures can stimulate seed sprouting in the same plant species (Shaban, 2013).

The most basic prerequisite for germination is water. The further stages of germination occur after the activation of the imbibition enzyme (Fenner and Thompson 2005). Temperature interaction, which occurs regularly throughout germination processes, determines germination and the speed of enzyme activity (Fenner and Thompson 2005). Physiological activities occur in seeds after water content spread crucial levels during imbibition. This period's duration fluctuates between 0 and 50 h that depends on seed size, plant species, and temperature (Fenner and Thompson 2005).

The majority of species are planted in the springtime in cold places where the continental climate is predominant. In this situation, fenugreek is a normal spring-sown plant. Due to snow melting and heavy precipitation at the end of the winter, sowing in early spring encountered low temperatures and excess water. It is critical to understand how fenugreek seed germination responds to lower temperatures and more water to produce a good stand. The goal of this research was to look at several germination features of fenugreek seeds at various temperatures and water levels (Fig. 19.4).

The optimum temperature is very important for the proper germination of fenugreek. There is scarcity of information about how fenugreek respond to cold or low temperature stress. Gullap et al. (2018) aimed to explore the response of excess water and low temperature applications on germination and seedling properties of fenugreek. They concluded that seed germination and seedling properties were increased with the increase in temperature. They concluded that low temperature has negative effect on the germination and seedling properties of fenugreek.

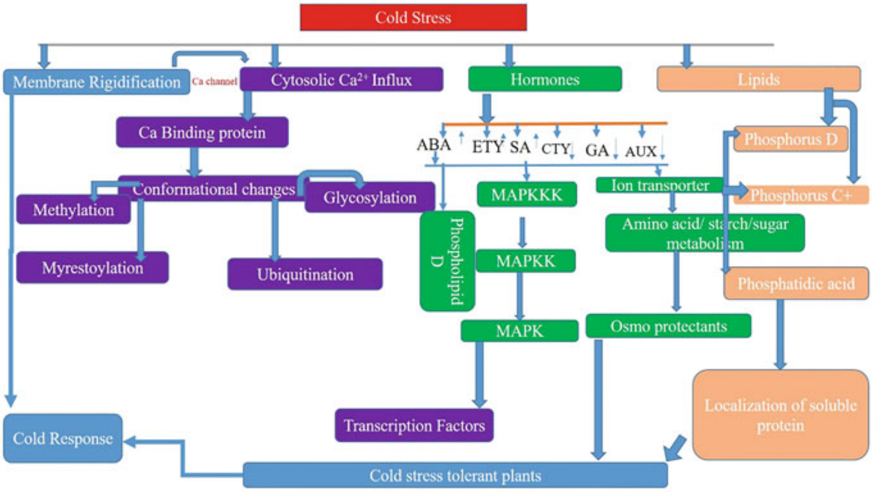

Fig. 19.4 Molecular mechanism of plants to cold stress

19.5 Future Recommendations

Fenugreek is one of the most important multipurpose medicinal plants. However, very less number of studies has been documented aiming to explore its response to various biotic stresses. Keeping in view the following suggestions are proposed:

1. Collection of fenugreek germplasm and its characterization at both phenotypic and molecular levels in aiming to identify novel variations that can be helpful for fenugreek breeding.
2. Multi-year/location experimentation of fenugreek germplasm aiming to understand genotype by environment interaction at performance of fenugreek germplasm.
3. Identification of genomic regions associated with environmental stress through genome-wide association studies.
4. Validation of identified markers and development of KASP markers for the speed breeding in fenugreek.
5. As genome editing is gaining focus of scientific community, it is highly recommended to conduct genome editing studies in fenugreek keeping in view the effect of environmental stresses on fenugreek.

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Chapter 20
Conservation Strategies for Medicinal Plants in the Face of Environmental Challenges

Navneet Kaur, Navdeep Kaur, and M. I. S. Saggoo

Abstract Medicinal plants are regarded as "chemical goldmines" as they are the source of valuable secondary metabolites and hence exploited at an alarming rate by herbalists and pharmacists worldwide. This overharvesting has led to terrible destruction of habitat and genetic diversity at a fast pace. Hence, sustainable management and conservation of medicinal plant species has become extremely crucial. The chapter discusses various strategies, viz., in situ methods, ex situ methods, biotechnological approaches, and role of government agencies and legal framework worldwide for intensive management and conservation of medicinal plants. Additionally, methods have been discussed for resource management and cultivation practices for improving yield of medicinal plants.

Keywords Medicinal plants · Conservation strategies · Sustainable use · Legal policies · Biotechnological approaches

20.1 Introduction

Medicinal plants are the plants containing bioactive constituents in concentration that exerts healing effect on an organism (Shahidullah and Haque 2015; Kaur and Ahmed 2021). With the advent of novel technologies, characterization and identification of these bioactive constituents in plants exerting beneficial effect have become less cumbersome, which has led more consumer inclination towards herbal and safe products in both developed and developing economies (Kaur and Ahmed 2021). In developed nations like Europe, nearly about 1300 plants with therapeutic

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significance are used, of which approximately 90 percent are obtained from wild reserves. Similarly, in the USA, out of topmost 150 drug prescriptions by medical practitioners, 118 are obtained from natural resources (Chen et al. 2016). However, in developing countries, rural communities solely rely on plant-based medicines as traditional healers are main health practitioners due to easy availability additionally herbal medicines are analogs to costly synthetic drugs (Kaur and Ahmed 2021). But this preference of consumers towards plant-based remedies over synthetic drugs has rapidly surged their global demand by 15–20% per annum which has further led to an alarming situation with hundred to thousand times increase in their extinction rate, with the world suffering the loss of minimum one prospective drug every 2 years (Shahidullah and Haque 2015; Chen et al. 2016).

The use of these healing plants is not limited to drugs only but they are also employed by other industries such as food, agriculture, perfumery, and cosmetics. Thus, they offer opportunities for employment, income, and foreign exchange in developing countries also. However, most of the species of medicinal plants used at commercial level and for trade purposes ranging from local to international scale are harvested from the wild and only few are cultivated and that too only on small scale. Their wide usage in various sectors has led to reckless collection and overharvesting of these plants from their native natural habitats which has resulted in their disappearance at a much faster pace than expected with strong bearing on biodiversity (Hamilton 2004; Shafi et al. 2021).

In third world countries herb-gatherers or poor people also collect curative and aromatic plants in their neighborhood forests to earn some money for sustaining their livelihoods. However, harvesting of plants with medicinal importance by such untrained gatherers not only leads to unsustainable harvesting and adulteration of plant material but also ultimately results in extinction of these plants at local or regional level (Hawkins 2008).

Thus, the escalating demand of medicinal plants has caused their “slaughter harvesting” and put them into endangered and vulnerable categories. As per data given by IUCN (International Union for Conservation of Nature) nearly about 50,000 to 80,000 Angiosperms are utilized for curative purposes only, on global level. Habitat destruction and overharvesting have brought nearly 15,000 of these species on the verge of extinction (Shafi et al. 2021). Thus, it has become paramount to hit equilibrium between protection and exploitation of these medicinal plants. The chapter is written with a motive to discuss mainly the strategies available for conserving these restorative plants along with policies and regulations set by government for their protection.

20.2 Need for Conservation of Medicinal Plants

The astounding variety of organisms forms an entangled and noteworthy part of our planet. Wide range of living entities surrounding us make world a pretty nice place to reside and share the resources. Biodiversity amplifies the productivity of ecosystem where each species, no matter how minute, has a crucial role. Homeostasis in the environment is directly influenced by the species abundance of an area. When we look at this biodiversity from the perspective of medicinal use, the IUCN and WWF (World Wildlife Fund) notice the significant number of angiosperms used for remedial purposes at world level. Medicinal herbs have a crucial part in supporting healthcare system, viz., Ayurveda, Homeopathy, Siddha, Unani, and even in Allopathy system of medicines. In Europe, 90% of the medicinally used plants come from wild while of the major 150 drugs prescribed by practitioners in the USA, 118 are obtained from plants (Balunas and Kinghorn 2005). Primary healthcare of 80% of the population of developing countries is based on herbal drugs. In developed countries of the total prescribed drugs, over 25% are from wild species. The contribution of medicinal plants to Western medicine can't be overlooked. They have either provided components for pharmaceutical drugs or have a pivotal role in drug discovery. They are the source of many novel bio-molecules (Kumar et al. 2011). Some presently used drugs of botanical origin are either directly obtained from plants or are modified version of these biochemicals drugs. Even those that are synthesized from inorganic materials have their origins in research into the bioactive constituents of plants. Herbal drugs are being prescribed in the conventional system of medicine though its rate of recommendation varies from one country to another. The extant of prescription of these plant-based drugs is much lower in UK or the USA as compared to Germany (Hamilton 2004). Further, the distribution of these plants is also not evenly distributed across the globe and various families of plant kingdom (Rafieian-Kopaei 2013). Some plant families include much more number of medicinal plants than the other families. The proportion of threatened species in these is also higher (Huang 2011). The demand for herbal products and secondary metabolites obtained from medicinal plants has increased tremendously in recent years at global level that is further oppressing our resources (Cole et al. 2007; Nalawade et al. 2003).

Loss of biodiversity brings about major disturbance in the ecosystems at global level in the form of loss of species or reduction in the number of species in natural habitat. Loss, particularly of plants with therapeutic use has serious social and economic costs besides the profound ethical and aesthetic implications. Many medicinal plants have now been included in Red Data Book of threatened species. It should be our top priority to take necessary measures for conserving the natural dwelling places of these immensely important medicinal plants. The United Nations Conference on Environment and Development (UNCED) held at Rio de Janeiro in 1992 helped in harnessing world's considerable attention towards this loss in biodiversity. Biodiversity loss has been recognized as one of the major problems of this century. The threat to survival or loss of a plant species may occur in different ways:

1. Direct ways that include overexploitation, deforestation, diseases and pests, commercial exploitation.

2. Indirect ways which include any type of modification of the natural habitats, habitat destruction by forest fires, urbanization and industrialization, draining and filling of wetlands, pollution and introduction of exotic species, etc.
3. Natural causes that are the product of climate change.

These changes disrupt the interactions among the species, either by killing or forcing out many species from the area. Overexploitation has led to reduction in the population size of the species hence propelling it towards extinction. The current rate of loss of plant species as estimated is much higher (between 100 and 1000 times) than the expected natural extinction rate (Pimm et al. 1995). Loss of medicinal species that are precious sources of new drugs is a cause of serious concern for human continuance (Chen et al. 2010, 2016; Chacko et al. 2010; Nalawade et al. 2003). Collections from wild and destruction of the natural habitat have resulted in pushing nearly 15,000 therapeutic plants species out of the total known on the verge of extinction (Bentley 2010). Increase in human population as well as surge in plant consumption has eroded 20% of the wild resources (Ross 2005). Only a small proportion of these plants that suffer from resource destruction and genetic erosion has been listed as threatened (Schippmann et al. 2005; Deeb et al. 2013). There is no authenticated source that can give exact number of threatened medicinal plants but according to Pimm and co-workers (1995), at current times, it is estimated that earth is facing the loss of at least one plant that has the capability to be used as major drug every 2 years. Species loss leads to loss of germplasm that influences stability of the plant populations by decreasing the variability in plants. Reduced variability increases the susceptibility of plants to diseases, pest, and natural disaster, hence further making it more prone to extinction. Once a species becomes extinct, the genetic resource of the species is lost forever; therefore, it becomes imperative to conserve these plants in the forest area (Biswas et al. 2017).

Prior to launch of any type of conservation technique it is important to identify species that are at high risk of elimination. The criteria of rare existence of the species and its difference from others are used to evaluate the extinction risk of medicinal plants (Figueiredo and Grelle 2009). Harvesting pressure does not affect all plants in a similar way (Andel and Havinga 2008; Wagh and Jain 2013). Though species rarity is affected by uncontrolled deforestation, indiscriminate collection, habitat destruction, and overexploitation, they are not enough indicators to measure species vulnerability to harvest pressure. Many biological features like population size, reproductive system, species diversity, distribution range, habitat specificity, and growth rate show correlation with extinction risk. Hence conservation of medicinal plant biodiversity is vital for economic growth and poverty reduction. It secures our future and is like an insurance policy that will sustain continuity of food chains along with tenable utilization of life support systems on earth. Besides this, the rural people in developing countries can engage in the sale of wild-collected herbs from wild resources and use them as a notable source of income. Although everyone benefits from medicinal plants, it is the economically weaker section of the society that are more closely associated with medicinal plants for their income, medicines, or culture (Hamilton 2004). Sustainable utilization of medicinal plants and their conservation has been the topic of research in many studies (Uprety et al. 2012; Larsen and Olsen 2007). Many suggestions regarding the conservation of these plants have been put together that includes preparing species inventory, monitoring the status, and the development of coordinated in situ and ex situ strategies of conservation (Hamilton 2004). Sustainable use of natural sources will be a more effective conservation alternative for the restorative plants that have limited supplies.

20.3 Conservation Strategies

Conservation may be defined as preservation of diverse organisms, their habitation, and the interdependence between them and their environment (Maxted 2001). Conservation of medicinal plants is quite tough as different plant species prefer variable habitat, growth, and geographical conditions. Even threats to their conservation and end use are diverse as their consumption is not only limited to local community but also includes urban populations of different countries. However, the objective of conservation of curative plants can be achieved by integrated approach involving traditional methods, scientific techniques (i.e., in situ and ex situ methods), cultivation practices, biotechnology methods, and policies and legislative framework set by government and world agencies (Hawkins 2008). The geographic distribution of medicinal plants along with their biological characteristics should be studied to guide conservation strategies, e.g. to judge if the species is to be protected in its natural surroundings or in a nursery. The prime objective of conservation is to focus on sustainable development. It can be ensured by using biological resources in ways that do not knock down principal ecosystems and habitats or shrink the world's diversity of species and genes. The details of the various approaches are discussed below.

20.4 Traditional Approaches

Medicinal plants can metaphorically be very significant in lives of many people such as they may hold a special position in their religious, cultural, and opinionated beliefs. This respect and value can be very beneficial for conservation practices as long as local communities are the stakeholders in it (Hamilton 2004). The role of these traditional beliefs and religious practices in conservation is quite evident in countries like India, Pakistan, Africa, and those falling in Southeast Asia.

In India, cultural and religious sentiments and practices have a crucial role in conservation of many plant species of medicinal importance. The idea behind sacred groves, sacred species, and sacred landscapes has been evolved to protect and safeguard the key biological assets including medicinal plants by traditional approach. The vegetation in sacred groves such as Devbhumi, Uttrakhand; Oran, Rajasthan; Kovilkadu, Tamil Nadu; Deovan, Himachal Pradesh; and Devarakadu, Karnataka, inhabits many plant species of medicinal value (Kala 2009; Akshay et al. 2014). India has a total of 13,720 sacred groves spread in its various states (Akshay et al. 2014). A survey was conducted on 79 sacred groves in India and it was identified that these groves constitute around 131 families, 340 genera, and 514 species. About 1.3% of out of the total area under these groves was undisturbed forest land, 42.1% was inhabited by dense forest, 26.3% had only limited canopy cover while 30.3% was open forest land. Precisely, the genetic diversity was quite significant in sacred groves than that of undisturbed forests (Rim-Rukeh et al. 2013). Thus, establishing these sites is one way to conserve species and genetic diversity of medicinal plants within their own natural habitats and environmental conditions (Kala 2009; Akshay et al. 2014).

Many medicinal plants are also epitomized as sacred and these sacred species not only hold religious importance but also exhibit numerous medicinal properties. This medico-religious thinking has made people worship these plants as gods or goddesses such as *Ocimum sanctum* L. is worshipped in India as local deity, *Saussurea obvallata* as lord Brahma, *Ficus religiosa* L. as lord Vishnu, *Ficus benghalensis* L. as lord Shiva, and *Sesamum orientale* L. as lord Saturn (Kala 2009).

Similarly in Africa, particularly Nigeria, people believe that gods and goddesses reside in nature on rocks, rivers, ponds, trees, plants, land, etc. as they aspire to live in community. These beliefs are further supported by rules and organizations strong enough that they are dutifully embraced by people. In Ethiopia, Nigeria, Okpagha and Ogriki trees are given very high regard as they are believed to belong to Aziza spirit, thought as deity of woods by the local people. The spirit is assumed to protect the fields, animals, and even people from their enemies. As sacred trees, plants growing in the vicinity of these trees are not allowed to cut and hence vegetation surrounding these trees is rare. The bark and wood of trees is only used for medicinal and religious purposes only and even the medicinal properties of trees and vegetation are kept as secret to prevent their overexploitation by outsiders. Local communities are not allowed to settle in these forest areas thus protecting the forest from deforestation and farming practices. Though the area inhabited by forest is quite small, the species diversity is fairly high (Rim-Rukeh et al. 2013).

In India, written ancient records in the form of “Vrikshayurveda” are present which not only highlight importance of plants but also include methods for conservation and protection of plants from diseases and external factors along with methods to improve yield. A chapter, “Bijoptivithi” deals with preservation practices followed for seeds. Another chapter, “Drumaraksha” accentuates details about protection of plants from external factors such as wind and storm (Shubhashree et al. 2018).

Thus, traditional beliefs, approaches, and methods have a critical role in conservation and preservation of herbal plants when coupled with modern technology.

20.5 Scientific Approaches

The two scientific fundamental strategies for safeguarding remedial plants are—in situ and ex situ conservation. These strategies further involve various techniques for maintaining species diversity under natural habitats and for sustainable development.

20.5.1 In Situ Techniques

In situ technique is the way of conserving the species in its natural surroundings by establishing protected areas that include forest parks, nature reserves, geological parks, scenic spots, wetland parks, marine special reserves, and marine parks (Wang et al. 2020). Protected areas play a key role in the maintenance of ecological security of the nation that forms the baseline of the in situ conservation system (Ma et al. 2012). Majority of the medicinal plants are endemic species, i.e. they are found growing in restricted areas that are mostly geographical and biologically isolated. Their medicinal properties are mainly attributed to their secondary metabolites that show best response to stimuli in wilds, and that may not be expressed to their full potential under culture conditions (Figueiredo and Grelle 2009; Coley et al. 2003). As many species of plants have curative properties so conservation of medicinal plant in a way is microcosm of floral conservation in totality (Hamilton 2004). Many medicinal plants have been recommended whose collection from the natural sources have been prohibited like *Aconitum* sp., *Aristolochia bracteolata*, *Acorus* spp., *Atropa acuminata*, *Berberis aristata*, *Chlorophytum* spp., *Commiphora wightii*, *Curculigo orchioides, Colchicum luteum, Concinum fariestatum*, *Dorsera* sp., *Didymocarpus pedicellata*, *Ephedra gerardiana*, *Gloriosa superba*, *Gentiana kurroa*, *Anchusa strigosa*, etc. (Lakshman 2016).

Conservation strategies that focus on in situ conservation serve dual purpose of saving the natural habitat of the species besides protecting the species. It is a convenient method of providing safety to large number of population. As the species are conserved in their home environment, the method is convenient and cost effective. The species is also saved from adjustment to new habitat. The in situ conservation attempts of the entire world targets at ecosystem-oriented setting up of protected areas rather than being focused on individual species (Ma et al. 2012). Rules, regulations, and potential compliance of therapeutic plants within natural habitats greatly influence the success of in situ conservation (Soule et al. 2005; Volis and Blecher 2010).

Various types of protected areas like biosphere reserves, wild nurseries, national parks, sacred groves, wetlands, and natural parks have been recognized. There have been other nation specific forms of in situ conservation measures like in China, they have Ecological Conservation Red Line (ECRL), the Non-Commercial Forest (NCF), Mini Natural Reserves (MNRs), and the Civil Protected Areas (CPAs)

(Gao 2019; Wang et al. 2020). In India, National Medicinal Plants Board has established Medicinal Plants Conservation and Development Areas to support in situ conservation of herbal plants (Biswas et al. 2017).

20.5.1.1 Biosphere Reserves

The primary reasons for loss of medicinal plants are degradation, fragmentation, and destruction of habitats (Camm et al. 2002). The secured localities of predominant wild resources designed to restore and save biodiversity are called natural reserves (Rodriguez et al. 2007; Chiarucci et al. 2001). Biosphere reserves are the categories of protected areas which may be land or coastal environment having people as an intrinsic component. UNESCO's MAB (Man and Biosphere) program started in 1975 gave the notion of Biosphere reserves as a part of the project that was concerned with conservation of ecosystems and genetic resources. The main target of these reserves is to bring about consolidated management of water (marine and freshwater) and land, along with living resources. This could be achieved by amalgamation of conservation and development by setting in place bioregional planning schemes that results in appropriate demarcation of core, buffer, and transition zones (Reyers 2013). At present, there are 714 biosphere reserves recognized at world level. They are spread in 129 countries that include 21 trans-boundary sites. These biosphere reserves merge three main "functions":

- Conserving diversity of living organisms and culture.
- Socio-culturally and environmentally supportable economic development.
- Providing logistic support and foster development through education, research, training, and monitoring.

The three well-separated zones, i.e. core area, buffer zone, and transition zone of biosphere reserve help in pursuing these functions (Fig. 20.1).

1. **Core Areas**: the innermost region of the natural reserve forms the core area. It constitutes a strictly guarded zone that contributes to the protection of genetic variation, landscapes, ecosystems, and species.
2. **Buffer Zones**: It skirts or adjoins the central core area, and is used for practices congenial with healthy ecological activities that can fortify scientific research, training, monitoring, and education.
3. **Transition Area**: It forms the outermost region of the biosphere reserve. Communities promote socio-culturally and ecologically viable economic and human activities in this area.

Protecting the habitat of the flora and fauna by establishing biosphere reserves forms an important strategy in conservation of wild resources. They include regions with representative geological conditions and dwelling places of some endangered flora and fauna. Careful planning is required for the efficient establishment of these reserves. It involves the genuine selection of sites from larger areas of potential sites with an aim of covering as many species as possible in the conserved area. The area

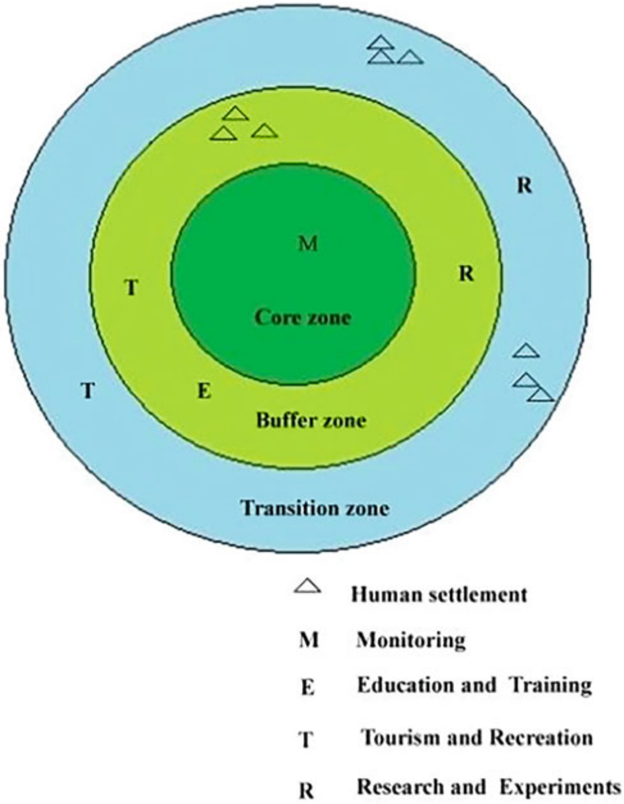

Fig. 20.1 Zones of biosphere reserve

covered under the natural reserve should contain sufficient habitats supporting viable population of each species at the specific place. There are more than 12,700 protected areas at global level. They cover 8.81% of the Earth's land surface, i.e.13.2 million square kilometer (Huang et al. 2002). Protecting therapeutic plants by securing vital wild habitats requires evaluating the ecosystem functions and contributions of discrete habitats (Liu et al. 2001). The primary target of biosphere reserves is safeguarding diversity of biological forms, but they are different from strictly protected areas as they allow human settlement as a part of the protected landscape. Natural resource harvesters and local communities play the major participatory role in these reserves hence forms the social, as well as spatial components of these reserves. Biosphere reserve's design and management may be outlaid with the involvement of major beneficiaries affected by the reserve. These natural reserves have helped in safeguarding the biodiversity in more scientific and systematic pattern that is economically and socially more admissible to mankind. At present, the major challenge that is faced by the world community is to add new sites for uplifting global coverage under biosphere reserves and ensuring that these reserves attain their assigned functions (Nations 2001).

20.5.1.2 National Parks

These are the conserved areas, whether within a sanctuary or not, on the basis of its ecological, floral, faunal, geo-morphological, or zoological association and relevance, needed for the purpose of safeguarding, multiplying, or evolving wildlife there in and its surroundings. The landscapes of these national parks together with their constituting animals and plants are kept in their natural form. Except for some of the activities for which the Chief Wildlife Warden of the state gives permission under the specific conditions no other human activity is permitted inside the national park. It is an area kept aside for the conservation of the natural environment by a national government and comprises sites that have ecosystems that are characteristic to an area. At global level, there are more than 12,700 established protected regions (Huang et al. 2002; Kadam and Pawar 2020). National Parks have a crucial role in safeguarding herbal plants (Lakshman 2016). Each country has its own norms for providing protection via establishing national parks. Canada and the USA target on protecting land and wildlife, Africa lays emphasis on conservation of animals while the United Kingdom aims primarily on the land via setting up national parks. Several other countries also have large areas reserved as national parks like Brazil, India, Japan, and Australia (Britannica 2021). The notion of national park, under state ownership was conceptualized in the USA in the year 1870 and Yellowstone National Park established in Wyoming was the world's first such park. However, some naturalists and others view Bogd Khan Mountain National Park in Mongolia that dates from as early as 1778 as the pioneer in the field. India alone has 101 national parks covering 1.23% of the total geographical area (40,564.00 km^2) of the country (National Wildlife Database, December, 2019). The importance of national parks in conserving medicinal plants could be well understood from the case study of Khadimnagar National Park, a protected area in northeastern Bangladesh (Rahman et al. 2011), case study of Ba Vi National Park, and case study of Ben En National park of Vietnam (On et al. 2001; Hoang et al. 2008).

20.5.1.3 Wildlife Sanctuary

A wildlife sanctuary is an area where animals and birds can live protected in their natural habitats, away from poaching or trafficking. These protected areas save the endangered species and protect them from humans and predator. Though they are mainly focusing on protection of animals, they also conserve wild plants as animals are to be inhabited in their natural habitat. Several species of medicinal plants with considerable value have been reported and provided protection in these reserved areas (Rana Man and Samant 2011; Kumar et al. 2015; Chen et al. 2016; Srivastava and Shukla 2018; Radha et al. 2020; Koti and Kotresha 2021). Barnawapara Wildlife

Sanctuary, India provides protection to 21 trees species, 11 herbs, 7 climbers, and 3 shrubs having remedial properties (Raj et al. 2016). Similarly Kedarnath Wildlife Sanctuary (KWLS), India conserves 152 medicinally significant plant species that represented 123 genera belonging to 61 families and comprises 103 herbs, 32 shrubs, and 17 tree species. These also include 18 species that belonged to the endangered (critically endangered), vulnerable, and rare categories of threatened plants (Bhat et al. 2013).

20.5.1.4 Wild Nurseries

The medicinal plants populations are under tremendous pressure due to the threat posed by invasive species, overexploitation, and habitat degradation. Wild nurseries are the effective and most effective practical approach for in situ protection of curative plants that are endemic and threatened but their demand in market is very in high. A wild nursery is an area established in a natural habitat, or a well-protected place nearby the locality having natural growth of the plants. These focus on species-intended domestication and cultivation of endangered therapeutic species (Chen et al. 2016; Kadam and Pawar 2020). As it is not feasible to designate every wild dwelling area of plant as a conserved area, setting up of wild life nurseries is more favorable and constructive.

20.5.1.5 Gene Sanctuary

A gene sanctuary is an area or a field where plants are conserved. Land, labor, and finances are required for germplasm conservation of annual or perennial replanting of forest trees as well as plants propagated by vegetative means in these life field gene banks (Lakshman 2016).

20.5.1.6 Wetlands

Wetland (such as marshes, swamps, and bogs) maintenance is focused on sustaining and preserving localities where water is present at or close by the Earth's surface. These wetlands are key ecosystem serving as important source of livelihoods for the people living within or around these sites. They are the source of many economically important plants including plants with medicinal value like *Colocasia esculenta, Desmodium triflorum, Eclipta prostrata, Heliotropium indicum, Hygrophila schulli*, etc. (Leaman 2016; Athira 2019). There is a need to compile a comprehensive world list of the distribution of herbal plants in various habitats. Without this knowledge, it is not possible to assess the relative significance of wetlands as a source of remedial plants. The present assessment of wetlands as source of therapeutically valuable plants banks upon well-known taxa only (Horwitz et al. 2012). Regional surveys of biological diversity conducted can provide thorough insights into the significance of wetlands as sources of therapeutic plants. They can also highlight their role in livelihood and health benefits along with the challenges faced in their conservation challenges (Leaman 2016).

20.5.1.7 Sacred Sites/Sacred Grove and Sacred Mountains

Care and regard for nature has been influenced by aboriginal belief and religious practices in India, as well as in parts of Asia and Africa. Sacred groves form the small or large specks of vegetation safeguarded mainly by tribal or local people based on their religious beliefs. They are conserved following ethnic and customary practices of the local people. These tribal areas are untouched and well protected by the locals because of their conventional and religious closeness with the area and their faith in the local deities (Behera et al. 2015; Mohanty et al. 2016; Singh et al. 2017). IUCN has contemplated these areas of rich floral diversity as "Sacred Natural Sites" and is a way of worshiping nature (Mohanty et al. 2016). These areas are rich repository of diverse flora with the medicinal plants and have lavish growth coverings of rare and endemic species (Chanda and Ramachandra 2019). Sacred groves as a rule are treated piously (Kandari et al. 2014). The livelihood of the tribal folks in the area is entirely dependent on forest resources. Their traditional practices help in conserving rich floral diversity that forms an important resource for food, shelter, fiber, or medicine. Sacred groves act as a repository and nursery for variety of folklore, tribal, and ayurvedic medicines (Bhakat and Pandit 2003). There are a total of 13,720 documented sacred groves reported in India (Lakshman 2016). These groves have an indispensable role in judicial use and protection of herbal plants. Involvement of the local people in the conservation and management of the traditionally known medicinal wealth of the sacred areas offers several advantages (Behera et al. 2015).

20.5.1.8 On-Farm Conservation

This is the method to provide protection to the medicinal plants within conventional agricultural system. The farmers cultivate land races of the crop plants developed by them for sowing on their lands. These plants are well acclimatized to the local environment of the area so doesn't cause any type of problem during their cultivation. Long-established crop cultivars (land races) or farming systems followed by agriculturist are safeguarded by carrying out conservation practices on the farmlands. (Lakshman 2016).

20.5.1.9 Home Gardens

Home gardens unintentionally serve as means of conservation of many plants or their races of therapeutic importance. Kitchen gardens are the way to provide on-farm conservation at much smaller scale. In urban or rural situations, these tend to accommodate a wide range of plant species such as vegetables, fruits, spices, and medicinal plants.

20.5.2 Ex Situ Techniques

Ex situ conservation can be defined as maintenance of biological diversity away from its original habitat (Murray 2017). Though, in situ conservation is regarded as the most suitable option for protection as it leads to random mating through genetic exchange and recombination, aggregation, and transfer of beneficial mutations among populations and allows for better adaptation along with greater genetic diversity through competition (Khan et al. 2012). Ex situ conservation may be used in case of critically over-exploited and endangered medicinal plant species which generally grow slow, are less abundant, and particularly vulnerable to replanting disease (Chen et al. 2016). The main goal of this type of conservation is to grow and nurture threatened or endangered species for their sustained persistence besides making plant material available to be used in large quantities for drug manufacture and medicinal purposes. This is achieved via sampling populations of species, cultivars, breeds, or varieties followed by their transfer and storage in the form of living collections in botanical gardens, field gene banks and arboreta or under special artificial conditions as samples of tissue explants, seeds, tubers, pollens, or DNA (Murray 2017).

20.5.2.1 Botanical Gardens

The concept of botanic gardens is quite ancient with countries and civilizations like China, Egypt, India, Mexico, Mesopotamia, and Rome involved in cultivation of plants with medicinal value, exotic trees, and spices in royal palaces and religious places. However, the modern "Physic Gardens" came into being in the 1500s in Italy, Europe with the building of Padua Botanic garden in 1545. At that time these gardens were purely built for academicians to study medicinal plants. But today, the definition of botanic gardens has changed tremendously as they have been found to cultivate more than six million plants which represent approximately 80,000 taxa of the world, which includes around 40% of endangered species along with those that are extinct in wild, thus playing crucial role in the study of world plant diversity and their ex situ conservation (Chen and Sun 2018; Westwood et al. 2020).

As per definition given by Botanic Garden Conservation International (BGCI), Botanic gardens may be defined as "Institutions possessing written records in the form of documents for living plants intended for use in scientific research, conservation purposes, display and education." In present times, botanic gardens harbor world-class facilities like seed banks, green houses, herb gardens, nurseries, and even research laboratories. Living plant facilities in these gardens are not only utilized for taxonomical or teaching purposes but also for scientific and conservation objectives (Westwood et al. 2020). The best example of conservation in botanical garden is provided by Botanic garden of Chinese Academy of Sciences as they successfully conserved 20,000 vascular plant species. It is 60% of Chinese native flora and represents about 90% of all the species present in all the botanical gardens of China (Chen and Sun 2018).

Though botanic gardens appear as convenient way of conserving sustainable genetic diversity, they are not free of challenges, as number of species conserved in botanical garden will always be scanty due to lack of space. Secondly, smaller population size restricts genetic swapping and stochastic processes which make them prone to deleterious genetic outcomes (Maxted 2001). The list of botanic gardens by country can be retrieved from the website, http://www.bgci.org.uk/ of Botanic Garden Conservation International.

20.5.2.2 Seed Banks

Seed banks offer an alternative ex situ strategy to conserve genetic diversity where botanic gardens fail to do so (Chen et al. 2016). Currently, 1750 seed banks operate worldwide which harbor around six million accessions. Out of the total 50,000–60,000 taxa present in germplasm banks, an estimated 45,000–50,000 are stored in conservation gene banks. One of the worth mentioning facilities for seed storage is Millennium Seed Bank consisting of thousands of seed samples from wild species (Hay and Probert 2013).

Seeds being complete organisms are highly adaptive towards harsh conditions which offer a convenient way to maintain and sustain plant germplasm (Walters and Pence 2020) except for certain plant species which either do not produce seeds and grow by vegetative propagation or in few plants the seeds produced has short life, they are referred to as "*recalcitrant.*" Recalcitrant seeds cannot resist drying above a certain threshold value without losing viability. However, seeds that can fairly be dehydrated to low moisture content, i.e., $\leq$ 3–7% are termed "*orthodox seeds.*" There are certain intermediate seed varieties (7–12% moisture content) also which can be dried to the level of orthodox seeds but are generally susceptible to low temperature storage used for orthodox seeds (Chen et al. 2016). For non-orthodox seeds, cryopreservation, i.e., storage below -196 °C is used (Hay and Probert 2013). Hence, the life span of seeds during storage depends upon conditions of relative humidity and temperature. Freeze storage is mainly used as an international standard for preserving seeds in seed banks. Orthodox seeds stored under proper conditions can remain viable up to 100–200 years under freeze storage.

Though seed banks appear cost-effective alternatives of conservation, the major issue they are facing is reintroduction and restoration of wild species in their natural habitats (Chen et al. 2016).

20.5.3 Biotechnology-based Approaches

Advancements in plant biotechnology have led to improved and powerful methods for the protection and maintenance of species diversity such as in-vitro techniques involving plant tissue culture and molecular biology approaches. Nowadays, biotechnological methods are more preferred over conventional methods for conservation as they allow rapid production of disease-free elite plants with high genetic diversity. In vitro methods for regeneration of clean disease-free plants are used in various sectors such as agriculture, floriculture, and pharmaceutical (Cruz-Cruz et al. 2013). These techniques are quite efficient in producing medicinal plants also as most of the herbal plant species are non-seed producing or their seeds are too small to germinate and hence are propagated vegetatively. Moreover, recalcitrant seeds of some medicinal plants cannot be dehydrated without losing viability. Some of the plants produce heterozygous seeds and hence, not suitable for conservation (Sharma et al. 2010).

20.5.3.1 Plant Tissue Culture

Plant tissue culture offers rapid, systematic, and season independent way of artificially propagating plants under sterile conditions. It's an effective method to regenerate disease-free elite plants (Pathak and Abido 2014). It is based on the fact that any plant part such as cell, tissue, organ, or even a cut out differentiated piece termed as explants has cellular totipotency to differentiate and form into whole plant. Plant tissue culture techniques offer various methods for rapid (e.g., micropropagation, somatic embryogenesis, or organogenesis), medium (e.g., restricted or slow growth), and long-term (e.g., cryopreservation) conservation process. The methods lead to unlimited plant multiplication, synthesis of secondary metabolites, and storage of plant material for longer period of time (Pant 2014).

Micropropagation/Clonal Propagation

Micropropagation or cloning or clonal propagation may be defined as the technique in which plants are propagated in vitro by vegetative means to produce genetically identical copies of cultivars. The technique is much preferred over traditional methods of propagation as it leads to mass production at fast multiplication rate, requires limited space, and is season independent (Pathak and Abido 2014; Pant 2014). Successful micropropagation involves various steps like selection and preparation of donor plant, establishment of aseptic cultures, multiplication, and development of roots in in vitro shoots and lastly, the transplantation of small plantlets in soil followed by acclimatization (El-Esavi 2016). Though the technique offers several advantages, it doesn't guarantee pathogen-free plants as viruses may hibernate in tissues without showing any symptoms and can proliferate during multiplication stage. To overcome such difficulties shoot tip or meristem cultures are employed as being young and having rapid division these tissues have viruses spread unevenly (Cruz-Cruz et al. 2013).

Micropropagation has been employed to regenerate many medicinal plant species such as *Allium chinense, Aloe vera, Artemisia annua-aremisin, Asparagus adsendens, Catharanthus roseus, Camellia sinensis, Phyllanthus amarus, Elettaria cardamomum, Stevia rebaudiana*, etc. (Pathak and Abido 2014; Pant 2014).

Somatic Embryogenesis/Organogenesis

Somatic embryogenesis may be defined as formation of embryo-like structures from somatic cells which are developed under special in-vitro conditions into whole plant. In this process, embryos are either produced directly from cell or group of cells such as pollen, style, etc. without formation of callus or from an explant, callus is formed from which embryo is generated using callus tissue or cell suspension culture. In either process, the composition of culture medium is very critical. Somatic embryogenesis offers several benefits over traditional micropropagation technique such as production of large number of somatic embryos, simultaneous development of root and shoots, easy scale-up, low labor cost, and long-term storage via somatic embryo dormancy. The only limitation of this method is induction of somaclonal variations which can lead to genetic changes, reduced plant viability, and regeneration capacity on long-term storage (Bhatia and Bera 2015).

Organogenesis, on the other hand, may be defined as formation of organs, i.e., roots, shoots, buds, etc. from cultured tissues. De novo organogenesis is mainly based on ratio of phytohormones (auxin and cytokinin), high cytokinin to auxin ratio results in induction of in-vitro shoots while high auxin to cytokinin ratio promotes in-vitro root development. Organogenesis mainly occurs in three stages, in the first stage explants respond to phytohormones provided, followed by the second stage in which dormant cells again enter cell cycle and fate of cells is decided. The final stage involves morphogenesis of in-vitro organs (Bhatia and Bera 2015).

Somatic embryogenesis and organogenesis have been successfully employed to regenerate endangered medicinal plants such as *Artesemia vulgaris, Baliospermum montanum, Calligonum comosum*, *Eleutherococcus senticosus, Hedychium coronarium*, *Heliotropium kotschyi*, *Lilium ledebourii*, *Psoralea corylifolia*, *Rauvolfia serpentine, Turbinicarpus pseudomacrochele*, and *Woodfordia fruticosa* (Pathak and Abido 2014).

Restricted Growth

Restricted or slow growth storage is a method used for mid-term protection of plant species. In this method the metabolic activity of in-vitro cultures is reduced leading to slow growth rate on either modified growth medium or in modified growth conditions. The aim of this method is to increase the time span between two in-vitro subcultures without affecting their re-growth potential, which is generally achieved via two main ways, one by altering the components of growth medium used and other by changing the culture conditions. The most accepted and widely recognized method is lowering the temperature in conjunction with reduced light intensity or by keeping culture under complete darkness. Though this method is extensively followed, for tropical plants mostly high temperature around 15–20° C is used as they are sensitive to cold conditions. These in-vitro cultures are maintained by periodic sub-culturing under standard conditions. This also avoids chances of contamination or deterioration of cultures. The altered growth media is achieved by changing concentration of minerals, sugars, plant growth regulators, plant growth inhibitors, osmotically active compounds, etc. which are either enhanced or reduced to minimize growth rate. Other parameters which influence the efficiency of procedure include type of explants, type of culture vessel, culture medium, and physical and chemical state of explant during storage (Chauhan et al. 2019).

Several medicinal plants are being conserved using this method such as *Elettaria cardamomum, Allium sativum, Garcinia indica, Colocasia esculenta*, Mentha spp., Musa spp., Saccharum spp., etc. Hence the method is widely used due to several advantages offered like it requires minimum space and reduced labor cost than other methods like cryopreservation. Thus, it allows clonal plant conservation for mid to long term, utilizing few sub-culturings (Chauhan et al. 2019).

20.5.3.2 Artificial Seeds

Artificial seeds are also known by different names like synthetic seeds, syn seeds, seed analog, or manufactured seeds. They usually enclose somatic embryos in a protective coating and can be sown in a similar way as natural seeds. In the past, only somatic embryos were utilized for development of synthetic seeds but today other tissues like shoot bud tips, embryonic masses (organogenetic or embryogenetic calli), protocorns or protocorn like bodies, etc. can be used (Pond and Cameron 2017).

Naked somatic embryos can also be used as synthetic seeds but they are quite prone to drying and attack by pathogens and micro-organisms under natural environmental conditions. Hence for large-scale plantation and to successfully enhance their germination in green houses or fields coating is preferred. Sodium alginate is mainly utilized as coating agent as it offers several benefits like ease of capsulation and being less toxic to embryos. Nutrition to embryo is provided by addition of growth regulators and nutrients in the encapsulation mixture. Further, to protect damage to embryos from environmental factors such as desiccation, contamination by microbes and pathogens or physical injury, various adjuvants are also added to coating material, viz. pesticides, fungicides, insecticides, charcoal, etc. Charcoal improves respiratory properties of embryos (Chauhan et al. 2019).

Hence, artificial seeds produced are used for both germplasm storage in case of recalcitrant seeds and also for the production of elite varieties with elite characteristics (Chauhan et al. 2019).

20.6 Cultivation Practices

Though medicinal plant population collected from wild resources is generally considered more effective in combating disease than cultivated varieties, still domestic cultivation is a widely accepted practice. The cultivation of medicinal plants under optimized conditions of nutrients, water, supplements, and environmental factors such as temperature, light, humidity, etc. offers numerous advantages over wild harvest, viz. stability in production and improved yield of bioactive compounds. In addition, it leads to conservation of wild medicinal plants by decreasing their harvest volume from wild reserves and availability of these plants in market at affordable prices (Chen et al. 2016).

20.6.1 Good Agriculture Practices (GAPs)

They have been designed keeping in mind the production, quality, and standardization of herbal drugs obtained from medicinal plants. These guidelines certify superior, secure, and pollution-free (free from chemical pesticides, insecticides, etc.) herbal drugs. As an approach GAPs include several elements like ecological surroundings of sites of production, detection of quality facet, authentication of plants at both macroscopic and microscopic levels, correct identification of bioactive constituents, and inspection and authentication protocol for final plant material. Several countries like China vigorously encourage execution of GAPs (Chen et al. 2016).

One of the methods of GAP is organic farming. It aims to produce medicinal plants having high quality and productivity along with the aim of conservation and sustainable use. It doesn't support use of chemical fertilizers, pesticides, herbicides as per organic certification levels in North America and Europe. On the other hand, it promotes use of organic manure and fertilizers which not only improve soil quality and stability but also drastically improve growth of therapeutic flora and production of bioactive compounds (Chen et al. 2016).

20.6.2 Good Harvesting Practices (GHPs)

GHPs must be formulated for conservation and tenable use of restorative plants. The wild collection of medicinal plant material is only possible if its collection per year doesn't exceed its annual natural increase in species population at particular location. However, if the collection rate increases than production rate, the probability of species becoming threatened increases proportionally. Healing plants with low abundance and slow growth can even become extinct through limitless harvesting practices that can lead to exhaustion at resource site. To prevent this, harvesting of leaves, shoots, flowers, and buds should be promoted in place of roots or whole plant wherever possible (Chen et al. 2016).

20.7 Policies and Regulatory Framework

As discussed earlier also in the chapter, medicinal plants are of immense importance as they play a significant part in the cultural, livelihood, or economic aspects of people's lives. Today, many medicinally important plants face severe genetic loss or are at the verge of extinction but the comprehensive facts are missing. Traditional communities, whose very existence is also under threat, have most of the information on the use of curative plants. Only fragment of the knowledge regarding these endangered medicinal plant species held by the tribal people has been recorded in a systematic manner. For majority of the countries of the world, there is not even a complete inventory of therapeutic plants and even for those that are known, in most cases, no conservation action has been taken at all (WHO, IUCN and WWF 1993; Lakshman 2016).

A set of suggestions have been compiled for safeguarding the curative plants by various associations, such as those associated with international conferences at Chiang Mai (Thailand) in 1988, Bangalore (India) in 1998 and the Forty-first World Health Assembly, 1988. The Chiang Mai Statement—"Saving Lives by Saving Plants" affirmed the significance of therapeutic plants. It emphasized inclusion of international organizations such as United Nations, its agencies and Member States, to take initiatives for the protection of herbal plants. The guidelines of World Health Organization (WHO) prepared by collaboration of IUCN (International Union for Conservation of Nature and Natural Resources), UNEP (United Nations Environment Progamme), WRI (World Resources Institute), and WWF (World Wide Fund for Nature) in 1993 gives an outline for the protection of remedial plants and their sustainable use in medicine. WWF in association with the Ministry of Indigenous Medicine in Sri Lanka is engaged in conservation of plants with medicinal significance through a joint project. Botanic Gardens Conservation International setup by IUCN is also encouraging botanic gardens to safeguard medicinal plants by using various techniques, particularly ex situ methods. UNESCO (United Nations Educational, Scientific and Cultural Organization) is also putting its efforts in conservation of medicinal plants through its Man and the Biosphere Programme. It utilizes its broad network of biosphere reserves for safeguarding herbal plants. FAO (Food and Agriculture Organization) is participating in its own way by being one of the three sponsors of a Newsletter that covers Medicinal and Aromatic Plants. The UN Industrial Development Organization (UNIDO) assists in transfer of technology for the genetic improvement of plants that hence contributing in conservation of healing plants. Majority of the countries of the world have listed their threatened flora in the form of "Red Data Books" and have used the criterion of the IUCN categories of threat. The countries are encouraged to prepare the list of RET (Rare, Endangered and threatened) and CITE (Convention on International Trade in

Endangered Species of Wild Fauna and Flora) plants that will help in managing and keeping track of the trade in these plants at international level. This will also help in checking any type of threat to the survival of these herbal populations in the wild.

Various guidelines and suggestions for conservation of therapeutic plants at international and national level have been given by WHO; (Hamilton 2004; Kathe 2006) like:

- Defining objectives.
- Collection of more information on the medicinal plant trade.
- Holding a regional and/or national workshop.
- Botanical institutions assigned the work of cataloguing all the plants used as medicine in the country along with their use, distribution as well as their population size.
- Recognition of threatened medicinal plants in the wild.
- Promoting research, identification, and distribution of medicinal plants.
- Establishment of national herbarium with a botanical library.
- Different subject experts to be brought together in order to assess the state of affair.
- To establish a system for preparing species inventory and monitoring status.
- Development of policies and programs by incorporation of viewpoint of community and gender.
- Setting priorities for conservation.
- Draw up a plan of action.
- Promoting the exchange of information, expertise. and technology within and between countries.
- Responsible business practices and sustainable production.
- Coordinated strategies for in situ and ex situ conservation.
- Broadening the scope of international organizations that deals with conservation of herbal plants.
- Development of common design of databases on the protection and sustainable use of curative plants.
- Promoting cultivation of the herbal plants as the source of supply.
- Emphasizing sustainable collection from wild.
- Conservation of wild flora and the Law (IUCN Environmental Policy and Law Paper no. 24, IUCN, 1990) critically assess all the laws that safeguard plants and supply comprehensive suggestions to law-makers to draft new laws for plant conservation.

In India as well as in many other countries, there are no distinct policies or laws for saving wild herbal plants. They are conserved according to the existing laws pertaining to forestry. The laws for conservation of forests formulated by Indian government directly or indirectly protect the natural flora of medicinal importance. These include Forest Act, 1927, Wildlife (Protection) Act, 1972; Environment Protection Act, 1986; Forest (Conservation) Act, 1980; National Forest Policy, 1988; National Biodiversity Act, 2002; Wildlife (Protection) Amendment Act

1991, and the Scheduled Tribes and Other Traditional Forest Dwellers Act, 2006 etc. (Lakshman 2016).

20.8 Conclusion

The overwhelming interest in use of medicinal plants and their ever-growing demand in various industries like cosmetic, food, pharmaceutical, and perfumery has led enormous pressure on their natural wild population. Uncontrolled destructive harvesting of plants from wild and the loss of their habitat are the major factors behind medicinal plant conservation and sustainable use. It has become paramount to integrate various conservation strategies and techniques and also different potential agencies and legal systems to put combine efforts for sustainable conservation of medicinal plants. It is of immense importance to encourage the techniques for large-scale cultivation of herbal plants. The laws and rules should be strictly implemented to prevent the loss of the medicinal plants from the environment. Hence, there is an urgent need for conservation of herbal plant wealth for the present and future generations, by adopting the acceptable strategy with most suitable method of protection.

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Chapter 21
Integration of Medicinal Plants into Comprehensive Supply Chains: The Threats and Opportunities of Environmental Devastation

Jameel R. Al-Obaidi, Shakinaz Desa, Khalid H. Alobaidi, A. B. Adibah, J. Azi Azeyanty, Syazwan Saidin, M. N. Nor Nafizah, and E. I. Ahmad Kamil

Abstract Herbs with medical value are of great importance as it is widely used in complementary and alternative healthcare and medical practices due to its alleged health benefits, easy availability, perceived effectiveness, and safety. It is a source of natural products with health value which claimed to treat various illness from minor cuts and general infections to post-partum care. Herbs are also consumed as a functional food and have high market values in both pharmaceutical and nutraceutical industries. Their pronounced efficacy has been attributed to their phytochemical constituents especially the bioactive compounds. Bioactive compounds from medicinal herbs found to be effective as anti-bacterial, anti-oxidants, anti-tumour, anti-cancer, anti-inflammatory, and numerous more. Therefore, plant-derived substances have recently become a subject of interest and very demanding as people are now in a trend going back to nature. However, they are affected by different environmental changes. The quality of alternative medicine usually depends on the secondary metabolites produced and usually, these metabolites are influenced by an environmental factor. Despite the complex nature of the biosynthesis of different plant secondary metabolites, research indications highlighted that their biosynthesis and accumulation remain under the control of the environment. Changes from environmental influence have been reported at genetic or protein level which caused profound alteration of the metabolite pool of the affected medicinal plants. This chapter aims to discuss the progress and scenarios of different strategies used by

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researchers worldwide to preserve the herbs under various adverse conditions that encompassing sustainable development approaches as well as how different environmental factors influence qualitatively and quantitatively the production of secondary metabolites of medicinal benefits which can be developed as detection tools to ensure medicinal quality in phytomedicines.

Keywords Environmental devastation · Medicinal plants · Bioactive compounds · Secondary metabolites

21.1 Towards a Sustainable Integration of Medicinal Plant into the Comprehensive Supply Chain

The current movement and practices towards sustainable development are displaying a positive sign of awareness. The implementation of the green economy has raised a significant understanding of the importance of a quality environment (Bedenik and Zidak 2019; Chen et al. 2020; Nwozor et al. 2021; Zazykina and Bukova 2021). Agricultural activities are also heading in the right direction in protecting the environment (Saitone and Sexton 2017; Chamberlain et al. 2019; Chudinov 2021; Kuzmich 2021; Panchenko et al. 2021). Education has been actively promoting the concept of sustainability in various subjects, for instance in vocational education (Dalyanto et al. 2021), music education (Varkøy and Rinholm 2020), physical education (Baena-Morales et al. 2021), inclusive education (Bucknor 2018), and science education (Sonetti et al. 2020). Moreover, many fields were seen to accept sustainable development as common practice. Among them are tourism (Ulfa et al. 2021; Zakharchenko et al. 2021), logistic (Filippova and Voronina 2021), minerals activities (Hushko et al. 2021), gender issues (Dewi 2021; Husein et al. 2021), and other social-related issues (Judiasih et al. 2020; Panchenko et al. 2021). Though witnessing the many efforts towards a sustainable world, we should not relax by those noble efforts, yet.

Economic growth and sustainability issues have been a long discussion in ensuring the balance of profit-making and minimizing the depletion of natural resources. Natural resources capital will always become the core to provide food, energy, water, and health. Many agree that the management principles should value conservation more than profit-making. Medicinal plants are an essential and valuable resource in health care in many countries, and this drives the “gold rush” for trading and business profit in the global market. Hence, exploitation becomes inevitable and deviates from sustainable development principles.

Indicators for green economy dimensions include ecosystem resilience, resource productivity, and social equity, which were developed to measure its implementation and also to support the decision in the natural biomass-rich environment (Timonen et al. 2021). They discovered that indicators must be context-dependent, which will contribute to the international and national level indicators. This indicates that the integration of sustainable concepts into the medicinal plant supply chain entails local sustainability indicators. The indicators must be able to inform and provide feedback to the governing bodies. This will enable them to make and control decisions in medicinal plant businesses. Hence, by understanding the why and how problems and performance may be assessed effectively.

A model on the green economy was introduced along with the discussion on sustainable development (Szetey et al. 2021). The concept of a green economy is to generate income for investors, promote positive social changes, and decrease the negative impact on the natural environment, ethically. This concept is intended to provide clear guidelines on how to manage the economy when dealing with natural resources. The Global Green Economy Index (GGEI) is another indicator that measures the green economy performance via four scopes: climate change management, efficiency sectors, marketplaces and investment, and the environment (Georgeson et al. 2017). The index offers information on benchmark performance, areas that need improvement, and how stakeholders can promote progress. It facilitates policymakers and private sectors to making decisions on policies and their investments to a greener economy. However, all countries don't need to involve, although at the moment 130 countries take part. Thus, having an index, be it locally or internationally must become an important component in a framework for the sustainable integration of medicinal plants into the global market.

Agriculture practices in sustainable development are another crucial component in planning a framework. The technologies of investment with social responsibility can be applied in agriculture but were hindered due to low awareness in the agribusiness community (Chudinov 2021). Therefore, to change the management philosophy of agricultural businesses, regulation and education will take the core centre in searching for meaningful and systematic agribusiness management, hence appreciating the importance of sustainable development for medicinal plants.

Without a doubt, rich natural resources offer medicinal plants that may promote good health and well-being, encourage research and development for drug discovery, and provide alternative healthcare. Traditional medicines are highly valued in some countries as their basic healthcare needs (Van Wyk and Prinsloo 2018; Ambu et al. 2020; Jalali et al. 2020; Mustofa et al. 2020; Papageorgiou et al. 2020; Petelka et al. 2020; Xiong et al. 2020). When the demand increases, it would change the perspective from medicinal values to profit values. Research discovery on medicinal plants' prospects is expanding very fast around the globe. We have been observing promising data that provide solutions to various health problems, thus, the value of medicinal plants gets its impacts. The market value is skyrocketed, in which the demand rate moves in the same direction (Ahmad et al. 2021; Al-Obaidi et al. 2021; Gong et al. 2021; Hashemi et al. 2021; Hossain et al. 2021; Lim et al. 2021; Shikov et al. 2021; Tang et al. 2021). The demand provides a positive profit signal to the business. Therefore, medicinal plants require higher resources and rigorous efforts to supply the demand. As a consequence, governing bodies need to set the correct purpose between competitive trade business, healthcare, conserving the natural environment, and ensuring legislative compliance.

Sustainable management of natural resources has emerged through many innovations. However, the ecological risks are still happening. The increase of uncontrolled purchasing and demand by global consumers resulted in overexploitation. The exploitation of medicinal plants resulted from the loss of trust and legitimacy and the failure of compliance and enforcement. Both of these factors are seen as derived from the controlling power, which is owned by an institutional of a country. There were linkages and integration found between the medicinal value chain and various factors. Among the factors were knowledge and ecological degradations. They concluded that the integrations require plans of action that should be made up of several separate approaches from several points of direction. Therefore, to supply a reasonable volume of medicinal plants into the global chain sustainably, we need to strike the right balance. At this point, what is reasonable? The potential solutions should be based on better collaboration and cooperation in addressing these global issues by sharing a common vision. Sustainable integration entails multi initiatives to cater to crucial factors. A deeper understanding of matching the initiative to the correct factor is compulsory. A conceptual framework was described to show the interaction of various contributing factors to the ecosystem and livelihood outcomes (Volenzo and Odiyo 2020). The framework summarizes the starter as knowledge, power, and agency, which initiate a one-way flow. The concept depicts the vulnerability of the environment when the process flow experiences incompetency and lack of knowledge. However, the concept did not indicate feedbacks, reversible actions, or loop actions to represent the interactions between factors or actions.

Medicinal plants will always be at the core of meeting healthcare needs. The limitation of plant materials due to over-harvesting, incompetency of regulation monitoring, lack of indicators to control medical plant's global supply chain, and imbalance of business behaviour and sustainable development require a comprehensive concept for global practices. Examples of common business models are global herbalism, small-medium enterprise, traditional herbalism, and hybrid innovative practitioners (Bejarano et al. 2020). These models' practitioners have a different perception of the challenges of the medicinal plant industry. Among significant perceptions are the vulnerability of the environment when dealing with mass production and the sustainability assessment for the business. In some countries, medicinal plant businesses are having challenges in searching for alternatives to products and processes. The intention is to enable enough harvest to sustain and maintain the business. Hence, sustainable production strategies should also be a crucial part of the sustainability framework throughout the value chain. The mission to conserve resources and lowering waste and emissions must be put forth as compulsory rules.

In pharmaceutical supply chain industries, similar issues were described (Settanni et al. 2017). Models lack in conceptualizing a structure and behaviour of a supply chain. Indicators for a given problem are not well developed, thus creating fewer options, inefficient decisions, lack of alternatives on products and processes. It seems all supply chain industries are having similar and unseen challenges. Economic evaluation should include a systematic approach that integrates sustainability practices.

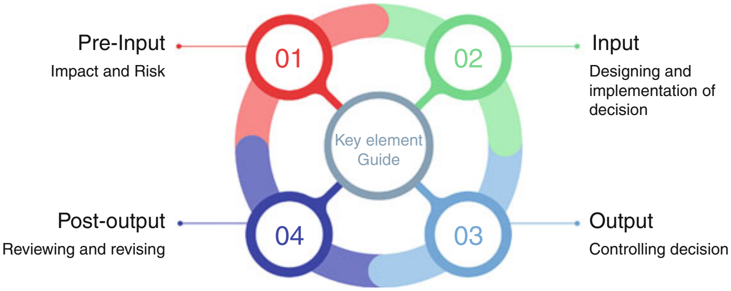

Fig. 21.1 In a framework, all four components should be represented by the key element

The framework is a concept to guide practitioners in managing medicinal plants into the global supply chain. To develop a conceptual framework, first, the framework itself must be sustainable. Sustainable in this sense is being able to communicate and signal the regulation of a process. This is to eliminate and avoid unwanted and inappropriate activities that may lead to waste and inefficient work. All elements must be interconnected and interacting. The key elements in the framework should be guided by four components: pre-input, input, process, output, and post-output. The component must be represented by at least one key element. This element is then further incorporated into the framework (Fig. 21.1).

Pre-input is the activities before deciding on preparing raw materials. Medicinal plant material should be prepared not solely on business demand, but to review the impact of over-harvesting and the risk of inadequate supply to the healthcare consumers. Input, on the other hand, is defined as activities such as decisions on regulations, policies, business models, and indicators. Process for sustainable integration is activities of educating, monitoring, assessment, evaluation, and providing feedback. Output is controlling the decision based on assessment, data sharing, and deep learning, local indicators, and regulation of the process. Post-output is reviewing and revising health and well-being, to determine other alternatives for medicinal plants. The post-output should be able to supply crucial decisions for the pre-input activities.

Second, each of the elements in the framework must include its own indicator, especially related to the local context. These indicators supply information for assessment and evaluation, provide feedbacks for regulation. Indicators must be able to supply information and signal Go or No-Go to all processes. Thus, the application of deep learning in this regulation of medicinal plants into the global supply chain is recommended. Third, as the world is at a constant change, predictions can be made. Future studies on the relationship between healthcare research findings and medicinal plant business should be done and reported. To see the connection and the balance between human health and human wealth, we should not compromise with environmental resources. Here, we suggest an outline to model the key elements into a sustainable framework (Fig. 21.2).

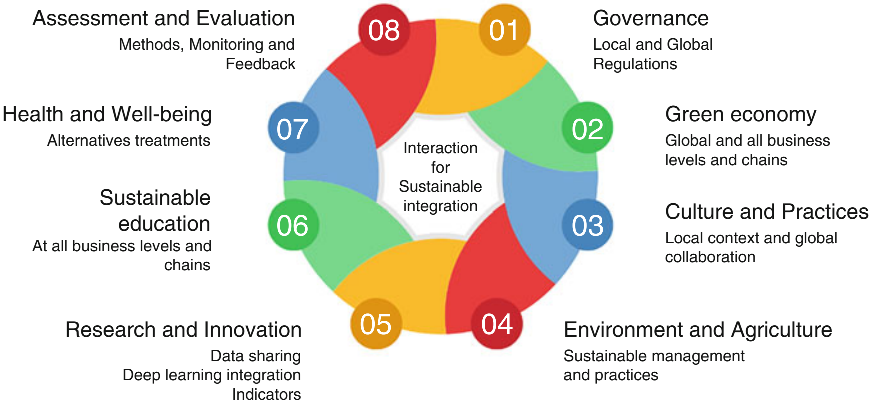

Fig. 21.2 Towards the integration of sustainable development into medicinal plants business, all components must be interconnected and possess their indicators

21.2 Sustainability of Global Medicinal Plant Sources Through Conservation Strategies

In parallel with various other typical economic transactions, as well as the interactions between different sources of power, be it in the government or corporate agency, the medicinal plant had evolved to become a multi-million-dollar industry with a worldwide distribution and consumption (McLeod 1999). This advancement had triggered the United States Congress to reclassify the herbs and medicinal plants as a dietary supplement in 1994, thus making it easier for companies to produce and trade medicinal plant products by minimizing the monitoring barriers (Robbins 1999).

Over the last few years there has been a huge increase in the global use of herbal medicines and, due to its alleged health benefits such as ready availability, efficacy, and safety, it is widely used in supplementary and alternative health care and medical practice and is seen as an integral part of the community culture (Razmovski-Naumovski et al. 2010). Herbal medicines are mostly being applied in developing countries as certain aspects of primary health care (WHO 2013) and more than two billion people are suspected of being highly dependent on herbal plants (Ganie et al. 2015). Governance systems have taken advantage of the hype and popularity of medicinal plants because most companies recognize the natural advantages and environmental merits of plant-based products and innovations and rely heavily on the purchasing power of healthy users (Robbins 1999). As the global market estimates reach USD 107 billion, international trading in herbal products is becoming a lucrative industry (Posadzki et al. 2013). This resulted in the production of a large amount of medicinal plant raw material mainly through the industrial wild flora (Nimachow et al. 2011; Goraya and Ved 2017; Seethapathy et al. 2019).

As a result of this fact, conservationists have been debating the ecological implications of increasing medicinal plant consumption for decades (Robbins 1999). By referring to the world's two largest medicinal plant providers, China and India, both countries have taken steps to reduce their reliance on raw medicinal plant sources while also conserving the products by promoting conservation through cultivation and regulation. The importance of plants in traditional medicines, particularly in developing countries, has been extensively documented; however, much remains unknown about the conservation needs of species exploited, leading to the extinction of some local medicinal plant species and the endangered status of others (McLeod 1999).

The aim of preserving global medicinal plant sources can contribute to the preservation of stable ecosystems for high-value medicinal products. Preserving these sources through the appraisal and use of medicine can help reduce global poverty and prevent local public health efforts in developing countries in particular. But demand for herbal medicines always goes hand in hand with the over-harvesting of medicinal plants, habitat loss, and farming invasion. Two types of biodiversity conservation strategies, in-situ and ex-situ technologies, are widely used to prevent such losses.

21.2.1 In-Situ Conservation of Global Medicinal Plant Sources

In-situ conservation of medicinal plant biodiversity refers to the conservation or recovery in the natural habitats of viable species where their unique characteristics have been established. Policy on land usage that enables such spots either to be found in protected zones or agricultural landscapes should be combined with in-situ conservation to ensure the preservation of medicinal plant biodiversity. A successful in-situ gene conservation programme must fulfil the three basic requirements as displayed in Fig. 21.3 below:

The survival of herbal medicinal species in situ must define its environment by various types of management and monitoring. Protection of such species requires the removal or at most containment of threats for endangered species. In-situ protection of target organisms should include interconnected practices, see Fig. 21.4:

21.2.2 Ex-Situ Conservation of Global Medicinal Plant Sources

Ex-situ conservation methods can include the cultivation of entire plants or seed storage in field gene banks for the conservation of diversity. Appropriate seed storage technologies must be developed for various species while ensuring that the plants produce comparable quality with the original stock.

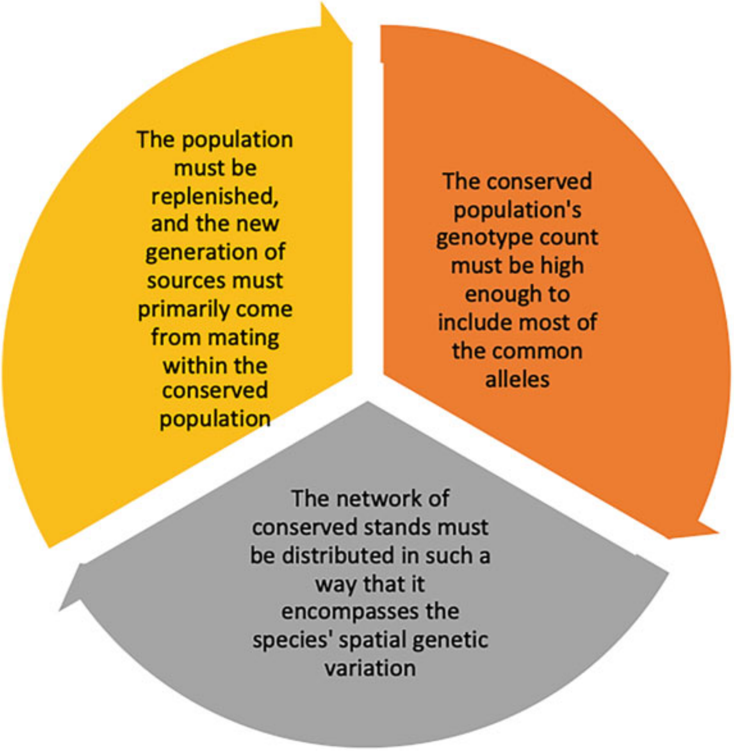

Fig. 21.3 Requirements for gene conservation program

One of the strategies that can be used is through propagation by vegetative means or by tissue culture, which is becoming increasingly popular with many species, particularly those propagated vegetatively and designated as endangered. Propagation techniques like tissue culture can usually be used in cases where the seed can be maintained and produced under dry and cool conditions. In addition, a reintroduction approach may also be used to identify extinct/endangered species in an area and the habitat in which they have been eliminated. The reintroduction of plants becomes more and more popular with several species and has proven successful in plant conservation and protected area management. The reintroduction, however, usually takes the form of an experiment. As a consequence, more plants may need to be planted in more than one location to achieve ecological restoration.

Cryopreservation is a useful method to be considered for germplasm preservation that involves storing plant material at ultra-low temperatures in liquid nitrogen usually at 196 °C. This is the only method available for the conservation of vegetative plants and those with recalcitrant seeds. This method is available for a long time. In recent years the number of plant species increased considerably, with the introduction of new cryogenic procedures such as cryopreserved plants and encapsulation dehydration. Several clonally propagated species/genotypes,

Fig. 21.4 Practices for in-situ protection

however, produce large seeds or otherwise require the involvement of in vitro methods for efficient cryopreservation.

21.3 Future Sustainable Strategy

Collectors of medicinal plants are untrained, and nearly half of the material collected by untrained labour is wasted. Consequently, methods for collecting medicinal plants from the wild must be developed sustainably. This includes teaching local collectors proper collection techniques, teaching people how to grow medicinal plants, and eliminating some of the middlemen in the trading chain. The fact that most people live below the poverty line and harvest natural resources mindlessly to supplement their meagre incomes is a major cause of medicinal biodiversity loss.

The medicinal plants and associated indigenous knowledge of a country require an aggressive ethnobotany study (Otimenyin 2021). As most medicinal plants are wild and harvested in preparation for remedies from their roots, the healers should not completely kill the medicinal plant species in consultation with governmental officials. These plants should be replaced by establishing nurseries for common medicinal plants to ensure sustainability, to reduce deforestation and its related consequences, such as erosion and soil fertility loss. Although there is still insufficient local effort to conserve medicinal plants, the traditional beliefs about folk medicine of long-standing populations are unintentionally important in conservation, management, and sustainable use. A major part of the conservation of medicinal plants shall be the active participation of local communities and stakeholders in the efficient monitoring of local community resources (Banerjee et al. 2021). Collaborative research projects involving local indigenous people and national and international partners and associated experts enable the improvement of the over-harvested population of natural medicinal plants.

A long-term, integrated, scientifically oriented action plan is required for the conservation and sustainable use of medicinal plants (Shafi et al. 2021). It is worth noting that biotechnology is opening up new avenues for the conservation of medicinal plants, enabling the speedy propagation and re-entry of endangered species into the environment, biodiversity evaluation and monitoring as a source of new conservation instruments and identifying potential new gene product uses. Medicinal plants are likely to become a natural resource for renewable energy. This would lead to a better utilization for the human well-being of those vital resources by preserving medicinal plant genetic resources.

21.4 Challenges and Potential Solutions in the Medicinal Plant Value Chain

21.4.1 Marketing Challenges in the Supply Chain for Medicinal Plants

To effectively commercialize medicinal products, an exhaustive understanding of demand and production processes and their value chain are needed (Volenzo and Odiyo 2020). In the last fifteen years, value chain analysis has been used in the production of medicinal plants as well as a variety of consumer products. By investigating the medicinal plant value chain, it is possible to obtain a better understanding of the role of various inputs in the chain, as well as their influence on chain management (Booker et al. 2012). Any action necessary to supply a product from the source to the end-user in a way that responds to their needs, tastes, and

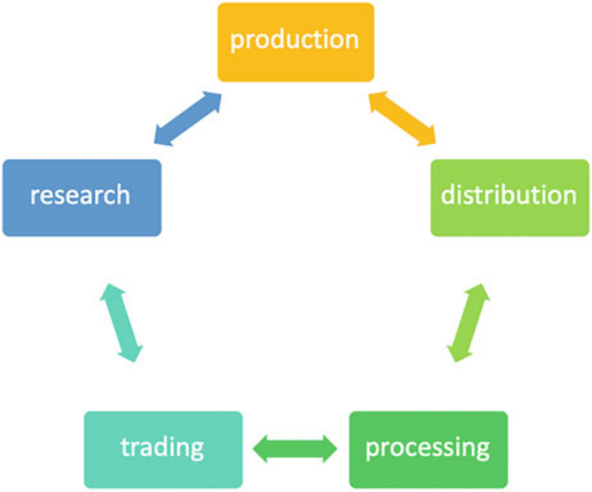

Fig. 21.5 Main steps for medicinal plants value chain

preferences is a value chain. This medicinal plant value chain includes several important aspects such as research, production, distribution, processing, and trading activities with everything designed to meet consumer requirements and preferences (Fig. 21.5) (Volenzo and Odiyo 2020).

Understanding the process is essential before proposing any meaningful improvement strategies; as a result, value chain analysis is one of the most valuable tools for understanding how markets for a specific good, such as plant-derived pharmaceuticals, work (Booker et al. 2012; Volenzo and Odiyo 2020). The medicinal plant value chain influences authority and governance among producers, retailers, and middlemen, and thus plays an important role in the long-term commercialization and amalgamation of medicinal plants (Giuliani et al. 2005; Volenzo and Odiyo 2020). The marketing system is largely ad hoc and undemocratic, with the majority of medicinal plant cultivators typically being low income. A middlemen-dominated medicinal plant value chain is typically lengthy and unfettered, magnifying discrimination and incompetence, as well as significantly decrease the margins to farmers and harvesters (Kala et al. 2006).

Those problems have increased the risk of piracy within the medicinal plant value chain and increased the problem of selling plant-based pharmaceuticals of native plant species without recognising and reimbursing countries or communities of traditional medicinal plant source knowledge (Volenzo and Odiyo 2020). The traditional knowledge retained by native communities serves as the foundation for the production of useful products, which can be passed down orally or through proper documentation; thus, a cultural custom is important in the conservation and sustainability management of biodiversity (Hill et al. 2020).

Management systems in most developing countries lack intellectual property rights and thus strengthen the susceptibility to piracy of genetic resources (Volenzo and Odiyo 2020). The emerging loss of genetic diversity due to unsustainable manipulation and habitat loss is one of the most serious threats to medicinal plants.

As the demand for medicinal plants has been expanding, a large number of economically important species have been depleted from the wild (Alam and Belt 2009). It was reported that approximately more than 4000 medicinal plant species are threatened globally, with a large percentage of over-harvesting to meet demand from drug manufacturers (Schippmann et al. 2002; Alam and Belt 2009). This number is increasing each year and species extinction has become a worldwide concern, which in turn can lead to serious negative significances for human livings as well as wildlife, economies, and health (Hamilton 2004; Alam and Belt 2009).

There is a thin line between medicinal plant species conservation and commercialization. Several developing countries have taken into their initiative to introduce medicinal plant cultivation through agricultural diversification (Piters et al. 2006). The initiative is being implemented through several government agencies as well as several research institutes. The initiative of domesticating medicinal plants, however, has constraints. This restriction includes, apart from the above mentioned, unlawful harvesting, prolonged maturity, unacceptable authorization, isolated producers, and non-existent links among producers, retailers, and intermediaries, as well as poor quality planting materials and unprocessed farming plots (Kala et al. 2006; Alam and Belt 2009; Volenzo and Odiyo 2020).

21.4.2 Possibilities for Increasing the Value of Medicinal Plants in the Value Chain

Even though medicinal plant products are very popular in the market, very few studies have looked at their value chain. There are only several published reports on the value chains of herbal medicinal plant products (Stewart 2003; Winkler 2008) in comparison with an abundance of reports on research and production of the plant products. Generally, three criteria have been discussed in previous research (Bryceson 2008; Menon 2008) on the strategy to increase the value of medicinal plants in the value chain which consists of demand in the market, quality of the product, and financial advantages (Fig. 21.6).

The challenges that might be of concern generally include growing, improving the value chain, quality assurance, and improving networking. The raw materials supply and agricultural practice inextricably linked to methods of post-harvest, appropriate market canals and supply chain. In the medicinal plant sector, improved qualities and market access from wild crops to end-users are challenging (Pauls and Franz 2013; Länger et al. 2018). For any herbal medicinal product to enter the global market, it must adhere to three sets of quality standards (WHO 2003; Leaman 2008; Robinson and Zhang 2011): the International Standard for Sustainable Wild Collection of Medicinal and Aromatic Plants (ISSC-MAP), Good Agricultural Practices (GAP), and Good Manufacturing Practices (GMP). It is unclear, however, the linkage between these international standards with local practices. The most difficult

Fig. 21.6 Medicinal plant value chain

part is when intermediaries tend to dominate the local chain (Sher et al. 2014). This dominance leads to price inflation and inequality of margins.

Furthermore, there are still limited prices research and most studies show that prices depend on the market and quality of the product. A market that is considered to be sufficient to ensure stable supply on the market needs to be well defined. There are still considerable weaknesses in today's literature. Therefore, Schreckenberg and Belcher (2007) suggest several factors to be considered to plan the marketing of herbal medicines such as:

1. the product features,
2. market trends,
3. demand factor,
4. market risks,
5. integrated value chain,
6. national government policy,
7. sustainable, and
8. quality and quantity improvement.

This list will improve understanding of the value chain and can help develop future intervention scenarios (Cunningham et al. 2018). In addition, the analysis of the value chain for medicinal plant research should be thoroughly investigated. Although the global focus of medicinal plant products continues to be strong, particularly about its multiple roles in alleviating poverty and supporting health services, the potential of the various medicinal plant production systems and methods of use, marketing and contribution to livelihoods remain scarce. These problems have, without doubt, current and future implications for the harvest, growth, use, and marketing of medicines.

21.5 Community as an Institution in Medicinal Plant Management

Huge demand for medicinal plant as medicinal resources is increasing very fast due to the acceptance of traditional medicine as an alternative in the treatment of various illnesses around the world. The WHO estimates that 70–80% of the population of many developed countries has used some form of alternative or complementary medicine (WHO 2013) This contributes to putting the high commercial value of medicinal plants as a commodity in the global value chain. The global market for herbal products is estimated to grow 7% per annum and reaching USD 5 trillion by the year 2050 (Ahmad and Othman 2013). Even though medicinal plants around the world are receiving continuous attention nationally and internationally, the main concern is the management of the medicinal and potentially medicinal plants, which is still fragmented (Astutik et al. 2019). Management of medicinal plants includes commercialization, the production system, and conservation of the plants. Overexploited and unsustainably managed medicinal species especially those threatened and endemic will lead to disappearing and over time towards extinction and finally diversity loss.

The commercialization of traditionally inherited medicinal plants into the global value chain has both advantages and disadvantages. It could negatively impact the plant genetic resources and ecology at large, as the environment and ecosystem vulnerability are influenced by the ability to access and supply the resources. The lesson should be learnt by examples, biodiversity loss of threatening, and endemic medicinal plants, *Prunus africanus* in Africa, and seriously threaten species of *Picrorhiza kurroa* Royle ex Benth. and *Swertia chirayta* in India (Uniyal et al. 2011). This is due to the plants being poorly managed. Therefore, conservation of plant resources especially those medicinal and highly demanded in global chain production needs critical attention ensuring the supply is not broken and at the same time benefiting the population without exhausting the natural plant resources.

Community-based conservation approaches to medicinal plant management need to be given great attention. This approach is found to be successful in many countries around the world especially the developing countries like India (Shukla and Gardner 2006), Africa (Lanata et al. 2013), Uganda (Acema et al. 2021) where it involves social interaction between the local communities and government bodies to hand in hand achieving the same goal. The approaches also attract the attention of the government, non-government organization, and also funding agencies around the globe (Shukla and Gardner 2006; Acema et al. 2021).

21.5.1 Community as Institution

An institution in a wider sense referring to families, households, villagers, formal and non-formal organizations, and associations. Institutions are the rules that assemble human interaction, including their enforcement features and sanctioning mechanisms and include any form of shared restriction that human beings devise to shape their daily interactions and transactions (Negi 2010). Such institutions are decentralized and managed by a community itself, where no external authority interference guarantee that social actors will abide by rules and procedures.

Formal institution constitutes written regulation and law practices that guide the organization and are highly associated with the structural complexity. Informal institutions include sanctions, taboo, custom, tradition, and code of conduct. Various institutions constitute a governance system that determines to various degree, the access to, the control, allocation and distribution of natural component and anthropogenic assets and the benefits. These entitlements define ownership, privilege, limitation in use of resources which collectively referred to as property rights, shape the usage pattern and motivation for sustainable strategies as well as sharing the benefits (Volenzo and Odiyo 2020).

In these recent decades, there are quite a several successful community-based organizations taking roles in natural resources management (Thakadu 2005; Conrad and Hilchey 2011; Musavengane and Kloppers 2020). Community-based organizations or associations are those run by the local community and their members are the community itself. Participation of the local community at any level from the smallest unit to well-structured organizations in medicinal plant management is important to ensure the market need is met and the resources are managed sustainably, where it also benefited the future generation. In other words, meeting the current generation necessary without compromising the need of the future generation. Interaction between the authorized government and the community is also crucial, the community has the right to voice out their necessity towards medicinal plants and the government can take into account, their view and voices in policymaking, execution, implementation, and enforcement. The demand for the medicinal plant in communities is undoubtedly essential since, before civilization, the community rely on plants as a source of food and medicine. The market demand arises also because of community needs for healthy and better living and ends up creating the supply and demand to continuously climbing not only locally but also internationally.

Countries around the globe practice community engagement as key to good governance including managing plant recourses sustainably. Sustainability is the best approach to manage medicinal plants as it gives less impact on the environment. This approach requires two-way interactions between the authority and community and must be clearly understood their roles in addressing sustainability in the medicinal plant's market. The interaction involves community's behaviour, motivation, and perception (through their action, reaction and responses) to be taken into consideration by the authority in making planning, implementation, enforcement, and policymaking and reviewing related to medicinal plant's market (Foo et al. 2016).

21.5.2 How Community Can Contribute to the Management of Medicinal Plants?

The smallest unit to well-structured community-based organizations has its role and responsibilities towards natural resources management. Community contribution includes the knowledge towards the medicinal plants that were kept for generations by the rural and indigenous community and is known as traditional medicinal knowledge. Traditional medicinal knowledge is practised in Asia since immemorial time, long in history, for example, Ayurveda, Jammu (Indonesia), Traditional Chinese Medicine, Sowa Rigpa (Bhutan), Kampo (Japan), Thai medicine, and Herbal Medicine (Bangladesh) (Astutik et al. 2019) and Unani or Islamic medicine (Shrivastava 2011). Their traditional management practices should be fortified and endorsed since they serve dual significant responsibility in conservation and primary health care component (Msuya and Kideghesho 2009). Europe, mid of the world population is depending on traditional and folk medicines in their healthcare (WHO 2003). This is due to easy access, availabilities, and low cost compared to modern medicine. The uncontrolled utilization of direct use of these medicinal plants has drawbacks where they are not being scientifically and clinically tested. Traditional and folk medicine rely on these plants natural compounds for the modern drugs development. In the last 20 years research on medicinal plant shows rapid progress and reaches its peak in 2010 then progresses instability. Most medicinal plants collaboratively research between the first world countries scientifically tested to confirm their effectiveness and safety of use. Most researched medicinal plants came from Asia and Africa which confirm the richness of medicinal plants in these areas (Salmerón-Manzano et al. 2020).

Inventory of medicinal plants has been carried out through literature to fulfil several contexts and conducted continuously from ancient time to the present. This helps to understand the medicinal importance and values towards local communities and the country as a whole. Therefore, researchers around the globe are continuously working on documentation medicinal plant within their smaller groups of local or indigenous communities (Msuya and Kideghesho 2009; Ripen and Noweg 2016). Bible and Quran mentioned over 240 species of pharmaceutical and nutraceuticals potentials (Hossain et al. 2016; Astutik et al. 2019). All plants used in traditional and folk medicinal documented or are considered crucial in biodiversity and need to be conserved to confirm the secured supply chain locally and globally are secured.

There are many innovative ways that allow the communities to contribute to medicinal plant biodiversity sustainibility and the fullfilment of the supply chain. Home garden is one of the good initiatives for a smaller unit of communities. Selected diversity of species are grown and domesticated, they considered to be important to subsistence and livelihoods and mostly focusing on the households' consumptions in everyday life. Collection of plants by different individuals, families or farmers and also depending on socio-cultural and economic values (Díaz-Reviriego et al. 2016; Whitney et al. 2018). The indigenous community can greatly contribute as most of the knowledge on the medicinal plant in most countries around the world come from them. Therefore, ethnobotanical research on indigenous people is encouraged rightfully between the research community and local indigenous to ensure their rights and benefit from the research and development are shared in an equitable way (Jamie and Vemulpad 2015).

Community-based and community-government botanical gardens are more effective in sustaining medicinal plant richness. Community-based organizations are driven by local needs but the recent trend shows some of these community-based organizations are evolving and responding to global and international global economic opportunity and challenges. Some successful community-based organizations around the world are now enterprises, for example: Thailand (CODI Community Organization Development Institute), Namibia CBNRM (Community-based Natural Resources Management) (Seixas and Berkes 2009), India RCMPCC Rural Communes Medicinal Plant Conservation Centre (Shukla and Gardner 2006).

CBNRM in Namibia participates in many program and conservation activities and has wide networking and partnership. CBNRM evolves from one simple community organization to a national NGO-government and currently receiving support and attention from multiple parties including international donors, higher institutions, and the related ministry. Ten community-based successful projects around the world were discussed by Seixas and Berkes (2009). These ten community-based organizations which initially driven by the local needs have shifted to higher performance in managing their local natural flora and fauna resources and receiving attention from national to global economic opportunities.

There are established and successful garden and park around the globe which greatly contribute to conserving and preserving the plant resources including those medicinal and other economic and ecological values. Botanic gardens and parks greatly involved in ex-situ and in-situ conservations. Ex-situ and in-situ conservations are complementing each other in sustaining the biodiversity of natural resources especially those overexploited and endangered medicinal plants (Chen et al. 2016). The main aim of ex-situ conservation is to cultivate and naturalize threatened species of threatening species to ensure their survival especially those overexploited or highly demanded in the market for the production of medicinal preparation or drugs (Wangkheirakpam 2018). The process involves the relocation of the mentioned species from their natural habitat to a protected area. Botanic gardens are major players in this role (Lanata et al. 2013) and can be expanded to undertaking programs of domestication and variety breeding and propagation (Chen et al. 2016).

On the other hand, national parks can play a big role in the plant diversity richness especially those endangered, threaten or endemic at their natural habitats. National parks around the world play a huge role as in-situ conservation in their respective countries (Amjad et al. 2015; Menale et al. 2016; Suba et al. 2019). Traditional management practices by rural and indigenous communities outside government gazette areas such as burial sites, farm, sacred forest, cultural forest, taboo are considered ideal places for in-situ conservation of plant biodiversity especially those medicinal (Msuya and Kideghesho 2009).

Integration of roles between the government, agencies, funders, and research institutions is crucial in granting success of the park and garden in their conservation agenda. Some conceptual frameworks need to be considered after understanding the contributions of the community from smaller entity to larger institutions in the management of medicinal plants, therefore the integration of community role can be clearly defined through the delocalization process of medicinal plants integration into commercialization and global value chain (Volenzo and Odiyo 2020).

21.6 The Knowledge, Power, and Subjectivity in Medicinal Plant Management

Due to the catastrophic climate change effect, plants are changing their ranges of distribution over new habitats, and to support the natural adaptation (evolutionary and ecological) of medicinal plants in the changing environment, the conservation of plant genetic resources in situ is very important (Wallingford et al. 2020). To apply different conservation measures to populations of medicinal plants at genetic reserve sites, a methodology was developed to ensure their long-term sustainability. This methodology was developed using an analysis of pertinent legal documents, literary sources, and databases, as well as the authors' personal experience and historical land use in the study area. It systematically outlines measures for the management and conservation of genetic reserve locations, to ensure the long-term viability of medicinal plant populations. It uses a scientific approach to the management and control parameters by gathering them into factor-specific and habitat-specific ones and follows the specific principles of biodiversity maintenance, like the notion of priority of in situ maintenance, regional approach, wariness and validity of decision-making, and ecosystem (Labokas and Karpavičienė 2021).

The strategy of the World Health Organization (WHO), 2014–2023, targeted to enhance the traditional medicine role, confirming the significance of promoting and including the exploitation of medicinal plants in the health care systems of its member countries (WHO 2013). A valid therapeutic benefit uses of medicinal plants has been revealed. However, the excessive uses of self-medication may increase the issue of lack of drug interactions and/or adverse reaction registration. Consumption of medicinal plants is a case to be taken into consideration in the control of pharmacological patients treatments. This will ensure quality, efficiency, and safety in the use of medicinal plants, thus constituting an integrated health care system (Sánchez et al. 2020).

The role played by indigenous knowledge on the conservation of medicinal plants is extensively acknowledged (Nimachow et al. 2011). According to a recent study, communities that rely heavily on traditional medicine and medicinal plants had a wealth of indigenous knowledge about medicinal plant conservation, as they could mention many conservation strategies. The conservation practices seemed to be fundamental in the conservation of medicinal plant species (Kibonde 2020).

The scientific approach to genetic reserve site conservation contributes to the design and implementation of involvement parameters in genetic reserve sites by the best understanding of the entire process concentrated on conservation goal (populations of target species) and factors (anthropogenic biotic and abiotic) affecting it. However, the recommended parameters must be considered in a flexible, active manner, taking into account potential impacts on the environment, different target species requirements, and local conditions by making ready individual site-specific arrangement plans. The individual plans, on the other hand, should be checked up and updated according to the results of periodic inventories and controlled according to information from the genetic resource users and protected area managers (Wallingford et al. 2020).

Local communities consider medicinal plants wild, lack knowledge, and have unattractive economical values. Therefore these local communities pay less attention to the management of medicinal plants. The mismanagement altogether with damage of habitat showed that traditional medicinal plant species used by traditional therapists are under critical intimidation which indicates the need for instant awareness towards their sustainable utilization, maintenance, and documentation (Birhane et al. 2011). Natural resource management is thus a complex socio-political system concerning how local and non-local actors engage to pursue their values around environmental systems, negotiate rights, and arrive at a workable model of collective action across scale (Ojha et al. 2016). Significant linkages have been identified between ecological degradation risks in the global supply, knowledge, power, agency, property rights, and medicinal value chains and demand of medicinal plants. A community is conceptualized as an entity that is concurrently entrenched in local, regional, and global networking on the delocalized community model scale (Ojha et al. 2016). Even though global value chains are important drivers in delocalization processes, scholarly opinions on the impact of delocalization on natural resource management outcomes are divided. Delocalization of community-based resource management systems, such as medicinal plants, may provide chances for income and employment, but it is biased in favour of dominant value chain actors that use Intellectual Property Rights (IPRs) governance systems to further their strategic goals. Because medicinal plant commercialization undermines locally established values and norms that restrict access to and control of Common Property Resources (CPRs), it encourages widespread incentives that exacerbate medicinal plant species' vulnerability to overexploitation and extinction. Furthermore, indigenous knowledge is generally seen as inferior to IPR in most judicial precedents, particularly where the legal and policy foundations are inadequate. IPRs, which are a typical characteristic of biotechnological advancements in the isolation, processing, and patenting of active compounds from medicinal plants, are expected to have far-reaching consequences for CPRs (Volenzo and Odiyo 2020).

Younger generations appear to have lost knowledge, as they cultivated fewer species and knew less about medicinal plant properties. Even though men cultivate more diverse gardens, women appear to play a critical role in the conservation of agrobiodiversity in home gardens, as they are identified as the primary source of traditional medicinal plant knowledge transmission. Our research emphasizes the necessity of integrated land use management that takes into account several social factors (such as culture, gender, health, and well-being) that are linked to biodiversity conservation and traditional knowledge in agroecosystems. Home gardens should be seen as major reservoirs of biological and cultural variety by policymakers. Traditional knowledge preservation necessitates a complete approach, which entails learning through emulation and context rather than formal instruction (Caballero-Serrano et al. 2019).

As pharmacopoeial requirements evolve and instrumental technology improves, we will be able to delve deeper into the chemical makeup of medicinal plants and create more advanced procedures for detecting and quantifying adulterants and contaminants. However, while technology advancements offer us this option, the conventional organoleptic analysis also provides us with important sensory information about medicinal plant quality. Many studies have documented the emergence and historical significance of complicated analytical techniques employed in medicinal plant analysis. Any analytical technique, on the other hand, can only provide a partial picture of complex multi-component preparations. As a result, future advancements in this field may not rely just on the development of ever more complicated analytical tools, but rather on the implementation of best practices at all phases of the manufacture and distribution of herbal medicines (Fitzgerald et al. 2020).

Environmental education should aim to critically examine and bring to light the interplay of power/knowledge connections, as well as its consequences in how knowledge is represented from knowledge-generating processes involving the interaction of modern institutions and local communities. This critical approach challenges the institutionalized knowledge-generating processes that appropriate local knowledge and incorporate them into Western science discourses. This opens up opportunities for indigenous views to be heard and represented in the academy, as well as in community development (Shava 2011).

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