Azamal Husen
Muhammad Iqbal *Editors*

Medicinal Plants

Their Response to Abiotic Stress

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ISBN 978-981-19-5610-2 ISBN 978-981-19-5611-9 (eBook)
https://doi.org/10.1007/978-981-19-5611-9

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Preface

Abiotic stresses, such as drought, waterlogging, salinity, temperature (heat and cold), mineral deficiency, metal toxicity, gaseous pollutants, and harmful radiations, significantly affect the overall growth, yield, and sometimes even the properties of medicinal plants. Current researches in this field are focused on understanding the mechanisms of tolerance and adaptation of medicinal plants to single as well as multiple abiotic stresses. Studies have shown that medicinal and aromatic plants regulate biosynthesis of their secondary metabolites (SMs) in order to survive under biotic and abiotic stresses. Their response depends on the type of stress, duration and intensity of stress, and the plant species. Some plant metabolic compounds released under the impact of biotic and abiotic stresses have miraculous therapeutic effects and are used in various herbal drug formulations. Changes in the nutritional and hormonal status of plants affect the SM production in plants. Efforts have been made to use biostimulants, phytoprotectants, and plant-growth-promoting rhizobacteria to alleviate the adverse effects of abiotic stresses and trigger the tolerance mechanism in medicinal plants. Application of such means and materials causes critical changes in metabolites, and the extracts of the treated plants are then tested in vitro, for their pharmacological and health-promoting potential. However, given the significant differences between in vitro and in vivo approaches, in vivo trials deserve special attention. It is known that some physiological mechanisms in plants help in extracting, immobilizing, or removing salts, metals, organic compounds, and radionuclides from soil and water and thus contribute to the process of phytoremediation. Certain medicinal plants have also shown great phytoremediation properties and can be used for removing contaminants from soil or water. However, it needs to be ascertained carefully whether such plants grown in contaminated soil are safe for human consumption and can still be used for therapeutic purposes.

In recent years, biotechnological approaches have been used to accelerate the production and extraction of secondary metabolites at the industrial level. The emerging omics technologies are gaining the ground and providing insights on the underlying molecular mechanisms involved in stress tolerance exhibited by plants. It has been noticed that metabolomics has a role in filling up the gap between genotype and phenotype, reviewing cell functions, and identifying the stress-related alteration in metabolites and expression of low molecular weight peptides in medicinal plants. The medicinal plants possess several biosynthetic pathways which are critical for alkaloid biosynthesis, and numerous alkaloids are used in herbal drug formulations. The recent commercial interest has triggered the exploration of techniques for altering the production of alkaloids. Several techniques, including the miRNA-mediated manipulation of secondary metabolites, gene editing by CRISPR/Cas9, and nanoparticle-based enhancement in SM biosynthesis, are being extensively used to stabilize and enhance alkaloid production for the large-scale use at the industrial level. All these aspects have been discussed in the book in hand.

Thus, the aim of this book is to present a fresh and consolidated view of recent researches on medicinal plants and their response to the various abiotic stresses, and to gather the scattered knowledge on these aspects at one place. The book is a compilation of 17 chapters having relevant text, tables, and illustration to describe experimental work on abiotic stress-induced responses in medicinal plants. All the chapters have been organized in a way to provide information to readers in a logical and systematic way. We hope that it serves the purpose for which it has been conceived and worked upon. It must be beneficial to students of plant science, environmental science, pharmacy, and toxicology right from the graduation to postdoctorate level.

We are grateful to all our contributors for readily accepting our invitation, sharing their knowledge in specialized areas of research, and readily adjusting the suggestions of reviewers and editors for improving the shape of their contributions. We also appreciate our reviewers, particularly Professors Altaf Ahmad, Nafees A. Khan, Zaki Anwar Siddiqui, and Dr Tariq Aftab of Aligarh Muslim University, and Professor M. Irfan Qureshi of Jamia Millia Islamia, for their precious help in updating and polishing the text of different chapters. We shall be happy to receive comments and criticism, if any, from subject experts and general readers of this book.

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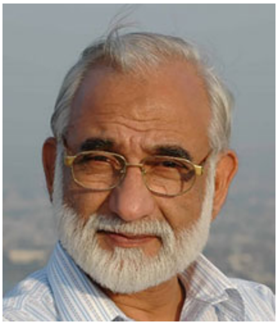
About the Editors

Azamal Husen currently a Foreign Delegate of Wolaita Sodo University, Wolaita, Ethiopia, has served the University of Gondar, Ethiopia, as a Full Professor of Biology, and worked there as the Coordinator of MSc Program and as the Head, Department of Biology. Earlier, he was a Visiting Faculty of the Forest Research Institute, and the Doon College of Agriculture and Forest at Dehra Dun, India. His research and teaching experience of 20 years involves studies of biogenic nanomaterial fabrication and application, plant responses to nanomaterials, plant adaptation to harsh environments at the physiological, biochemical, and molecular levels, herbal medicine, and clonal propagation for improvement of tree species.

He has conducted several research projects sponsored by various funding agencies, including the World Bank (FREEP), the National Agricultural Technology Project (NATP), the Indian Council of Agriculture Research (ICAR), the Indian Council of Forest Research Education (ICFRE), and the Japan Bank for International Cooperation (JBIC). He received four fellowships from India and a recognition award from the University of Gondar, Ethiopia, for excellent teaching, research, and community service. Dr Husen has been on the editorial board and the panel of reviewers of several reputed journals published by Elsevier, Frontiers Media, Taylor & Francis, Springer Nature, RSC, Oxford University Press, Sciendo, The Royal Society, CSIRO, PLOS, MDPI, and John Wiley & Sons. He is on the advisory board of Cambridge Scholars Publishing, UK. He is a Fellow of the Plantae group of the American Society of Plant Biologists, and a Member of the International Society of Root Research, Asian Council of Science Editors, and INPST. Over 200 publications go to his credit. He is Editor-in-Chief of the *American Journal of Plant Physiology*, and a Series Editor of *Exploring Medicinal Plants* (Taylor & Francis Group, USA); *Plant Biology, Sustainability, and Climate Change* (Elsevier, USA); and *Smart Nanomaterials Technology* (Springer Nature, Singapore).



Muhammad Iqbal former Head of Botany, Dean of Science, Chairman of Admission Committee, and the Vice-Chancellor of Jamia Hamdard (New Delhi, India), specializes in Developmental, Environmental, and Medicinal Botany. He worked as a Visiting Scientist in South Korea and Poland and as a Visiting Professor at King Saud University of Riyadh, Saudi Arabia. He has made over 450 publications, including 10 books, 290 original research papers, 78 book chapters and numerous technical reports, book reviews, and popular articles. In addition, over 220 abstracts of papers presented in botanical conferences go to his credit. He has guided research of 4 MPhil, 36 PhD, and 6 Postdoctoral scholars. His research interests include radial growth in vascular plants, plant-pollution interaction, heavy metals influence on plants, stress crop physiology, phytoremediation, and traditional herbal medicine.

He was an Invited Speaker or Sectional Chairman in numerous botanical conferences held in 16 different countries. He has been on the editorial boards of 20 research journals, is a Series Editor with Elsevier, USA, and has acted as a reviewer for over 65 journals and as a subject expert for various institutions, commissions, councils, and ministries of India, Croatia, Hungary, and Oman. He was President of the Academy of Environmental Biology (2006–09) and Vice-President of the Indian Botanical Society (2003–04) and the International Society of Environmental Botanists (2013–18). He is an elected Fellow of eight prestigious scientific organizations, including the Linnean Society of London (FLS) and the National Academy of Sciences, India (FNASc). He has received over a dozen academic awards, including the H. L. Chakravarty Award of

Indian Science Congress Association, Best Scientist Award of National Environmental Science Academy, Professor V. Puri Medal of Indian Botanical Society, Excellent Researcher Award of King Saud University, Riyadh, Bharat Jyoti Samman of India International Friendship Society, and Vigyan Gaurav Award of the UP Council of Science & Technology. The Prime Minister of India nominated him to the Prize Committee of the coveted "*Indira Gandhi Paryavaran Puraskar* (2006)." He is included in several biographical directories (Who's Who) published from India, UK, and USA.

Medicinal Plants and Abiotic Stress: An Overview

Shakeelur Rahman, Muhammad Iqbal, and Azamal Husen

Abstract Medicinal plants, like others, are affected by biotic as well as abiotic stress. The latter category includes a variety of stressors such as drought, flooding, salinity, temperature extremes (hot as well as cold), UV radiations, gaseous pollutants, heavy metals & metalloids, nutrient deficiency, and pesticides, among others. Low doses of these stresses stimulate metabolic activities in plants but higher doses negatively affect the overall plant performance by limiting the genetic potential, growth, photosynthesis, metabolic capacity and yield. These stresses also produce free radicals, such as superoxide and peroxide ions, which is a source of injury to the various plant systems. Almost all abiotic stresses damage the photosynthetic device at various levels of its organization such as chloroplast ultrastructure, and the pigments, lipids and protein composition. Production and relative distribution of photosynthate influence the growth and development, morpho-anatomical traits, and secondary metabolites (SMs) biosynthesis in the affected plants. Variation in the quality and concentration of SMs means a lot in medicinal plants, because the therapeutic efficacy of these plants is in fact dependent on these metabolites. However, most of the plants defy the adverse effect of the stressors by means of adequate defense mechanisms and tolerance potential through integrated cellular and molecular reactions. This chapter reviews the overall impact of abiotic stresses on growth features, developmental processes, cytological and physiological parameters, and SMs production in medicinal plants.

Keywords Abiotic stress · Climate change · Growth performance · Medicinal plants · Secondary metabolites

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A. Husen, M. Iqbal (eds.), *Medicinal Plants*,
https://doi.org/10.1007/978-981-19-5611-9_1

1 Introduction

Phytodiversity, encompassing the medicinal plants, plays a key role in the nutrition, health and livelihood of humans and animals. Man has been trying from times immemorial to benefit from plants for fighting against diseases and physical disorders (Anis et al. 2000; Atique et al. 1985a, b, 1986). Today, he is keen on improving their productivity and efficacy using different nutritional, genetical, agricultural and phytochemical techniques (Iqbal and Ahmad 2014; Aftab and Hakeem 2020; Beshah et al. 2020; Sharma et al. 2020; Husen et al. 2021; Husen 2022a; Asfaw et al. 2022). However, consequent upon rapid industrialization and construction works in the recent past, changes in climatic condition and ever-increasing environmental pollution have caused a direct threat to various plant species, including those of medicinal importance (Ahmad et al. 2011; Husen 2021a, 2022b). Over the coming decades, degradation of medicinal plants' diversity is likely to be faster due to climate changes. Plants are highly sensitive; they tend to resist the stress and do not generally adapt rapidly to the changed environment (Iqbal et al. 1996, 2018; Pimm 2009). Thus, the climate change may have an indirect effect on the traditional healing systems that are based primarily on medicinal plants and herbal drugs (Arya et al. 2022, Beigh et al. 2002b; Parveen et al. 2020a, b, 2022; Rahman and Husen 2021, Husen 2022a, b, c). Medicinal plants exposed to hostile environmental conditions. For instance, light stress, low or high temperature, ultraviolet (UV) radiation, salinity, drought, air pollutants, nutrient deficiency and heavy metals stress results normally in stunted plant growth, altered metabolite production, emission of hydrogen peroxide, superoxide and hydroxyl radicals, and activation of defence mechanisms (Iqbal et al. 2011a, b; Qureshi et al. 2011; Wani et al. 2016; Husen 2021b, c).

The unfavorable environmental conditions have tremendous impacts on medicinal plants at cellular, molecular and the overall physiological levels. Futther, the global rise of temperature due to climate change has to give rise to visible impacts on life cycles and the pattern of distribution of various species of medicinal and aromatic plants. Additionally, the raised level of gases, like ozone, and oxides of carbon, sulphur and nitrogen in the atmosphere, is also inflencing the quality and productivity of medicinal plants species, causing changes in their active ingredients (Idso et al. 2000; Iqbal et al. 2018; Deepti et al. 2022a; Rahman and Husen 2022).

The medicinal and aromatic plants control biosynthesis of their secondary metabolites (SMs) to be able to survive under various biotic and abiotic stress situations. These plants use different mechanisms to deal with the stressful situations. Their responses depend on types of stress, duration of stress, and plant species. They synthesize a variety of SMs with varied chemical composition, from primary metabolites, viz. amino acid, carbohydrates, and lipids. These metabolites, which are involved in the defense of plants against environmental stresses, pathogens and herbivores, are also beneficial to humans due to their pharmaceutical, nutritive and cosmetic value (Seigler 1998; Bachheti et al. 2021; Husen and Iqbal 2021). Taken together, this chapter reports the influence of abiotic stress on growth and

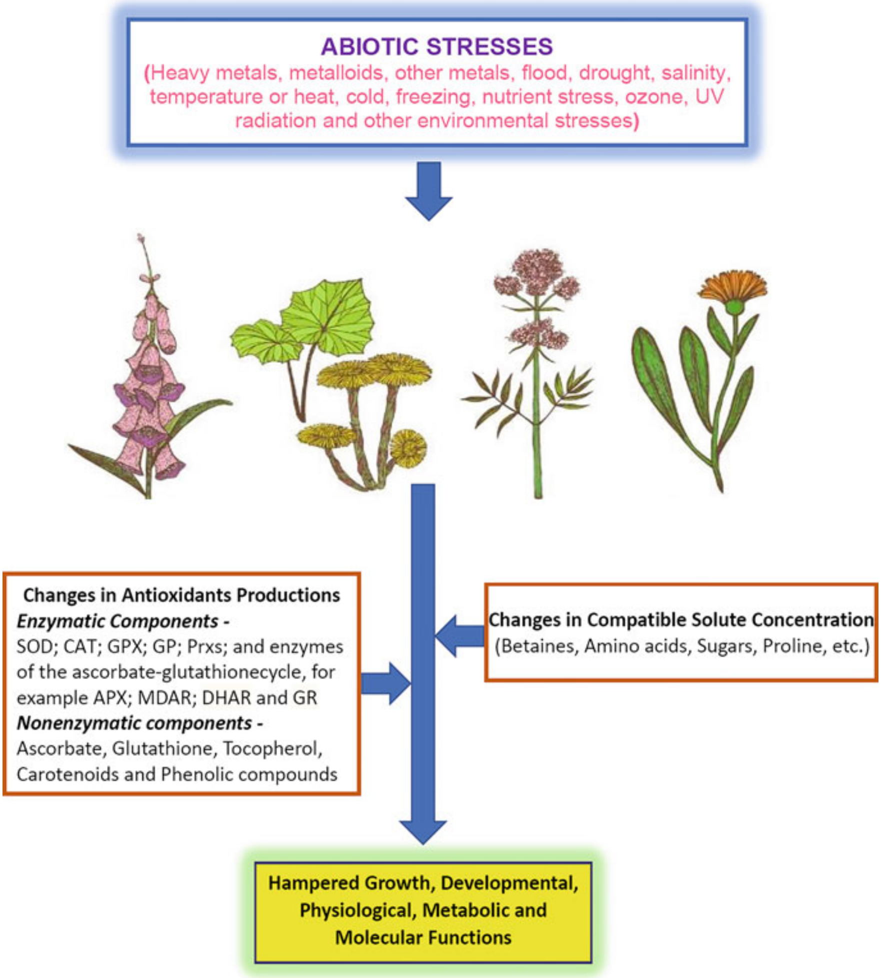

Fig. 1 Effect of abiotic stress on medicinal plants (where SOD, CAT, GPX, GP, Prxs; APX, MDAR, DHAR, and GR stand for superoxide dismutase, catalase, glutathione peroxidase, guaiacol peroxidase, peroxiredoxins, ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase, respectively)

development, cytological, biochemical and physiological mechanisms and production of SMs in medicinal and aromatic plants. The overall impact of abiotic stresses on these plants is presented in Fig. 1.

2 Abiotic Stresses and Medicinal Plants

2.1 Temperature Stress

Temperature is a main abiotic factor that decides the level of photosynthesis, and hence of plant growth, and other physiological processes. Not only that the growth parameters in the primary plant body are affected by environmental temperature, but even the secondary growth cycle in woody plants is regulated by changes in temperature (Ajmal and Iqbal 1987; Fahn and Werker 1990; Iqbal and Ghouse 1980, 1985; Iqbal 1994, 1995), and the structural pattens of the secondary tissues are determined largely by the thermal condition of the habitat, as is evident, for instance, from the ring-porous and diffuse-porous texture of wood (Ghouse and Iqbal 1982; Ajmal and Iqbal 1988; Gizińska et al. 2015). Cold, chilling, frost and heat come under the temperature stress. Several reports have scrutinized the impacts of enhanced temperatures on the production of plant secondary metabolites. Temperature affects the plant ontology and metabolic activity, and brings premature leaf senescence (Peerzada and Iqbal 2021). Thermal treatments influenced the contents of carotenoids, including β-carotene, in Brassicaceae, and the concentration of steroidal furostanol and spirostanol saponins increased due to low soil temperatures (Szakiel et al. 2011). Fluctuations in temperature have a manifold influence on plant growth, metabolic regulation, rate of intracellular reactions and permeability in plant cell cultures (Morison and Lawlor 1999). Leaf senescence and concentrations of root SM were increased by elevated temperatures in *Panax quinquefolius*. An increase in temperature (by 5 °C) decreased the rate of photosynthesis and production of biomass significantly (Jochum et al. 2007). The temperature and light-quality effects on the production of ginsenoside in hairy root culture of *Panax ginseng* were observed by Yu et al. (2005). Increased temperature changed the timing of crop flowering and seed development, which are critical developmental phases in the life cycle of majority of medicinal plants. Jagadish et al. (2008) have shown that the temperature levels (>32–36 °C) significantly decrease the seed set and crop yield. Reports have shown that various plants are at a high risk due to high temperature and strong heat waves with respect to their growth and development, and have to devise various adaptive strategies (Cleland et al. 2012; Bhatla and Tripathi 2014; Noor et al. 2019). High temperature hampers the vegetative growth, metabolite production and yield of medicinal plants (Hatfield and Prueger 2015).

Plants can observe even 1 °C increase and or decrease in temperature due to their natural sophisticated mechanism (Kumar and Wigge 2010). Mittler et al. (2012) have suggested that there are a number of pathways, regulatory networks and cellular components engaged in the defence of plant system against heat stress. Bita and Gerats (2013) have also reported that the levels of several hormones like ethylene, ABA and salicylic acid (SA) are enhanced due to heat stress, as they control the unfavorable impacts of abiotic stress situations in plants. SA regulates important plant physiological activities like proline metabolism, nitrogen metabolism, antioxidant defense system, photosynthesis, and plant-water relations under abiotic stresses (Khan et al. 2015). Further, the kinds of thermotolerance decide the requirement for different hormones and their response to signaling. Plant tolerance to heat stress improves the SA content in *Ocimum basilicum* (Clarke et al. 2004). Hasanuzzaman et al. (2013) have reported that the heat stress hinders the rate of photosynthesis due to decrease of soluble proteins and Rubisco-binding proteins. Heat enforces harmful effects on leaves (e.g., decreased leaf area, leaf water potential and pre-mature leaf senescence), which cause adverse impacts on the rate of photosynthesis (Peerzada and Iqbal 2021). Decreased activities of ADP-glucose pyrophosphorylase, invertase and sucrose phosphate synthase also influenced the synthesis of starch and sucrose (Rodríguez et al. 2005).

Heat stress also affects sexual reproduction and flowering processes because high temperature is fatal to the flowering stage and bud initiation, thus decreasing the productivity of crop plants ultimately (Thakur et al. 2010). Perhaps, this is due to a decreased water and nutrient transport during reproductive phase. Heat stress causes the down-regulation of sucrose synthase and a number of cell wall and vacuolar invertases in the developing pollen grains. It disrupts the sucrose and starch yield and reduces the soluble carbohydrates deposition (Sato et al. 2006).

Alterations in physiological features and metabolic perfomances in *Portulaca oleracea* due to heat, drought and combined stresses include a higher malondialdehyde level, peroxidase and superoxide dismutase activities, amino acid asparagine content and electrolyte leakage (Jin et al. 2015). On the contrary, chlorophyll content declined. Glycine betaine plays a vital role in plants exposed to high temperatures (Sakamoto and Murata 2002). The deposition of anthocyanin due to heat stress decreases the osmotic potential of the leaf, which in turns accelerates the uptake of water and decreases the rate of transpiration. Zhong and Yoshida (1993) have noticed a significant decrease in anthocycnins concentrations in *Perilla frutescens* suspension cultures, when temperature was augmented to 28 ° C, whereas the pigment productivity was optimal at 25 ° C. Release of the anthocyanin pigment due to distinct temperatures was reported from hairy root cultures of *Beta vulgaris* (Thimmaraju et al. 2003). Meiri et al. (2010) have shown that *Arabidopsis* overexpress the chyB gene that codes for β-carotene hydroxylase, which participated in the biosynthetic pathway of zeaxanthin. Zeaxanthin causes tolerance to augmented temperature and prevents membranes from oxidative damage (Meiri et al. 2010).

Temperature has immense impact on SM production in plants (Ramakrishna and Ravishankar 2011). Reda and Mandoura (2011) have reported that high-temperature stress reduces chlorophyll biosynthesis in plastids. Plant metabolism creates heat-stress tolerance by producing energy and the SMs, which is essential for cellular homeostasis. Saponins found in *Panax ginseng* leaves, stems, bulbs, flowers, fruit and roots get affected by different abiotic factors (Szakiel et al. 2011). Lin et al. (2009) have suggested that the upsurge of saponins in reproductive organs plays a role in chemical protection of plants in the adverse environmental situations. The American ginseng has a higher root ginsenoside content in plants exposed to long-period sunlight than in those exposed to shorter duration of direct sunlight (Li et al. 1996).

Beigh et al. (2002a) studied the effect of various treatments, including the hot water treatment and chilling for different durations (15–120 days), on *Aconitum heterophyllum* seed germination and seedling survival. The percentage of seed germination and the seedling-survival rate were appreciably enhanced by all treatments under laboratory as well as field conditions, but pre-chilling was most effective. Low temperature brings morphological and structural variations in medicinal plants, altering the membrane fluidity and the cell osmotic potential, and triggering changes in the macromolecules' activity. Photosynthetic apparatus exhibits CO_2 assimilation inhibition, and photoinhibition of photosystem I (PSI), PSII and diverse enzymatic activities due to cold stress. Also, there occurs a rise in the ROS production that stimulates damage to membranes, lipids, proteins, DNA and RNA (Sevillano et al. 2009).

Plant metabolism in temperate regions is redirected for synthesis of cryoprotectant molecules such as sugar alcohols, soluble sugars and nitrogenous compounds (low-molecular weight) in excessive winters (Janska et al. 2010). Phenolic production is increased due to cold stress. The lignin and suberin depositions increase resistance to cold temperatures, thus protecting plants from freeze damage (Griffith and Yaish 2004). Anthocyanins are also deposited during cold stress (Christie et al. 1994). In *Pinus pinaster*, the water and cold stresses produce changes in endogenous jasmonates (Pedranzani et al. 2003). Zhao et al. (2011) suggested that melatonin ensures the survival of cryopreserved *Rhodiola crenulata* callus. Cold stress directly affects the SMs of *Ocimum tenuiflorum*, thus reducing the eugenol content (Rastogi et al. 2019). Plant growth in *Datura stramonium* was affected due to temperature stress. However, increase in the enzymatic and non-enzymatic antioxidants indicates that the plant can control the level of cellular reactive oxygen species (ROS) and grow effectively under stressful conditions. At high and low temperatures, alkaloidal content of *D. stramonium* increases the tolerance ability with strong antioxidant defense mechanism (Shriya et al. 2019). The temperature and water stresses inhibit vegetative growth of *Ocimum basilicum* and cause proline deposition in leaves, which works as an osmolyte for osmotic adjustment in the stressful conditions. Glycine betaine also accumulates against the exposure of temperature and water stress in basil plants. The other metabolites, namely carotenoids or total soluble sugars, decline in *O. basilicum* leaves against the stress (Al-Huqail et al. 2020). The stimulation of SMs due to heat stress could perform as a noticeable mechanism of cross-protection against stresses (Arbona et al. 2013).

Viability of seeds in tropical plants is known to be negatively influenced by chilling temperatures. The stored seeds of Neem (*Azadirachta indica*) are sensitive to chilling when their moisture content is ≥10%. This limits the possibility of their preservation for long duration (Tompsett 1994). *Zingiber officinale* exposed to chilling stress may characteristically exhibit structural damages and undergo to decomposition of metabolic compounds. Chilling stress may inhibit enzymatic and photochemical activities and generate reactive oxygen, like hydroxyl radicals, hydrogen peroxide and superoxide, causing severe oxidative damage (Li et al. 2014).

Composition of lipid is a major factor in membrane stabilizations, hence plays the main role in developing the stress tolerance (Anjum et al. 2015). Unsaturated fatty acids are related with the cold tolerance, as these are found in greater amount in plastid membrane of the cold-resistant than of the cold-sensitive plants. Plant membranes experience transition from a liquid crystalline to a gel-like phase with a reduced fluidity (at low temperatures); this is accompanied with leakage of ions and membrane-integrated proteins deactivation (Upchurch 2008). Some of the medicinal plants like *Cistus incanus, Phlomis fruticose, Satureja thymbra, Teucrium polium* and *Thymus sibthorpii* exhibit seasonal dimorphism (Lianopoulou and Bosabalidis 2014) by evolving mechanical and chemical defensive obstacles to manage the stress under cold conditions. These plants develop structural, physiological, and biochemical defense mechanisms, and these changes are quite often facilitated by phytohormones.

Lianopoulou and Bosabalidis (2014) have reported that *Origanum dictamnus* induced structural and functional variations under cold conditions, affecting the shape, size and distribution of leaves. Mesophyll cells developed large intracellular spaces for ample air deposition. *O. dictamnus* also accelarates the development of a thick and dense layer of non-glandular trichomes as a defensive armor, together with a thick cuticle layer on the leaf epidermis under stress. Essential oils are more profusely released by glandular hairs under cold stress, p-Cymene (60%) being the most important constituent during the winter while carvacrol (42%) during the summer.

Salvia sclarea, an important medicinal herb, exhibited a decrease in leaf area but an increase in spikes' length and number, and a longer inflorescence with more essential oils in cold stress situations (Kaur et al. 2015). *Teucrium polium* and *Thymus sibthorpii* are also influenced due to chilling, as leaves are smaller and thicker and have a higher number of stomata and glandular hairs, whereas mesophyll and epidermal cells contain dark phenolic and calcium oxalate crystals in their vacuoles in winter season (Lianopoulou et al. 2014a, b).

Phytohormones regulate all growth features; generally, ABA is responsible for stomatal closure, thus slowing down the rate of photosynthesis, as seen in leaves of *O. dictamnus* in winter season (Lianopoulou and Bosabalidis 2014). However, an elevated rate of photosynthesis and stomatal conductance was observed in winter leaves of *Thymus sibthorpii* and *Teucrium polium* (Lianopoulou et al. 2014a, b), indicating that the physiological changes also vary with plant species.

It has been observed that certain abiotic stress situations are associated with a higher deposition of antioxidant compounds in various medicinal plants (Qureshi et al. 2013; Saema et al. 2016), and often increase the medicinal or nutritional value of those plants (Saba et al. 1999; Mir et al. 2015). Production of biomass, chlorophyll and β-carotene content and also the antioxidant activity enhanced in response to cold stress in the growing seedlings of *Foeniculum vulgare* (Nourimand et al. 2012). The leaves of Indian ginseng demonstrated an upsurge in the deposition of withanolide, a key bioactive compound, in response to cold stress. However, cold stress slightly reduced the withanolide concentration in root tissues (Kumar et al. 2012; Mir et al. 2015), indicating that various plants and their parts have evolved a specific kind of mechanisms against cold stress. Cold is also related to rise in antioxidant enzymatic activity, as noticed in the leaves of Indian ginseng (Mir et al. 2015) and *Thymus sibthorpii* (Lianopoulou et al. 2014b). This provides protection to the aerial tissues of plants from cold-caused damage. The transfer of WsSGTL1 gene in *Arabidopsis thaliana* resulted in increased tolerance against cold and a quicker formation of sterol glycosides coupled with a higher enzymatic activity in transgenic plants (Mishra et al. 2013). In addition, the gene overexpression improved tolerance to cold stress together with an elevated stomatal conductance, a better photosynthetic performance and regulation of PSI and PSII (Saema et al. 2016).

Serotonin is an indoleamine neurohormone in vertebrate animals. It is also found in several plants, and helps in different physiological functions such as protection against pathogenic infection and environmental stress. It has a defensive role against ROS, causing suspension in senescence (Ramakrishna et al. 2011a, b). According to Murch et al. (2009), serotonin works as an antioxidant to protect the young reproductive tissues from harsh environmental conditions in *Datura metal*. The cold stress exposure augmented the concentrations of serotonin in *D. metal* (Murch et al. 2009).

2.2 Drought Stress

Drought stress affects the plant development and growth processes; even the secondary growth phenomena, including the cambial periodicity and wood-formation pattern undergo alterations (Aref et al. 2014). Tolerance to drought stress is noticed in all plants, but its impact varies from one plant species to another. It occurs due to water shortage, often accompanied by high temperatures and solar radiation (Xu et al. 2010). Drought frequently causes oxidative stress and increases the antioxidant enzymes activity, non-enzymatic defence materials, flavonoids and phenolic acids (Larson 1988; Anjum et al. 2008; Aref et al. 2013a, b). It changes the chlorophyll a and b ratio, and carotenoids content (Anjum et al. 2003). A decline in chlorophyll content, photosynthesis and metabolite synthesis has been reported in several plants such as *Catharanthus roseus*, *Brassica carinata*, *Sorghum bicolor*, *Pisum sativum* and *Vicia faba* under drought stress (Osman et al. 2007; Getnet et al. 2015; Embiale et al. 2016; Husen et al. 2014, 2017). *Chenopodium quinoa* plants growing under low water-deficit condition showed that the saponin content decreased by 0.46% (dry weight) as against 0.38% under high water-deficit condition (Soliz-Guerrero et al. 2002). Anthocyanins (a class of water-soluble flavonoids present in all tissues of higher plants) are known to deposit in plant tissues under drought and cold temperatures (Chalker-Scott 1999). In fact, flavonoids provide protection to plants growing in soils contaminated with toxic metals (Winkel-Shirley 2001). The heat and drought stress severely affected the growth and metabolism of *Artemisia sieberialba* by decreasing water uptake and use efficiency. Differential expression of the heat and drought stress responsive genes reflects a sort of dual functioning under the collective effect of heat and drought stresses. Their interactive tolerance mechanism at the biochemical and molecular levels is not clear (Haifa and Alhaithloul 2019).

The impact of drought on the SMs of different medicinal plant species, such as glycosides of rosmarinic acid in *Salvia miltiorrhiza (*Liu et al. 2011) and *Scrophularia ningpoensis* (Wang et al. 2010); morphine alkaloids of *Papaver somniferum* (Szabo et al. 2003); chinolizidin alkaloids of *Lupinus angustifolius* (Christiansen et al. 1997); epicatechins of *Camellia sinensis* (Hernández et al. 2006); chlorogenic acid of *Helianthus annuus* (Del Moral 1972) and betulinic acid of *Hypericum Brasiliense* (Nacif de Abreu and Mazaferra 2005), have been recorded. Drought stress caused an upsurge in the monoterpene concentrations in *Salvia officinalis* (Nowak et al. 2010). Analogous research with *Petroselinum crispum* has discovered a drought-caused enrichment of monoterpenes concentration in leaves (Petropoulos et al. 2008). Likewise, phenolic compounds concentration was significantly improved in *Hypericum brasiliense*. Although the stressed *H. brasiliense* plants were relatively smaller in comparison to the well-watered controls, the biomass as well as the concentration of phenol was considerably more in the stressed plants due to increased phenolic compounds concentrations (de Abreu and Mazzafera 2005).

2.3 Salinity Stress

Salinity is responsible for cellular dehydration that leads to osmotic stress and elimination of water from the cytoplasm, thus, decreased cytosolic and vacuolar volumes are noticed. Salinity stress produces species of reactive oxygen and causes oxidative stress (Alharby et al. 2019a, b). It frequently creates ionic and osmotic stress in plants, leading to severe impacts on specific SMs (Ali et al. 1999a; Arshi et al. 2002, 2006a; Mahajan and Tuteja 2005). Salinity hinders the rate of photosynthesis and plant growth by disrupting the nutrient-uptake equilibrium maintained by the plant (Arshi et al. 2004). Availability, partitioning and transport of nutrients and photosynthate are often affected, resulting in functional as well as structural alterations (Ali et al. 1999b; Mahmooduzzafar et al. 2003). This is owing to the competition of Na^+ and Cl^- ions with the nutrient ions like K^+, $Ca_2{}^+$ and $NO_3{}^-$. Abundance of Na^+ and Cl^- ions disturbs the ionic balance which directly acts upon the biophysical and metabolic components of the plant (Banerjee et al. 2016). Higher Na^+ and Cl^- during salinity result in reduced levels of N, P, K^+, Ca^{2+} and Mg^{2+} in plants, like fennel, lemon, peppermint, *Achillea fragratissima, Matricaria recutita*, *Trachyspermum ammi*, and *Verbena* spp. (Queslati et al. 2010).

Seedling is considered to be the most susceptible stage in the plant's life cycle. Salinity was found to cause a remarkable decrease in *Thymus maroccanus* seedling growth by hindering reserve food mobilization, suspending cell division, enlarging and injuring hypocotyls. Comparable observations are also noticed for basil, chamomile and marjoram (Aziz et al. 2008; Said-Al Ahl and Omer 2011). Anthocyanins and polyphenol contents are reported to increase in a number of plants under salinity

(Parida and Das 2005). In contrast, anthocyanin level was found to decrease in the salt-sensitive species of potato (Daneshmand et al. 2010). The salt-tolerant alfalfa plants quickly doubled root proline content, however in salt-sensitive plants the increase was slow (Petrusa and Winicov 1997). Proline accumulation showed a correlation with salt tolerance in *Aegiceras corniculatum* (Aziz et al. 1998), whereas total phenolics content increased with a moderate saline stress in red peppers (Navarro et al. 2006). Plant polyamines seem to be involved in plant response to salt stress. Salt stress-induced changes of free and bound polyamine levels have been observed in the roots of *Helianthus annuus* (Mutlu and Bozcuk 2007). Salinity affects different SMs of medicinal plants such as sorbitol of *Lycopersicon esculentum* (Tari et al. 2010); GABA of *Sesamum indicum* (Bor et al. 2009); tropane alkaloids of *Datura innoxia (*Brachet and Cosson 1986) and polyphenol of *Cakile maritime* (Ksouri et al. 2007).

Salinity shows adverse effect on growth and development of plant parts including the length and dry weight of root and shoot of *Artemisia annua*. Photosynthetic parameters and total chlorophyll content are normally reduced under salinity stress (Hussein et al. 2017). It considerably increases the electrolyte leakage and proline content (Aftab et al. 2010). Proline and activity of antioxidant enzyme significantly increase under different salt stress concentrations and enhance the inhibitory effect on growth and photosynthetic features (Qureshi et al. 2013; Li et al. 2014; Yousuf et al. 2017). Similarly, salinity stress inhibits growth, development, biochemical properties and SM accumulation in *Mentha piperita*. Antioxidant enzyme activity, lipid peroxidation and proline content increase significantly, while essential oil content decreases as the salinity increases (Coba and Baydar 2016). Salinity resulted in reduced number of leaves, leaf area and leaf biomass in *Mentha piperita* var. *officinalis* and *Lipia citriodora* var. *verbena* (Tabatabaie and Nazari 2007). *Solanum nigrum* showed enhanced production of solasodine content, a steroidal alkaloid, when grown under salinity stress. Solasodine is used as progenitor for the commercial steroidal drug production (Bhat et al. 2008). Salt stress also induced disorder in the mineral nutrition and affects the growth, antioxidant properties, physiological activities and phenolic content of *Trigonella foenum-graecum* (Baatour et al. 2010). Reduction in foliage, root growth and dry matter was also observed in *Aloe vera* under salinity (at 2, 4, 6 and 8 ds m^{-1}) (Moghbeli et al. 2012). Similarly, higher concentration of salt levels decreased the number of tillers in *Citronella java* (Chauhan and Kumar 2014). Different salt concentrations inhibited the fresh and dry weights and reduced the chlorophyll *a* and *b*, and β-carotene contents of the *Foeniculum vulgare* seedlings (Nourimand et al. 2012).

Salt stress influences seed germination. For instance, reduced seed germination was recorded in *Eruca sativa*, *Ocimum basilicum*, *Petroselinum hortense* and *Thymus maroccanus*, when seeds were sown in the salt-contaminated soil (Miceli et al. 2003). Growth features were found to be suppressed under salt stress in *Achillea fragratissima*, *Bacopa monniera*, *Cassia angustifolia*, *Catharanthus roseus*, *Chamomilla recutita*, *Nigella sativa*, *Ocimum* spp., *Salvia officinalis*, *Thymus vulgaris* and *Withania somnifera* (Ali et al. 1999b; Arshi et al. 2004; Jaleel et al. 2008a, b; Hussain et al. 2009). Accelarated salinity unavoidably affected the overall productivity of certain medicinal plants, including fennel, cumin, milk thistle, *Ammi majus* and *Trachyspermum ammi*. The fruit yield and the number of umbels per plant significantly decreased in the stress-exposed *Trachyspermum ammi*, the ajwain plant (Ashraf and Orooj 2006).

Quite often, abiotic stress affects the photosynthetic features of plants. This physiological function is the most essential for plant growth performance and survival (Iqbal et al. 2000; Bashir et al. 2015; Husen 2021a, b). It has been reported by Said-Al Ahl and Omer (2011) that the chlorophyll *a* and *b*, and the total chlorophyll content, were decreased in several plants, such as *Centaurium erythraea, Satureja hortensis, Teucrium polium*, *Thymus vulgaris*, *Zataria multiflora* and *Ziziphora clinopodioides*. This reduction in the the content of various photosynthetic pigmants is mostly due to the chlorophyll synthesis inhibition together with an augmented chlorophyll degradation.

Under salinity stress, the oil yield was reduced in the *Ricinus communis* roots, but increased in shoots (Ali et al. 2008a). In *Coriandrum sativum* leaves, the total fatty acid content was considerably decreased due to exposure of salt stress condition. Increase in NaCl concentrations caused a significant decline in the unsaturated to saturated fatty acid ratio, which accelerated the formation of rigid membrane (Neffati and Marzouk 2009). However, Anitha and Ranjitha Kumari (2006) have reported increased concentration of reserpine in *Rauvolfia tetraphylla* under salt stress condition. In *Ricinus communis*, amount of ricinine alkaloids declined in roots under salt stress condition, though their amounts were higher in shoots (Ali et al. 2008a). A study of the root proteome from a culture of hairy roots of *Panax ginseng* indicated that a combination of internal sequencing and expressed sequence tag database analysis was a nice identification method for proteome analysis of plants having incomplete genome data like *P. ginseng*. Further, study also suggested that expression of certain proteins may be exclusive for different tissues according to the specific cellular functions (Kim et al. 2003). Differential modulation of spinach proteome was observed in response to salinity stress and cadmium stress applied singly as well as in combination (Bagheri et al. 2015; Qureshi et al. 2015). Yousuf et al. (2016b) observed a differential expression of 21 proteins in shoots of a salt-tolerant genotype and a salt-sensitive genotype of *Brassica juncea* (Indian mustard) grown under salt stress. These proteins were linked to various physiological functions. Likewise, analysis of expression of chloroplast proteins in these genotypes exposed to salt stress could enable identification of proteins related to a variety of chloroplast-associated molecular processes, including the oxygen-evolving process, PS I and PS II functioning, Calvin cycle, and redox homeostasis. Further, Yousuf et al. (2016a) confirmed the above reults using the expression analysis of genes encoding the differentially-expressed proteins through real time PCR. Deposition of phenolics with higher salt concentration was also noticed in *Mentha pulegium and Nigella sativa*. The biosynthesis of phenolic compounds like trans-cinnamic acid, quercetin and apigenin was enhanced in *Nigella* grown on a highly saline soil (Bourgou et al. 2010). Cardenolide is a steroid derivative present in some plants such as *Digitalis purpurea*. Extract of *D. purpurea* is used to treat cardiac failures. Cardenolides occur mostly in the form of glycosides containing structural groups derived from sugars. An elevated cardenolide level was recorded in *D. purpurea* roots and leaves under a moderate salt stress (Morales et al. 1993).

The economically valuable essential oils are especially present in mint species such as *Mentha piperita*, *M. pulegium* and *M. suaveolens*. Salnity reduced the yield of essential oils (monoterpenes) in all mint species, but the percentage of one essential oil (menthone) increased with the increase in salt concentration condition. Poorer yield of essential oils under saline condition was also observed in Basil, Fennel, *Trachyspermum ammi* and *Thymus maroccanus* (Ashraf and Orooj 2006). The salinity decreased even the anethole content in fennel (Abd El-Wahab 2006). Moreover, several essential oil compounds like a-bisabolonoxide A, a-bisabololoxide B, chamazulene, a-bisabolol oxide A, trans-b-farnesene and a-bisabolol were found to increase under saline condition (Baghalian et al. 2008). Effort has also been made to understand the impact of salinity stress on plants through the study of proteome (Hakeem et al. 2012, 2013). Intensity of the impact of NaCl may be alleviated by application of certain other ions such as calcium (Arshi et al. 2005, 2006b; Yousuf et al. 2015), potassium (Umar et al. 2008, 2010; Yousuf et al. 2015) and sulphur (Anjana et al. 2006; Saifullah et al. 2016) in addition to hormones, like indole acetic acid, SA and jasmonates (Husen et al. 2016, 2018, 2019; Siddiqi and Husen 2019).

2.4 Heavy Metal Stress

Different toxic heavy metals (HMs) such as chromium (Cr), nickel (Ni), arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) are rigorously released in the environment from sources like fertilizers, pesticides, metal smelters and industrial effluent, and influence the plant health and performance (Qureshi et al. 2005; Gauba et al. 2007; Diwan et al. 2010a; Rasool et al. 2013). These HMs are present in the soil as metal complexes in soluble form, exchangeable metal ions, and insoluble oxides, free metal ions, hydroxides, carbonates, or ingredient of structural silicates (Rai et al. 2004; Umar et al. 2005; Ansari et al. 2012). They affect not only the physiological and biochemical characteristics but also the developmental and morphological aspects of plants (Mehindirata et al. 2000; Khudsar et al. 2001; Ahmad et al. 2005; Anjana et al. 2006). Medicinal plants grown in HM-polluted environment tend to change their SM profile through stimulation or suppression of the secondary bioactive compounds (Iqbal and Khudsar 2000). Exposure to HM is a source of generation of oxidative stress in plants, activating the formation of highly active signaling molecules that affect the SM production, which influences the medicinal potency of the plants concerned (Nasim and Dhir 2010).

Higher euginol as well as proline content in *Ocimum tenuiflorum* was found under chromium stress, although photosynthetic pigments, protein, cysteine, ascorbic acid and non-protein thiol contents were reduced. Besides, chromium also accelerated lipid peroxidation coupled with potassium leakage (Rai et al. 2004). Increased accumulation of chromium and cadmium on the leaves of *Phyllanthus* *amarus* enhanced the contents of phyllanthin and hypophyllanthin, which are therapeutically essential SMs of the plant (Rai and Mehrotra 2008; Rai et al. 2005). Zhou et al. (2016) reported that the cadmium treatment increased the biosynthesis of arteannuin B, artemisinin and artemisinic acid in *Artemisia annua*. Application of nickel to *Hypericum perforatum* reduced the production of pseudohypericin and hypericin; capability of the plant to synthesize hyperforin was not noticed (Murch and Saxena 2002). Cao et al. (2009) reported that the accumulation and uptake of arsenic in *Sculellaria baicalensis* inhibit the formaton of baicalin and wogoninside, but the generation of baicalein, wogonin and oroxylin A was increased.

The elevated concentrations of trace metal nickle hamper plant growth (Hagemeyer 1999), and decrease the anthocyanin levels (Hawrylak et al. 2007). Co^{2+} and Cu^{2+} cause stimulatory effect on the SMs production. Cu^{2+} accelerated the betalains production in *Beta vulgaris* (Trejo-Tapia et al. 2001). However, Obrenovic (1990) observed stimulatory effects of Cu^{2+} on betacyanins accumulation in *Amaranthus caudatus* callus cultures. Under Cd^{2+} or Cu^{2+} exposure, sunflower leaf disks showed a remarkable decrease in spermidine content but no variation in spermine level was noticed (Groppa et al. 2001).

Chemical wastes, including those containing nanoparticles (NPs), are released from the household and the industrial and medical products, and accumulate in two major environmental sinks, *viz.* soil and water. As the plants are stationary with these natural substrata (soil and water), they cannot get away from the severe effects of the chemical pollutants present therein. Nanoparticles have been found to stimulate the generation of ROS and must therefore affect the secondary metabolism of plants (Marslin et al. 2017). However, some NPs are also known to favour plant growth and metabolism (Husen and Iqbal 2019). For instance, increase in the amount of an important SM (artemisinin) has been reported in the *A. annua* hairy-root cultures under the exposure of silver NPs. This increase can be linked to the signaling molecule production (Zhang et al. 2013). Silver NPs also have shown a positive impact on synthesis of anthocyanin and flavonoid in *Arabidopsis* plants, as the expression level of genes responsible for their synthesis exhibits up-regulation (Garcia-Sanchez et al. 2015). Enhancement in the content of a steroidal diosgenin and saponin in *Trigonella foenum-graecum* was found after application of silver NPs (Jasim et al. 2017). The higher accumulation of flavonoids and phenolics along with increased callus induction was observed in *Prunella vulgaris,* known for its antiviral features, when cultivated in a medium fortified with NAA along with gold or silver NPs (Fazal et al. 2016). Melatonin, an environment-friendly molecule with significant antioxidant capacity, is present in water hyacinth. This aquatic plant is tolerant to the stress caused by chemical pollutants of water and soil (Tan et al. 2007; Arnao and Hernandez-Ruiz 2006). Moreover, A wide range of chemicals, including fungicides, herbicides and pesticides, also influence the morpho-physiological traits of plants, normally causing negative effects (Bashir et al. 2007a, b; 2014; Bashir and Iqbal 2014; Majid et al. 2013, 2014).

Many plants are capable to withstand the HM contamination of air, water or soil and can thrive well in the polluted environment. Based on their extraordinary capability to absorb and accumulate heavy metals in their tissues, some hyperaccumulator plant species have been identified (Iqbal et al. 2015; Memon 2016). Such plants are being used for phytoremediation, a technique to clean contaminated substrata by using plants. This cost-effective and least disruptive technique of remediation is rapidly gaining ground for removing metal ions from contaminated soil or groundwater in an environment-friendly manner (Jabeen et al. 2009; Vamerali et al. 2010; Ansari et al. 2015). Numerous plants have been studied to assess their capacity to remove heavy metals such as chromium (Diwan et al. 2010b, 2012), arsenic, cadmium, copper, mercury and nickel (Ansari et al. 2013a, b, 2015, 2018, 2021) from the contaminated sites.

2.5 Air Pollutant Stress

Air pollutants together with greenhouse gases constitute a key environmental challenge for medicinal plants. Among the gaseous pollutants, CO_2 and SO_2 are most prominent, having shown a remarkable rise in atmosphere since the industrialization has taken place (Yunus and Iqbal 1996; Yunus et al. 1996; Iqbal et al. 2000a, b; Husen 2021a, 2022d). The issue of the impact of air pollution on medicinal plants began attracting serious attention of researchers early in 1970s. In India, preliminary studies of air pollution versus plant performance were undertaken during the 1980s, dealing with morpho-anatomical changes caused by air pollution in the open-grown herbs and shrubs such as *Achyranthus aspera*, *Cajanus cajan*, *Cassia occidentalis*, *C. tora*, *Cleome viscosa*, *Datura innoxia*, *Lantana camara*, *Phyllanthus rhamnoides* and *Sida spinosa* (Iqbal et al. 1986, 1987a, b; Ahmad et al. 1987; Mahmooduzzafar et al. 1987; Ghouse et al. 1989). Later investigations focused primarily on functional and biochemical analysis of a number of species including *Achyranthus aspera, Croton bonplandianum, Datura innoxia, Peristrophe bicalyculata*, *Phyllanthus rhamnoides* and *Ruellia tuberosa* grown under the load of coal-smoke (with SO_2 as its major constituent) released from thermal power stations (Mahmooduzzafar et al. 1992; Dhir et al. 1999; Husen et al. 1999; Nighat et al. 1999; Ahmad et al. 2004; Husen and Iqbal 2004). Special emphasis was laid on foliar features including the leaf growth, trichome density, stomatal behaviour, chlorophyll biosynthesis, and the net photosynthetic rate (Nighat et al. 2000, 2008; Dhir et al. 2001; Trag et al. 2001, 2002; Aquil et al. 2003; Wali et al. 2004, 2007; Verma et al. 2006; Iqbal et al. 2000a, 2010b), because leaves are affected by the pollutants maximally and almost invariably. However, the major concern about the medicinal plants relates to the quality and quantity of their secondary metabolites as affected by environmental pollution. Production of hyoscyamine in *Datura innoxia* (Singh et al. 2000) and psoralen in *Psoralea corylifolia* (Ali et al. 2008b) was adversely affected by coal-smoke pollution. Composition of seed oils of *Peristrophe bicalyculata* and *Ruellia tuberosa* was changed due to an altered ratio of the component fatty acids under the polluted condition, suggesting thereby that not only the quantity but even the quality of herbal drugs is likely to be affected by the polluted environment (Iqbal et al.

2011b). Efforts have been made to characterize the medicinal plants with controversial botanical identity on the basis of their active ingredients and evaluate the impact of pollution stress on these molecules of therapeutic value (Iqbal et al. 2011a).

Increased CO_2 accelarated the photosynthetic carbon assimilation rates (~31%) across 40 plant species. In C3 species, it caused 20% increase in the above-ground biomass on an average (Ainsworth and Long 2005; Law et al. 2001). However, too high concentrations of CO_2 are injurious for plant health. CO_2 reduced the concentration of nitrogen in vegetative plant parts as well as in seeds and grains, subsequently a decrease of protein levels, increase of total phenolics, tannins and the monoterpene a-pinene were noticed (Idso et al. 2000; Williams et al. 1994). In the case of *Papaver setigerum*, increased level of CO_2 exhibited the enrichment of four alkaloids, namely. Noscapine, morphine, codeine and papaverine. Additionally, increase in CO_2 may result in high plant carbon-nutrient ratios producing surplus of non-structural carbohydrates (Ziska et al. 2008). SO_2, a major air pollutant, has the capability to enter into the plant through roots and stomatal openings during respiration and photosynthesis. It may cause damage to photosystems (Swanepoel et al. 2007), affects stomatal density and perturbations in the efficiency of C-fixation (Chung et al. 2011). On the other hand, sulphur deficiency also hampers plant productivity, as it reduces the uptake and assimilation of nitrate (Kaur et al. 2011). Sulphur transport system in plants has a role in modulating S efficiency (Ahmad et al. 2005a), and even the timing of application of S-fertilizer to plants affects their growth and yield; split doses of S applied at different stages of plant development ensure the best outcome (Ahmad et al. 2005b).

Since the medicinal plants are rich in SMs, they have a significant plasticity to adapt to the changing environments (Bachheti et al. 2021). This may affect the production of SMs, which generally form the basis for the medicinal properties of plants (Mishra 2016). For intance, *Digitalis lanata*, which is used mostly in heart diseases (Rahimtoola 2004), goes richer in digoxin, a cardenolide glycoside, when treated with high CO_2 while the concentration of other three glycosides, namely, digitoxin, digitoxigenin and digoxin-mono-digitoxoside, decreases (Stuhlfauth et al. 1987; Stuhlfauth and Fock 1990). *Hymenocallis littoralis*, normally used as an antineoplastic and antiviral, showed a rise in the concentration of three alkaloids (pancratistatin, 7-deoxynarciclasine and 7-deoxy-trans dihydronarciclasin) in the Ist year and a decline during the subsequent years, when exposed to elevated CO_2 (Idso et al. 2000). In *Ginkgo biloba*, used in dementia and Alzheimer's disease (Weinmann et al. 2010), the flavonoids concentration is affected by the increased CO_2 and O_3, the impact of CO_2 being more dominant. Elevated CO_2 reduced the concentrations of isorhamnetin aglycon and keampferol aglycon. The combined treatment of elevated CO_2 and O_3 also gave similar results, although the elevated O_3 alone caused a decline in isorhamnetin aglycon concentration and a rise in quercetin aglycon concentration (Huang et al. 2010).

The air pollution effect on plant performance is not confined to the primary plant body and its metabolic activities but also affects the secondary growth of woody plants. Periodicity of the lateral meristem (the vascular cambium) may be altered, *i. e.*, the prescribed schedule of the cambial activity and dormancy in a species and, consequently, the pattern of the production of secondary vascular tissues (bark and wood) are changed under the pollution stress (Iqbal et al. 2000b, 2010a, c). It was observed that the production of secondary tissues is not correlated necessarily to the rate of carbon assimilation (photosynthesis) or the amount of photosynthate produced but to the pattern of carbon partitioning, *i.e.*, distribution of photosynthate (Iqbal et al. 2000b, c, 2005; Mahmooduzzafar et al. 2003). The amount of annual wood production as well as the gross structure of wood may change under the stress of air pollution (Gupta and Iqbal 2005; Mahmooduzzafar et al. 2010).

2.6 Nutrient Deficiency Stress

Carbon, nitrogen, calcium, phosphorus and potassium are considered as the major essential mineral nutrients required by plants. These nutrients help plants in maintaining the physical organization, energy generation and molecules production, participate in protoplasm repair, and regulate the metabolic activities and other functions of living cells. These nutrients play important roles in improving the crop yield and maintaining the soil fertility. Nutrient stress has a noticeable effect on the checmical compostion and growth of plant tissues. Production of many secondary plant products is dependent on the growing conditions or the environment that affect the various metabolic pathways responsible for the deposition of the concerned natural products (Ganai et al. 2020). In a study of *Brassica juncea* grown inside the free air CO_2 enrichment rings, elevated [CO_2] showed insignificant impact on the minimal chlorophyll fluorescence (F_0), but the quantum efficiency of photosystem II increased by 3% (Ruhil et al. 2015). The electron-transport rate, photosystem I, photosystem II, and the whole-chain electron-transport rates were enhanced by 8%, while the net photosynthesis rate increased by ≈50%. Moreover, the metabolic pathways of carbon and nitrogen, the two essential elements for plant growth and development, influence each other and affect the gene expression, but information about genes or the mechanism affected by carbon and nitrogen interaction is limited. Attempt has been made to recognize proteins and the encoding genes of the interaction between carbon and nitrogen in Indian mustard. Identification of proteins like PII-like protein, cyclophilin, elongation factor-TU, oxygen-evolving enhancer protein and rubisco activase has provided hints about how the N-efficient cultivars of Indian mustard adapt to low N supply under elevated [CO_2] conditions (Yousuf et al. 2016c). Further, as a macronutrient, calcium contributes to the structure and functions of plants. It helps in maintaining the normal function of membranes and the growth of meristematic tissues and leaf primordia, and in sending signals in response to internal and external indications (Dordas 2009; Price et al. 1994; Naeem et al. 2009). Calcium is supportive in the regulation of plant responses to a range of environmental stresses by contributing either directly or indirectly in plant defense mechanisms. Khan et al. (2010) have reported that the exogenous exposure of calcium improves plant resistance against the drought, heat and salt stresses by regulating the antioxidant enzyme activities, and reducing the membrane lipid peroxidation, thus helping plant cells to survive under a range of environmental stresses.

Phosphorus deficiency hampers plant growth in many ways. P inputs significantly increased the biomass production and the P and Zn accumulation in high- as well as low-Zn-accumulating genotypes of chickpea (*Cicer arietinum* L.). However, higher concentration of P had a negative effect on the features studied, but helped in resisting the low P availability in the soil (Siddiqui et al. 2015a). Zn supply to the soil caused significant increase in growth parameters, although a too high ZN dose was inhibitive. Improvement in growth parameters was evident from pre-flowering stage to post-flowering stage of plant life, except for the leaf area index, which showed a decline in the post-flowering stage. High Zn-accumulating genotype (HZnG) performed better than the low Zn-accumulating genotype (LZnG) at deficient levels of zinc supply (Siddiqui et al. 2016). In another study of chickpea, the HZnG was found to maintain a significantly higher level of chlorophyll, protein, nitrate, leghemoglobin, nitrate reductase, superoxide dismutase, and carbonic anhydrase, in comparison to LZnG under zinc-limiting condition. Zinc supply to the soil improved the situation in both genotypes, more effectively in HZnG (Siddiqui et al. 2015b).

Potassium also affects the metabolic activities of plants. It increased nitrogen assimilation and yield in the case of *Lepedium sativum* (Dhawan et al. 2011). Likewise, sulphur has a role in maintaining plant performance. Cultivar Pusa Jai Kisan of *Brassica juncea* was found to be more sensitive to S deprivation than cv. Pusa Bold (Anjum et al. 2011). Further, based on the study of *Arabidopsis thaliana*, Wadhwa et al. (2012) reported that sulphur metabolites regulate the uptake of sulphate.

2.7 Radiation Stress

Light or radiation is a physical factor, which can influence or stimulate the metabolic activities in plants. Light intensity shows a correlation with the level of phenolics. In the case of *Zingiber officinale* callus, it stimulates production of gingerol and zingiberene (Anasori and Asghari 2008). The impact of diverse environmental situations, like light intensity and irradiance, was observed on cell biomass and production of anthocyanin in *Melastoma malabathricum* culture (Chan et al. 2010). Light irradiation affects artemisinin biosynthesis in hairy roots of *Artemisia annua* and digitoxin formation in *Digitalis purpurea* (Hagimori et al. 1982; Verma et al. 2018). Exposure of *Datura innoxia* to low (5 Gy) dose of gamma radiation caused stimulatory effect on germination of seed and increased the growth rate of root and shoot, stomatal conductance, chlorophyll and carotenoids contents and the net photosynthetic rate. Higher doses proved inhibitory for all these parameters; the negative impact being positively correlated to increase in radiation intensity. However, hyoscyamine content exhibited only irregular and non-significant variation (Aref et al. 2016).

UV-C part of sunlight is totally absorbed by the stratospheric zone layer. Most of UV-B is also absorbed there, and only a small part of this radiation reaches the Earth surface. UV-A radiations are not injurious to living beings. Plants sense UV-B radiation with the help of UVR8 photoreceptor but UV-A might be sensed by cryptochromes and phototropins. Overdoses of UV-B and UV-C radiations negatively influence the plant growth, development and photosynthetic features. It causes overproduction of ROS and development of oxidative stress that can decrease cell viability and lead to cell death. However, low UV-B or UV-C quantities may activate plant acclimatization, including the induced biosynthesis of SMs.

Application of UV-B to *Catharanthus roseus* causes a significant increase in vincristine and vinblastine production, which are used in the treatment of lymphoma and leukemia. Also, the UV-B radiation increases the contents of flavonoid and phenylalanine ammonia-lyase, which are correlated to a decrease in chlorophyll content (Liang et al. 2006). The photoperiod influences endogenous indoleamines (melatonin and serotonin) in cultured *Dunaliella bardawil* (Ramakrishna et al. 2011a, b). O_3 exposures increase the phenolic concentrations in conifers (Rosemann et al. 1991), whereas low O_3 has no effect on the concentration of monoterpene and resin acid (Kainulainen et al. 1998). In *Ginkgo biloba,* O_3 fumigation accelerated terpenes concentrations and reduced those of phenolics in leaves (He et al. 2009). The elevated O_3 reduced the isorhamnetin aglycon concentrations, but increased the quercetin aglycon concentration (Huang et al. 2010).

The commercial value of a medicinal or aromatic plant is reflected by the yield and composition of its SMs or essential oils. Several reports have shown a favourable impact of UV-B radiation on aromatic plants, fetching improvement in the volatile aroma production and inducing changes in the essential oils' chemical composition (Agrawal et al. 2009; Deepti et al. 2022b). Such results have been recorded for many specie including *Mentha spicata* (Karousou et al. 1998) and *Ocimum basilicum* (Chang et al. 2009). Dolzhenko et al. (2010) investigated that application of UV-B was responsible for high contents of menthol and phenolic in peppermint, as the modulation of expression of some genes participating in the essential oil biogenesis was up-regulated by UV-B irradiation. Moderate and low dose UV-B supplementation also enhanced the essential oil yield in *Acorus calamus* and improved its medicinal value by decreasing the content of its potentially toxic constituent, β-asarone (Kumari et al. 2009a). *Cymbopogon citratus* exhibited enhancement in its medicinal value due to higher concetration of z-citral in the essential oil and a larger yield under UV-B irradiation (Kumari et al. 2009b).

3 Conclusion

A large number of abiotic stresses adversely affect plant species, including those with medicinal importance. The symptoms of stress vary in form and intensity. The affected plants undergo a variety of changes, which may cause antagonistic effects on growth features and various other developmental, physiological and metabolic processes of plants. The medicinal plants faced with abiotic stress may have a low relative water content, accelerated ROS production, increased stress injury and cell electrolyte leakage, and a reduction in photosynthetic pigment, root and shoot length, and yield, etc. They may then undergo several morpho-physiological, biochemical and molecular alterations to overcome the negative effects. This includes the biosynthesis of numerous SMs, which is often directly linked to the medicinal importance of the plant. The effect of climate change on medicinal and herbal plants is also important. It is highly desirable to investigate the accumulation of SMs of therapeutic significance under adverse situations. Further, the use of plant cell culture (in vitro) for the production of chemicals and pharmaceuticals has made its significance felt. Moreover, the use of genetic tools in regulation of pathways for secondary metabolism may offer a wider basis for commercial production of SMs. Research on medicinal plants with respect to abiotic stresses and climate change is random and insignificant in comparison with other commercial crop plants. The herbal wealth of great medicinal value deserves a greater attention, as it is a potential source of bio-molecules and nutraceuticals.

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Medicinal Plants Proteomics in Response to Abiotic Stresses

Phaniendra Alugoju and Tewin Tencomnao

Abstract Medicinal plants are the richest sources of a diverse range of phytochemicals which can be validated for their efficacy to develop new therapeutic molecules against a spectrum of human diseases. Despite the fact that these phytochemicals are present at low concentrations, their abundance is influenced by the dynamic metabolic pathways which are modulated at the genome and proteome level. A variety of environmental factors including biotic (e.g., pathogen infection and herbivore attack) and abiotic factors (e.g., light, temperature, drought, salinity, heavy metals and other toxic chemicals) influence these metabolic pathways and other cellular processes, often posing a threat to the yield of crops including medicinal plants crops. Emerging omics technologies have gained interest in the recent years to provide insights on the underlying stress tolerance molecular mechanisms exhibited by plants to cope with different environmental stresses at system biology level. Proteomics, bridging the gap between genomics and transcriptomics, offers a useful approach to study and characterize the proteome (the total set of proteins present in a cell). Mass-spectroscopy-based proteomics has been widely used for characterization and quantification of stress effect at protein and subproteome levels to gain insights on the key biomarker proteins and their post translation modification, which subsequently can be used for developing new breeding strategies to modulate the plant stress tolerance. This chapter discusses the medicinal plants' proteomics in response to a variety of stress factors including light (UV-B and UV-D), temperature (high temperature and low temperature), drought (water deficit), salinity (salt stress) and heavy metals (lead, cadmium, chromium, and Copper).

Keywords Medicinal plants · Proteomics · Mass spectrometry · Abiotic stress · Temperature stress · Salt stress · Drought stress · Metal stress · UV stress

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A. Husen, M. Iqbal (eds.), *Medicinal Plants*,
https://doi.org/10.1007/978-981-19-5611-9_2

Abbreviations

2DE	Two-dimensional electrophoresis
2D-DIGE-Fluorescence	2D difference gel electrophoresis
ESI	Electrospray ionization
HT	High temperature
ICAT	Isotope-coded affinity tag
iTRAQ	Isobaric tag for relative and absolute quantitation
LC	Liquid chromatography
LT	Low temperature
MS	Mass spectroscopy
MALDI	Matrix assisted laser desorption/ionization
MudPIT	Multidimensional protein identification technology
PTMs	Post-translational modifications
ROS	Reactive oxygen species
SILAC	Stable isotope labeling with amino acids in cell culture
TOF	time-of-flight
UV	Ultraviolet

1 Introduction

1.1 Medicinal Plants

Medicinal plants are a rich source of highly diverse specialized secondary metabolites with important pharmacological properties for the generation of leads in the drug development process. They play a vital role in health maintenance and prevention of chronic degenerative diseases (Iqbal and Ahmad 2014), and therefore, interest in medicinal plant research is growing significantly to discover new and more biologically compatible phytomedicines (Hassan et al. 2017). Traditional medicinal plants have long been exploited widely in various regions across the continents (Briskin 2000; Brusotti et al. 2014; Husen 2021a, 2022a), even for treating chronic ailments like arthritis, gastritis, diabetes, psoriasis, and hypertension (Atique et al. 1985a, b; Atique and Iqbal 1992; Singh et al. 2008; Beigh et al. 2014; Husen and Iqbal 2021). The growing interest of consumers in the herbal drugs raises the need for extensive research to detect the low levels of secondary metabolites in medicinal plants (Sharma and Shrivastava 2016). However, with the growing population, there is also a significant change in the environmental climatic conditions, which hampers the plant growth, productivity and quality (Iqbal et al. 2000b, 2005, 2011; Husen 2021b, c, d, 2022b; Husen et al. 2014, 2016, 2017, 2018, 2019; Getnet et al. 2015; Embiale et al. 2016; Hussein et al. 2017). The dynamic environmental stress factors (Fig. 1) including biotic factors such as pathogen infection and herbivore attack, and abiotic factors such as temperature (high or low), drought

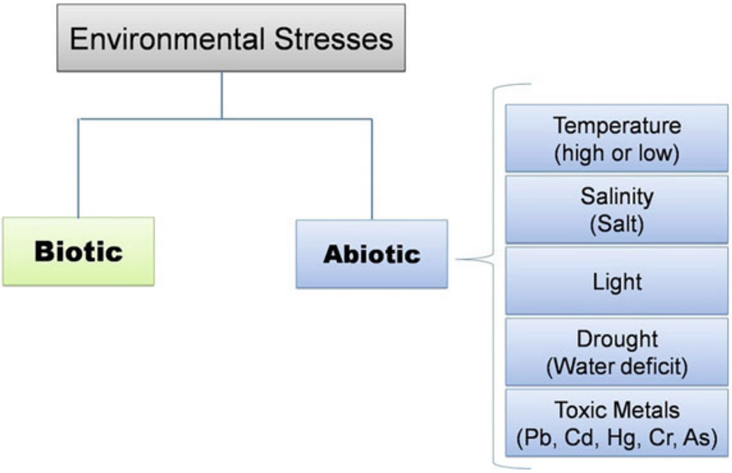

Fig. 1 Environmental stress factors including biotic and abiotic stress factors

(water deficit), soil salinity (salt concentration), and excess heavy metals (Pb, Cd, Cr and Cu) can adversely affect the plant growth and limit the productivity and quality of agriculture produce (Umar and Moinuddin 2005; Zhu 2016; Zhang et al. 2018b; Gong et al. 2020). In fact, about 50% of the crop loss is attributable to abiotic stresses. Plants have developed different mechanisms to face the environmental challenges, especially, plants produce secondary metabolites that play multiple protective roles in response to changing environment, affects biomass production and biosynthesis of plant secondary metabolites (Isah 2019). Plants synthesize enormous number of secondary metabolites from primary metabolites, but they do not play much of significant role in the primary life of plants. However, these secondary metabolites are important for the plant to interact with its environment for adaptation and defense (Ramakrishna and Ravishankar 2011). Besides, secondary metabolites are associated with a wide array of biological functions which render these metabolites a unique source for pharmaceuticals, food additives, flavors, and industrially important biochemicals (Ramakrishna and Ravishankar 2011; Ncube and Van Staden 2015). Though the secondary metabolites are produced only at very low concentrations (less than 1% dry weight) in plant cells, yet they determine the plant's adaptive capacity to cope with conditions of abiotic stress (Rao and Ravishankar 2002; Edreva et al. 2007). Abiotic stress factors can cause a myriad of changes at physiological, molecular and biochemical levels in plants by affecting the proteomes so as to cause alterations in protein relative abundance, cellular localization, post-transcriptional and post-translational modifications, protein-protein interactions, and protein functions (Kosová et al. 2018). Comprehensive knowledge on stress-responsive proteins and the corresponding proteome alterations is necessary to explain the processes of stress tolerance in plants and to implement new breeding strategies for developing stress-tolerant plant species to cope with the dynamic environmental changes. Thus, the proteomics study offers a new approach to discover key metabolic proteins involved in stress tolerance and metabolic pathways associated with crop physiological and stress responses (Hakeem et al. 2013; Ahmad et al. 2016).

1.2 Omics

OMICS technologies focus on the global analyses of biological samples (DNA, RNA, proteins and metabolites) using high through-put analytical techniques and bioinformatics and may provide new insights into biological phenomena (Vailati-Riboni et al. 2017). The word "omics" in biology refers to the field of studies that ends with *-omics* such as genomics (study of genome, the complete set of genetic information of an organism), transcriptomics (study of transcriptome, a full range of mRNA molecules expressed by an organism), proteomics (study of proteome, the complete set of proteins expressed by an organism) and metabolomics (study of metabolome, the global collection of all low molecular weight metabolites) (Fig. 2) (Aizat et al. 2018). The promising modern "multi-omics" platforms are high-throughput valuable tools for comprehensive analyses of the genome, transcriptome, proteome, and metabolome of medicinal plants so as to reveal the novel connected genetic pathways, regulator sequences, proteins, and secondary metabolite

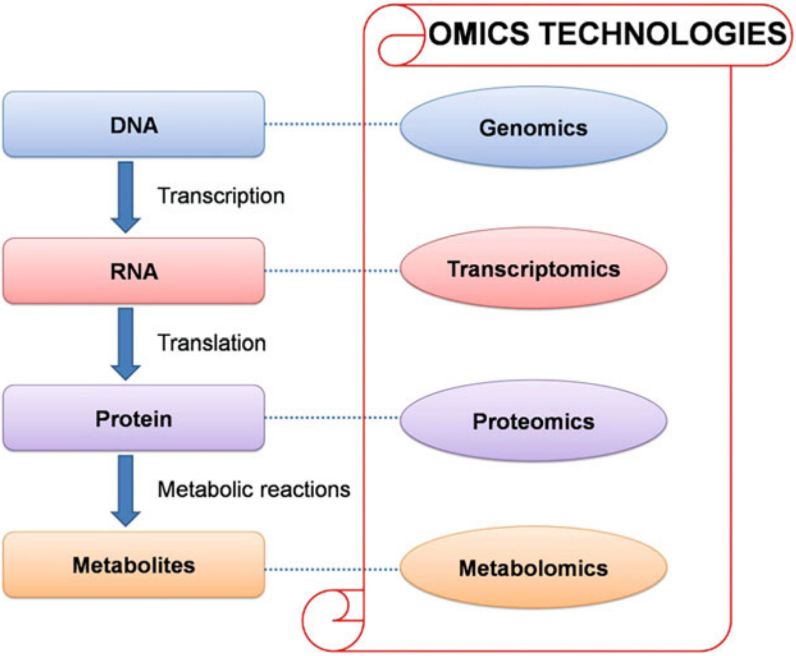

Fig. 2 OMICS technologies

biosynthetic pathways of a variety of plants with therapeutic significance (Sahu et al. 2014; Ma et al. 2020; Pandita et al. 2021). Therefore, integration of the omics data is extremely important for mechanistic studies on plant metabolism and bioengineering of metabolic pathways in the medicinal plants.

1.3 Proteomics

Proteomics plays a major role in comprehensive perception of phenotypes, because proteins are the biomolecules that can perform various biological functions in a cell (Correia et al. 2016). The term “Proteome” was first introduced by Marck Wilkins in 1996 to denote the “PROTein complement of a genOME”. Proteomics is the characterization of proteome including structure, functions, expressions, interactions and modifications of proteins at any stage. Proteome fluctuates from time to time, cell to cell and in response to external stimuli. Genomics alone may not tell the whole story of how organism functions and responds to environment (Aizat et al. 2018). Although in many cases changes in protein abundance and function can be attributed to altered gene expression, multiple post-transcriptional and post-translational mechanisms can affect protein abundance and function as well. Therefore, proteomes are often much more complex than the corresponding genomes. It is also very important to note that changes in transcriptome are not always correlated with changes in the abundance of the corresponding protein species. Therefore, proteomics approach presents numerous advantages, as compared to transcriptomics. Indeed, proteomics offers complementary information to genomics and transcriptomics essential for molecular level understanding of complex biochemical processes. Proteomics approach provides significant information regarding post-transcriptional and post-translational regulation mechanisms, mRNA localization and transport, translation rates, transcript and protein stability, and intercellular protein trafficking (Wang et al. 2013a; Passamani et al. 2017). In fact, proteomics provides the missing link between genome/transcriptome and metabolome, allowing for the identification and quantification of stress-tolerance-associated proteins and therefore, serving as a useful tool to study stress response in plants (Pinheiro et al. 2014; Bagheri et al. 2015). The identification of stress-related proteins enables the use of protein markers to improve selection of elite genotypes with high levels of tolerance to stress, conferring fitness advantages in a climate change scenario (Lippert et al. 2005). Plant adaptation to stress is associated with profound changes in proteome composition. Since proteins are directly involved in plant stress response, proteomics studies can help to the explore the relationship between protein abundance and plant stress and to understand the mechanism of proteome adaptation to abiotic stresses (Kosová et al. 2011; Qureshi et al. 2015).

The goal of high-throughput proteomics is to decrease sample analysis time while increasing the depth of proteome coverage (Zhang et al. 2014b). There are two approaches in proteomic analysis differentiated by upstream sample preparation methods. The first and most common approach is bottom-up proteomics or peptide-level proteomics in which protein samples are digested into peptides prior to mass spectrometry (MS) analysis. In the second approach, known as top-down proteomics, intact proteins are directly analyzed by MS (Zhang et al. 2014b). The available proteomic technologies are categorized as "gel-based" methods and "gel free" methods that can be coupled to MS for the analysis of proteomes. The gel-based proteomic methods include Two-dimensional electrophoresis (2DE) and Fluorescence 2D Difference Gel Electrophoresis (2D-DIGE)), whereas the gel-free methods include Isotope-Coded Affinity Tag (ICAT), Stable Isotope Labeling with Amino Acids in Cell Culture (SILAC), 18O Stable Isotope Labeling, Isobaric Tag for Relative and Absolute Quantitation (iTRAQ), Multidimensional Protein Identification Technology (MudPIT) and Protein Microarray Technology. It is important to note that, regardless of the choice of a given proteomic separation technique, gel-based or gel-free, a mass spectrometry (MS) is always the primary tool for protein identification and proteome analysis. MS-based proteomics is gaining interest in the recent years due to developments in instrumentation, sample preparation and computational analysis that subsequently owe to analyze proteomes for a more comprehensive understanding (Altelaar et al. 2013). Let us now have a look on the medicinal plant proteomics in response to various abiotic stresses.

2 Medicinal Plants Proteomics in Response to Temperature Stress

Temperature is the most important abiotic stress and is an important limiting factor for plants' growth and productivity when there is a random environmental fluctuation due to global climate change (Zinn et al. 2010; Parankusam et al. 2017). As a consequence of climate change, temperature stress is becoming a major concern in plant research. Both high and low temperature stresses are detrimental to plant growth dramatically, as they alter a variety of cellular phenomena such as membrane fluidity, nucleic acid and protein structures, metabolite and osmolyte concentrations, enzyme activities, photosynthesis, carbon assimilation, DNA/RNA stability, transcription and translation rates, cytoskeleton dynamics, and redox status (Wang et al. 2003; Apel and Hirt 2004; Chinnusamy et al. 2007; Niu and Xiang 2018).

2.1 High Temperature (Heat) Stress

High-temperature (HT) stress (or heat stress) is defined as the temperatures above normal optimum and is a major cause for physiological alteration and yield loss in many crops. Most noticeably, it inhibits photosynthesis mainly by affecting chlorophyll content, leaf firing, and tassel blast (Noor et al. 2019). High-temperatures damage the OEC (oxygen evolving complex) of PSII (photosystem II) (Strasser

1997), reducing the activity of RuBisCo (Ribulose-1,5-bisphosphate carboxylase-oxygenase, an enzyme involved in the CO_2 fixation) (Law and Crafts-Brandner 1999), and causing disorganization of thylakoid membranes. Protein metabolism, involving protein synthesis and degradation, is one of the most sensitive processes to HT stress. Changes in the level and expression pattern of some proteins may play an important role in plant adaptation to heat stress. HT stress may induce or enhance protein expression or protein degradation. The induction of heat-responsive proteins, particularly heat shock proteins (HSPs), plays a key role in plant tolerance to HT stress. Protein degradation by proteases is also important in regulating plant responses to HT stress (Huang and Xu 2008). Several protein groups related to heat-stress responses have been identified, which include antioxidative enzymes, heat shock proteins (HSPs), proteins related to energy and carbohydrate metabolism, redox homeostasis, protein synthesis and degradation, signal transduction, and transcription factors (Escandón et al. 2017; Parankusam et al. 2017).

Proteomics studies have been conducted to investigate the impact of HT stress on medicinal plants such as *Brassica campestris* (Yuan et al. 2019), *Pinus radiata* (Lamelas et al. 2020; Castander-Olarieta et al. 2021), *Solanum lycopersicum* (Jegadeesan et al. 2018), *Glycine max* (Wang et al. 2012a; Wei et al. 2020), *Portulaca oleracea* (Yang et al. 2012c), *Cicer arietinum* (Parankusam et al. 2017), *Lepidium meyenii* (Wang et al. 2020b), *Panax ginseng* (Kim et al. 2019), *Pinellia ternate* (Zhu et al. 2013), *Avena sativa* (Chen et al. 2016), *Spinacia oleracea* (Zhao et al. 2018; Li et al. 2019), and *Xanthoceras sorbifolium Bunge* (Du et al. 2021). Several plant parts including the somatic embryos (Castander-Olarieta et al. 2021), seeds and seedlings (Wang et al. 2012a; Zhu et al. 2013; Chen et al. 2016), leaves (Zhao et al. 2018; Kim et al. 2019; Wei et al. 2020; Du et al. 2021), cotyledons (Wei et al. 2020), pod, and pollen (Jegadeesan et al. 2018) have been studied to unravel the impact of the HT stress on proteomes of these medicinal plants. A list of proteomic studies conducted on medicinal plants in response to HT stress is given as Table 1.

Temperature stress can reduce yield and edible quality of *Brassica campestris* (Yuan et al. 2016a). Genotype WS-1 exhibited higher tolerance to high and low temperatures than other genotypes. Under high-temperature, WS-1 had a greater net photosynthesis rate, antioxidative capacity, and carbon–nitrogen assimilation efficiency than other genotypes (Zou et al. 2016; Yuan et al. 2017). Proteomics studies revealed that differentially expressed proteins (DEPs) were enriched in different pathways including redox homeostasis, photosynthesis, carbohydrate metabolism, heat-shockprotein, and chaperones and signal transduction pathways which are associated with temperature stress tolerance in wucai cabbage (Yuan et al. 2019).

Somatic embryogenesis (SE) has long been used as a model to study the different physiological, developmental and biochemical mechanisms underpinning the stress response in plants. A short-gel LC-MS proteomics approach revealed the impact of high temperatures on the proteomics in somatic embryos of *Pinus radiata* during initiation of embryonal masses. High temperature (40 °C) exposure led to a significant decrease in the initiation rate of embryonal masses and an increasing tendency in the production of somatic embryos. High temperature provoked a significant

Table 1 A list of proteomic studies conducted in medicinal plants in response to high temperature (HT) or low temperature (LT) stress

Plant name	Part studied	Stress treatment	Proteomic approach	References
Cicer arietinum	Leaves	HT (42 °C)	LC-MS-MS/MS	Parankusam et al. (2017)
Glycine max	Cotyledon, embryo, leaf, and pod	HT (40 °C)	iTRAQ technology coupled with LC-MS/MS	Wei et al. (2020)
Glycine max	Soybean	HT (40 °C)	2DE coupled to MALDI-TOF-MS	Wang et al. (2012a)
Lepidium meyenii	Leaves from seedlings	HT (42 °C)	TMT based LC-MS/MS	Wang et al. (2020b)
Panax ginseng	Leaves	HT (35 °C)	LC-MS/MS	Kim et al. (2019)
Pinellia ternata	Seedlings	HT (38 °C)	2DE coupled to MALDI-TOF/TOF MS	Zhu et al. (2013)
Pinus radiata	Somatic embryos	HT (40 °C and 60 °C)	2DE coupled nanoLC-MS/MS	Castander-Olarieta et al. (2021)
Pinus radiata	Seedlings	HT (45 °C)	1D nanoLC-MS/MS	Lamelas et al. (2020)
Pinus radiata	Needles from seedlings	HT (40 °C)	GeLC-Orbitrap/MS	Escandón et al. (2017)
Avena sativa	Seed	HT (35, 45, and 50 °C)	2DE coupled with nano LC-MS/MS	Chen et al. (2016)
Portulaca oleracea	Leaves	HT (35 °C)	2DE coupled MALDITOF/TOF	Yang et al. (2012c)
Solanum lycopersicum	Pollen and leaves	HT (50 °C)	1D nano-flow LC-MS/MS	Jegadeesan et al. (2018)
Spinacia oleracea	Leaves	HT (37 °C)	iTRAQ coupled LC-MS/MS	Zhao et al. (2018)
Spinacia oleracea	Leaves	HT (37 °C)	2DE based and iTRAQ coupled MALDI TOF/TOF MS	Li et al. (2019)
Xanthoceras sorbifolium	Seedlings	HT (38 °C)	iTRAQ coupled nanoLC-MS/MS	Du et al. (2021)
Brassica campestris	Seedlings	Both HT (40/30 °C) and LT (3/8 °C)	Tandem mass tag (TMT) coupled with LC-MS/MS	Yuan et al. (2019)
Actinidia arguta	Kiwifruits	LT (4 °C)	LC-MS/MS	Zhang et al. (2021)
Allium sativum	“Seed” bulbs	LT (5 °C)	2DE coupled with LC-ESI-MS/MS	Dufoo-Hurtado et al. (2015)
Brassica napus	Leaves	LT (−4 °C)	Nano-HPLC-MS/MS	Mi et al. (2021)
Butea superba	Tubers	Seasonal changes on tubers (winter,	2DE coupled with nanoLC-coupled to	Leelahawong et al. (2016)

(continued)

Table 1 (continued)

Plant name	Part studied	Stress treatment	Proteomic approach	References
		summer and rainy season)	ESI-quadrupole-TOF-MS/MS	
Camellia sinensis	Leaves	LT (4 °C)	iTRAQ coupled triple TOF MS	Zhu et al. (2019)
Crocus sativus	Plant buds	LT (16 °C)	iTRAQ coupled to LC-MS/MS	Chen et al. (2021)
Cucumis melo	Cantaloupe	LT (0.5 °C)	iTRAQ coupled to LC-MS/MS	Song et al. (2020)
Cynodon dactylon	Leaves	Chilling (<20 °C) and freezing (<0 °C)	2DE-coupled with MALDI-TOF-MS	Shi et al. (2014)
Eriobotrya japonica	Leaves	LT (4 °C)	(iTRAQ) coupled LC-MS/MS	Lou et al. (2018)
Hippophae rhamnoides	Seedlings	Freezing stress (−5 °C)	2DE coupled with nano LC-MS/MS	Gupta and Deswal (2012)
Ipomoea batatas	Tuberous roots	LT (4 °C)	1-De coupled to LC-MS/MS	Cui et al. (2020)
Lathyrus sativus	Leaves of seedlings	LT (4 °C)	2DE coupled to LC-TOF-MS	Chattopadhyay et al. (2011)
Musa paradisiaca	Seedlings	LT (8 °C)	iTRAQ coupled to nano LC-MS/MS	Yang et al. (2012b)
Phaseolus vulgaris	Roots of germinating seeds	LT (10 °C)	2DE coupled with MALDI-TOF-MS	Badowiec and Weidner (2014)
Picrorhiza kurroa	Rhizome	HT (2–3 °C above Ambient temperature)	MALDI-TOF/TOF-MS	Kumar et al. (2020)
Pisum sativum	Chloroplast	LT	MALDI TOF/TOF-MS/MS	Grimaud et al. (2013)
Sonneratia apetala	Seedlings	LT	LC-MS/MS	Shen et al. (2021)

increase in the number of structural constituents of ribosomes, together with the down-regulation of the enzyme methionine-tRNA ligase, suggesting a heat stress induced readjustment of the protein-synthesis machinery. Besides, high temperature in somatic embryos led to elevated levels of heat shock proteins and chaperones, transmembrane transport proteins, proteins related to post-transcriptional regulation and enzymes involved in the synthesis of fatty acids, specific sugars (myo-inositol), and carbohydrates of the cell wall. However, the enzymes of the glycolytic pathway, nitrogen assimilation, and oxidative stress response were found at lower levels in somatic embryos treated with HT stress (Castander-Olarieta et al. 2021).

Exposure to non-lethal stress, referred to as priming, can allow plants to survive subsequent and otherwise lethal conditions (i.e., induced HT or thermotolerance). Plants acquire HT-stress tolerance through priming, which establishes stress memory during mild or severe transient heat stress. Priming and the establishment of stress memory help to maintain metabolic homeostasis under HT stress. Short-term exposure of *Pinus radiata* to HT stress resulted in the expression of several differentially accumulated proteins related to different pathways such as lipid metabolism, heat stress, transport, and major and minor carbohydrate metabolism, covering primary and secondary metabolism (Escandón et al. 2017). As the nucleus is involved in the regulation of several critical cellular functions such as gene expression, cell proliferation, and cell survival, studies on nuclear proteomics may help in understanding the underlying molecular mechanisms of plant response to abiotic stresses (Yin and Komatsu 2016). Lamelas et al. (2020) studied the impact of HT stress (at 45 °C) on the nuclear proteomics of *P. radiata* seedlings. They suggested that epigenetic mechanisms possibly play a key role in thermopriming and heat stress tolerance. Several differentially-expressed proteins related to heat-shock proteins, several clusters involved in epigenomic-driven gene regulation, some transcription factors, and a variety of RNA-associated functions were identified in the seedlings *P. radiata* (Lamelas et al. 2020).

Pollen grains are highly sensitive to HT stress; consequently, impaired pollen development greatly reduces the productivity of a wide range of plants upon exposure to HT stress (Noor et al. 2019). Proteomic studies conducted in tomato plants revealed that exposure of developing pollen grains to heat stress caused a significant downregulation of proteins involved in protein synthesis, TCA cycle, and protein translation and degradation machinery, suggesting the downregulation of subsequent protein homeostasis in tomato pollen grains. In addition, proteins involved in cellular functions, like cell cycle, cell division, and transport as well as proteins involved in transcription regulation were significantly down-regulated in tomato pollen grains upon exposure to HT stress (Jegadeesan et al. 2018).

HT stress can cause severe damage to soybean seed development and maturity. To unravel how proteins and associated pathways are involved in the deterioration of developing soybean seed upon exposure to HT stress, Wang and co-workers analyzed the comparative proteome composition of developing seed of a pre-harvest seed deterioration sensitive soybean cultivar with its corresponding control. A total of 31 DEPs were identified which were involved in several cellular responses and metabolic processes including carbohydrate metabolism, signal transduction, protein biosynthesis, photosynthesis, protein folding and assembly, energy pathway, cell rescue and defense, cell cycle, nitrogen metabolism, lipid metabolism, amino acid metabolism, transcription regulation, and secondary metabolite biosynthesis (Wang et al. 2012a). Another comparative proteomic analysis of *Pinellia ternata* seedlings revealed that 20 spots were up-regulated and 7 spots were down-regulated. Of these, about 24 proteins were found to be mostly small heat shock proteins (e.g., cytosolic class I and II small HSPs as well as mitochondrial small HSPs). Further functional characterization of these proteins showed that differentially expressed proteins are enriched in RNA processing, protein degradation and defense, photosynthesis, and chlorophyll biosynthetic processes (Zhu et al. 2013). High temperature exposure and moisture content can cause loss of seed viability. It is emphasized that protein metabolism and expression play a critical role in seed resistance to heat stress. ATP synthases play a critical role in the mobilization of carbohydrates and energy and the balance between synthesis and degradation of other proteins during seed deterioration. Therefore, a proteomic approach was conducted in oat seeds to understand mechanisms of heat-stress acclimation and tolerance, following exposure to high temperatures (35, 45, and 50 °C) and it was unveiled that a total of 19 significantly different proteins were down-regulated (notably heat shock proteins and two ATP synthases) while two were up-regulated, which could be linked to the significant reduction in the seed vigor with increased temperature from 35 to 50 °C. It was also observed in seeds that a significant up-regulation of argininosuccinate synthase, a key protein involved in maintaining reactive oxygen species (ROS) homeostasis for the resistance of heat stress. Functional characterization of DEPs revealed their involvement in different pathways such as nucleotide transport and metabolism, carbohydrate transport and metabolism, amino acid metabolism and transport, posttranslational modification, protein turnover, chaperones, translation, ribosomal structure, and biogenesis (Chen et al. 2016).

Soybean (*Glycine max*) is an important legume crops and has a major impact on the global economies. Soybean pod skin plays a key role in the protection of seeds and also provides nutrients for seed growth and development through photosynthesis. Thus, it is imperative to study the proteomics of leaf and pod under HT stress. A recent study performed proteomic analyses in cotyledon, embryo, leaf, and pod of two cultivars of *G. max* (Ningzhen 1 and Xiangdou 3). iTRAQ coupled LC-MS/MS analysis identified 247, 179, and 517 DAPs in cotyledon, embryo, and leaf of cultivar Xiangdou 3 under HT stress, while 235, 366, and 479 DAPs were identified in cotyledon, embryo, and the leaf of cultivar Ningzhen 1. Moreover, 120, 144, and 438 differentially abundant proteins (DAPs) between the two cultivars were identified in cotyledon, embryo, and leaf under HT stress, respectively, and these DAPs were enriched in major metabolic pathways (tricarboxylic acid cycle, fatty acid metabolism, photosynthesis, starch, and sucrose metabolism) and cellular processes (protein processing, folding and assembly, protein biosynthesis or degradation, plant-pathogen interaction, signal transduction, and oxidative stress response) (Wei et al. 2020).

Understanding the molecular differences in plant genotypes with contrasting heat sensitivity can provide useful insights into the mechanisms that confer heat tolerance in plants. Therefore, Parankusam et al. (2017) conducted proteomic analyses in the leaves of heat-sensitive (ICC16374) and heat-tolerant (JG14) genotypes of chickpea under HT stress. Comparative proteomic analyses identified heat shock proteins, other proteins such as acetyl-CoA carboxylase, pyrroline-5-carboxylate synthase (P5CS), ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), phenylalanine ammonia-lyase (PAL) 2, ATP synthase, glycosyltransferase, sucrose synthase, and the late embryogenesis abundant (LEA) proteins, which were strongly associated with heat tolerance in the tolerant genotype. Several crucial proteins were induced by heat exclusively in the heat-tolerant genotype. Comparative pathway analysis revealed that accumulation of osmoprotectants, synthesis of ribosome and secondary metabolites, activation of antioxidant and defense compounds, amino acid biosynthesis, and hormonal modulation are the mitigating strategies that might play key roles in chickpea heat tolerance (Parankusam et al. 2017).

The rare and endangered plant species are particularly more vulnerable to change in global climatic conditions. *Picrorhiza kurroa* is an endangered medicinal plant of the Himalayan region having a great medicinal potential due to the presence of iridoid glycosides. Kumar et al. (2020) performed an MS-based proteomic approach to understand the adaptive heat stress responses in the leaves and rhizomes of *P. kurroa*. It was found that DAPs were related to signaling, antioxidant, stress-responsive, and chromatin remodeling proteins, as well as cytoskeleton proteins were elevated upon HT stress exposure in *P. kurroa* (Kumar et al. 2020). Another proteomic study conducted on the leaves of *P. oleracea* also revealed that a total of 51 DEPs identified were functionally related to material and energy metabolism, the antioxidant defense responses, protein destination and storage, and transcriptional regulation (Yang et al. 2012c). In *P. ginseng,* high temperature (35 °C) treatment hampers photosynthetic efficiency. Proteome analysis led to the identification of 847 DEPs in response to HT stress and functional pathway analysis indicated that increased abundance of DEPs was associated mainly with antioxidant and translation-regulating activities, whereas the proteins related to the receptor and structural-binding activities exhibited decrease in abundance. Besides, many other DEPs related to chaperones, G-proteins, calcium-signaling proteins, transcription factors, and transfer/carrier proteins were specifically downregulated in the leaves of *P. ginseng* (Kim et al. 2019). Similarly proteomic studies were conducted on spinach, which is a cold-tolerant but heat-sensitive green leafy vegetable, to study the heat tolerance of varieties Sp73 and Sp75. It was found that HT stress inhibited photosynthesis in both varieties. A total of 911 and 257 heat-responsive proteins were noted in spinach varieties Sp73 and Sp75, respectively. Functional pathway analyses showed that diverse primary and secondary metabolic pathways such as glycolysis, pentose phosphate pathway, amino acid metabolism, fatty acid metabolism, nucleotide metabolism, vitamin metabolism, and isoprenoid biosynthesis were promoted in Sp75 (Zhao et al. 2018), whereas in Sp73, ROS-scavenging pathways, stress-defense pathways, protein synthesis and turnover, carbohydrate and amino acid metabolism, and protein folding and turnover were promoted, suggesting a conservative and ROS-scavenging strategy as being critical for plant response to HT stress (Li et al. 2019). It is speculated that heat stress can hinder the synthesis of riboflavin, reducing NADH dehydrogenase content, which might further have an impact on energy utilization. *Xanthoceras sorbifolium* Bunge is rich in oleanane type and barrigenol-like triterpenes and possesses neuroprotective properties (Li et al. 2016). Comparative proteomic analysis of leaves exposed to HT stress revealed that abundance of most of the proteins associated with oxidative phosphorylation, NADH dehydrogenase, and superoxide dismutase was reduced and the DEPs were found to be associated with photosynthesis enzymes, indicating that stress had different effects on photosystem I and photosystem II (Du et al. 2021).

Studies have suggested that HT stress can induce genes/proteins related to protein processing in the endoplasmic reticulum. This makes the endoplasmic reticulum, play a crucial role in plant responses to high temperatures. A recent proteomic study unveiled about 300 proteins showing significant alterations in expression in the leaves, following HT exposure of *Lepidium meyenii.* Further, pathway analyses indicated that protein processing in the endoplasmic reticulum was the most significantly up-regulated metabolic pathway following the application of HT stress on *L. meyenii* (Wang et al. 2020b).

2.2 Low Temperature (Cold) Stress

Low temperature (LT) is also an important environmental stress, influencing plant performance and distribution. Proteins are the major players in most cellular events and are directly involved in plant responses, and proteome analysis often uncovers additional novel proteins associated with stress tolerance. LT can modulate different pathways/proteins involved in carbohydrate metabolism, ROS scavenging, redox balance, cell-wall remodeling, cytoskeletal rearrangements, cryoprotection, and defense/detoxification (Janmohammadi et al. 2015). Proteomic approaches have investigated the impact of LT stress on the protein profiles of medicinal plants such as *Picrorhiza kurroa* Royle ex Benth. (Kumar et al. 2020), *Camellia sinensis* (L.) Kuntze (Tea plant) (Zhu et al. 2019), *Hippophae rhamnoides* L. (Sea buckthorn, a Himalayan wonder shrub) (Gupta and Deswal 2012), *Lathyrus sativus* L. (Grass pea) (Chattopadhyay et al. 2011), *Ipomoea batatas* L. (Lam or sweet potato) (Cui et al. 2020), *Butea superba* Roxb (Red KwaoKrua, a native Thai herbal plant) (Leelahawong et al. 2016), *Cynodon dactylon* (L.) Pers. (Bermuda grass) (Shi et al. 2014), *Cucumis melo* L. (Cantaloupe) (Song et al. 2020), *Musa paradisiaca* L. (Plantain) (Yang et al. 2012b), *Actinidia arguta* (Siebold & Zucc.) Planch. ex Miq. (Kiwi fruit) (Zhang et al. 2021), *Pisum sativum* L. (Pea) (Grimaud et al. 2013), *Allium sativum* L. (Garlic) (Dufoo-Hurtado et al. 2015), *Crocus sativus* L. (Saffron) (Chen et al. 2021), *Sonneratia apetala* Buch.-Ham. (Shen et al. 2021), *Medicago sativa* L. (Alfalfa) (Chen et al. 2015), *Phaseolus vulgaris* L. (Common beans) (Badowiec and Weidner 2014), *Eriobotrya japonica* Lindl. (Loquat) (Lou et al. 2018), and *Brassica napus* L. (Rapeseed) (Mi et al. 2021). A list of proteomic studies conducted with medicinal plants in response to LT stress is given in Table 1.

Tea plants are the richest source of bioactive flavonols such as quercetin, rutin, myricetin, etc. Low-temperature can dramatically affect growth, survival and geographical distribution of tea plants (Wang et al. 2012b). Endogenous γ-Aminobutyric Acid (GABA) is thought to play a crucial role in the stress response of tea plants and GABA interaction with stress response substances may is supposed to be involved in the regulation of DAPs associated with optimum temperature and cold stress. To validate this, tea plants, supplied with or without GABA, were subjected to optimum or cold temperatures and proteomics was investigated. DAPs were found for protein metabolism and nucleotide metabolism, energy, amino acid transport and metabolism, other biological processes, inorganic ion transport and metabolism, lipid metabolism, carbohydrate transport and metabolism, biosynthesis of secondary metabolites (polyamines and anthocyanins), antioxidant and stress defense. Thus, the role of GABA-induced altered levels of the stress response is associated with improved cold tolerance. In addition, protein-protein interaction analysis confirms that alteration of endogenous GABA and stress response substances induce interactions among photosynthesis, amino acid biosynthesis, and carbon and nitrogen metabolisms, and the corresponding differences may contribute to improved cold tolerance of tea plants (Zhu et al. 2019).

Cynodon dactylon (Bermuda grass), a creeping grass, has been traditionally used to treat epilepsy, dropsy, wound infections, piles, and snakebite (Murali et al. 2015). It is tolerant to drought, salt and heat stresses, but sensitive to LT stress. Low temperature (cold stress), which includes chilling (<20 °C) and freezing (<0 °C) temperatures, can cause severe disruption of cellular homeostasis which subsequently leads to cell damage and drastically impairs plant development and growth (Yang et al. 2006). There is a positive correlation between exogenous $CaCl_2$ treatment and ROS detoxification system, which together contribute to the increased freezing tolerance in plants. To further confirm the role of calcium in plant cold stress response, proteomics studies were conducted on bermudagrass leaves to identify changes in DEPs induced by cold stress and exogenous calcium. About 51 DEPs were induced by $CaCl_2$ treatment under control and chilling stress conditions and functional analysis of these proteins indicated that most of the DEPs were enriched in redox regulation, tricarboxylic acid cycle, glycolysis, photosynthesis, oxidative pentose phosphate pathway, and amino acid metabolisms. Enrichment of DEPs in redox regulation further confirmed the positive link between exogenous $CaCl_2$ and ROS-detoxification system, which might have contributed to superior freezing tolerance in bermudagrass (Shi et al. 2014).

Fruits of *Sonneratia apetala*, a woody mangrove species distributed in the tropical coastal areas, are typically used as food and medicine in tropical coastal countries (Yi et al. 2020). The low temperature in winter was the most crucial abiotic stress that limits the mangrove afforestation. Proteomic analyses of *S. apetala* leaves subjected to chilling stress revealed an increase in DAPs related to ROS scavenging, photosynthesis, and energy metabolism, carbohydrate metabolism, cofactor biosynthesis, and protein folding, suggesting the crucial roles of these DEPs in improving the cold tolerance of *S. apetala* (Shen et al. 2021). Sea buckthorn (*Hippophae rhamnoides*) berries, fruits, and leaves are widely used in folk medicine. This plant is an important medicinal plant and is extensively grown in Asia, Europe, and Canada. Plants' distribution and productivity are negatively affected by LT stress. Proteomics of Sea buckthorn seedlings subjected to low stress led to the identification of 61 low temperature-responsive proteins. Functional categorization revealed that 34 up-regulated DEPs were enriched mostly in signaling. Other proteins are related to redox regulation and defense associated proteins as well as putative antifreeze proteins (e.g., thaumatin-like protein and Chitinase) (Gupta and Deswal 2012).

Plantain (*Musa paradisiaca*) exhibits greater cold tolerance, compared to banana. It is interesting to understand the molecular mechanisms and responses of plantain to cold stress for developing cold-tolerant banana cultivars. Using a comparative proteomics approach, responses of plantain to cold stress were investigated in seedlings and it was found that a total of 809 DEPs showed differential expression. These DEPs were mostly implicated in redox, whereas others were associated with photosynthesis, photorespiration, and primary metabolism (carbohydrate metabolism and fatty acid beta-oxidation). An increased antioxidant status and decreased lipid peroxidation might contribute to molecular mechanisms for the increased cold tolerance in plantain (Yang et al. 2012b).

The tuberous roots of *Ipomoea batatas* (Sweet potato) form a delicious food with a high nutritional value and medicinal benefits. Cold stress often causes altered protein-expression pattern and substance contents in the tuberous roots of sweet potato during low-temperature storage (Cui et al. 2020). Proteomics approach revealed that most of the DEPs were implicated in metabolic pathways related to phenylpropanoids, starch and sucrose metabolism. Enzymes L-ascorbate peroxidase 3 and catalase were down-regulated during low temperature storage. α-amylase, sucrose synthase, and fructokinase were significantly up-regulated in starch and sucrose metabolism, while β-glucosidase, glucose-1-phosphate adenylyl-transferase, and starch synthase did the opposite (Cui et al. 2020). Seasonal variations greatly impact the stress tolerance of plants. Indeed, seasonal changes are major factors affecting environmental conditions which induce multiple stresses in plants, leading to changes in proteins relative abundance in the complex cellular plant metabolic pathways. Therefore, it is significant to understand the molecular basis of plant proteomes and stress-tolerance mechanisms during seasonal changes. Besides, such studies might help to unveil potential protein markers in plants during different seasons. Effect of seasonal variations (winter, summer, and rainy season) was explored on the proteomics of tuberous roots of *B. superba* Rox., a Thai herbal plant widely used in traditional medicine as an anti-aging therapy. A total of 191 DEPs were identified and most of them were involved in carbohydrate and energy metabolism and defense to stress. Winter harvested samples exhibited the highest numbers of DEPs. It was also shown that changes in the protein levels were due mainly to temperature stress during summer and to water stress during winter (Leelahawong et al. 2016).

Refrigeration is an important method to extend the shelf life of fruits, but the inappropriate storage temperature can lead to chilling injury in the fruits. Therefore, prevention of LT stress-induced damage in fruits is an important storage industry. However, quality of the fruit will deteriorate after long-term cold storage and the concerned biological activities in fruits are regulated by proteins. It is, therefore, significant to study the proteomics of fruits to reveal the key proteins involved in the cold induced damage in fruits. *Cucumis melo* (Cantaloupe) has been reported to possess potent anti-inflammatory, anti-diabetic, cardioprotective, and neuroprotective properties (Saddi et al. 2018; Ezzat et al. 2019). Cantaloupe is highly susceptible to cold stress when it is stored at low temperatures, resulting in the loss of edible and commercial quality. Proteomic analysis of a cold-sensitive cultivar, Golden Empress-308 (GE) and a cold-tolerant cultivar, Jia Shi-310 (JS) of cantaloupe indicated that the number of DEPs changed remarkably during the chilling treatment. JS expressed cold-responsive proteins more rapidly and mobilized more groups of proteins than GE. The DEPs found were related mainly to carbohydrate and energy metabolism, structural proteins, ROS scavenging, amino acids metabolism, and signal transduction (Song et al. 2020). In another study, proteomics of Kiwi fruit under cold stress identified a total of 244 DEPs, among which 113 were up-regulated and 131 were down-regulated. Further, Kyoto Encyclopedia of Genes and Genomes shows that the DEPs are implicated mainly in TCA cycle, oxidative phosphorylation, fatty acid biosynthesis, and starch and sucrose metabolism (Zhang et al. 2021). Loquat (*Eriobotrya japonica* Lind.) is a subtropical fruit tree with high medicinal value, and used historically as folk medicines (Liu et al. 2016). Cold and freezing stress often causes dynamic loss to loquat fruits. Proteomic investigations in loquat leaves under 4 °C treatment identified 300 DEPs, which are functionally categorized mostly to metabolic pathways and biosynthesis of secondary metabolites (Lou et al. 2018).

Pisum sativum L. (Pea) is an important source of high-quality protein in the human diet and pea proteins are known for their potent health benefits. Pea productivity depends on LT during fall and winter. The chilling tolerance might be related to an increase in proteins related to soluble sugar synthesis, antioxidant potential, regulation of mRNA transcription, and translation through the chloroplast (Grimaud et al. 2013). *Phaseolus vulgaris* L. (Common beans) is also the richest source of polyphenolic compounds with numerous health-promoting properties (Hayat et al. 2014). In beans, germination under continuous chilling causes alterations in the accumulation of the proteins involved in stress response, energy production, translation, vesicle transport, secondary metabolism, and protein degradation (Badowiec and Weidner 2014). MS-based proteomics identified 48 stress-responsive proteins (SRPs) in grass pea (*Lathyrus sativus*) seedlings subjected to LT stress. These stress response proteins presumably involved in metabolism, signal transduction, protein biogenesis and degradation, and cell defense and rescue (Chattopadhyay et al. 2011).

The ROS versus antioxidant balance plays an important role in deciding plant response to cold stress. Garlic has diverse bioactive compounds and is the most widely consumed spice in the world. LC-ESI-MS/MS proteomics revealed that LT stress in garlic “seed” cloves alters the accumulation of proteins related to stress response, regulation of transcription, transport of macromolecules, protein folding, photosynthesis, carbohydrate metabolism, nucleotide metabolism, and redox (antioxidative/oxidative) state (Dufoo-Hurtado et al. 2015). Similarly, *Crocus sativus* (Saffron) is widely used as a natural dietary spice as well as a popular traditional medicine. Several studies have described anticancer activity, antidepressant activity, and cytotoxic effects of this plant (Kashani et al. 2018; Moradzadeh et al. 2018; Krishnaswamy et al. 2020). It is an expensive and valuable species that exhibits preventive and curative effects. Chen et al. (2021) screened the key proteins affecting the floral initiation of saffron under cold stress. They identified 201 DAPs between flowering and non-flowering saffron buds, and the upregulated DAPs were involved in the sucrose metabolic process, lipid transport, glutathione metabolic process, and gene silencing by RNA, while the downregulated DAPs were significantly involved in starch biosynthetic process and several oxidative stress-response pathways (Chen et al. 2021).

Rapeseed (*Brassica napus*) is an anti-oxidant plant; its extracts (especially the cooked one) may be beneficial for diabetic patients (Akbari et al. 2016). Rapeseed oil is the second most abundantly produced edible oil in the world and the quality of rapeseed oil has attracted global attention. Cold-pressed rapeseed oil appeared to be a preferred choice than refined oil due to no solvent and less processing involved in the cold-pressing. Cold-pressed rapeseed oil offers health benefits due to its preserved fatty acid profile and bioactive compounds (Chew 2020). Proteomic profiles were screened in the leaves of two winter rapeseed cultivars, “NS” (cold tolerant) and “NF” (cold-sensitive) after low temperature (−4 °C) stress treatment. Antioxidant activity and osmotic adjustment ability were higher, and plasma membrane injury was less obvious, in NS than in NF under cold stress. About 911 cold-stress-response proteins were more abundant in NS only after cold treatment, compared to NF. These DEPs were rapidly accumulated to scavenge the ROS through four metabolic pathways: lysine degradation; phenylalanine, tyrosine and tryptophan; flavonoid biosynthesis; and ubiquinone and other terpenoid-quinone biosynthesis (Mi et al. 2021).

3 Medicinal Plant Proteomics in Response to Drought Stress

Drought stress is the key environmental stress that occurs due to temperature dynamics, light intensity, and low rainfall (Seleiman et al. 2021). Drought-responsive proteins exhibit various patterns depending on plant species, genotypes, and stress intensity. Proteomic analyses show that dominant changes occur in sensing and signal transduction, ROS scavenging, osmotic regulation, gene expression, protein synthesis/turnover, cell structure modulation, and carbohydrate and energy metabolism (Wang et al. 2016a). The proteomic studies of plant drought response have been widely investigated in model plants, crops, and woody plants including those with various medicinal properties, such as *Arachis hypogaea* L. (Peanut) (Kottapalli et al. 2013; Katam et al. 2016), *Brassica napus L.* (Rapeseed) (Mohammadi et al. 2012; Koh et al. 2015), *Camellia sinensis* (L.) O. Kuntze (Tea plant) (Wang et al. 2017b; Gu et al. 2020), *Carthamus tinctorius L.* (Safflower) (Çulha Erdal et al. 2021), *Cicer arietinum L.* (Chickpea) (Tamburino et al. 2017; Gupta et al. 2020), *Cucumis sativus L.* (Cucumber) (Cui et al. 2019), *Eleusine coracana* (L.) Gaertn. (Finger millet) (Li et al. 2021), *Glycine max* L. (Soybean) (Oh and Komatsu 2015; Das et al. 2016; Wang et al. 2016b, 2017a; Yahoueian et al. 2021), *Helianthus annuus* L. (Sunflower) (Fulda et al. 2011; Ghaffari et al. 2013), *Hippophae rhamnoides L.* (Sea buckthorn) (He et al. 2016), *Hordeum vulgare* L. (Barley) (Chmielewska et al. 2016; Gołębiowska-Pikania et al. 2017; Kausar et al. 2013), *Humulus lupulus* L. (Hop plant) (Kolenc et al. 2016), *Medicago sativa* L. (Alfalfa) (Ma et al. 2017; Wang et al. 2020a), *Morus alba* L. (Mulberry) (Liu et al. 2019a), *Olea europaea* L. (Olive) (Ben Abdallah et al. 2018), *Phaseolus vulgaris* L. (Common bean) (Zadražnik et al. 2013), *Phoenix dactylifera L.* (Date palm) (El Rabey et al. 2015), *Pinus halepensis Mill* (Aleppo pine) (Taïbi et al. 2017), *Pisum sativum* L. (Pea) (Castillejo et al. 2016), *Quercus ilex L.* (Holm oak)

(Echevarría-Zomeño et al. 2009), *Selaginella tamariscina* (Beauv.) (Wang et al. 2010), *Setaria italica* L. P. Beauv (Foxtail millet) (Pan et al. 2018), *Vicia faba L.* (Faba bean) (Li et al. 2018a), *Vignata radiata* L. (Mung bean) (green gram) (Sengupta et al. 2011), and *Vitis vinifera* L. (Grapevine) (Król and Weidner 2017) (Table 2).

Drought can strongly influence growth, development, and yield of grain legumes in arid and semi-arid areas of the world. For instance, drought stress frequently occurs during the seedling stage and finally affects the yield of faba bean (commonly referred to as broad bean), which has been reported to possess diverse health-promoting properties including neuroprotection (Mejri et al. 2018; Eskandrani 2021). MS-based quantitative leaf proteomic analysis detected 25 DEPs that were downregulated and five DEPs that were upregulated in the leaves of drought-tolerant faba bean plant subjected to water-deficit conditions. These DEPs were functionally annotated to regulatory proteins (46.7%), energy metabolism (23.3%), cell cytoskeleton (6.7%), other functions (20%) and unknown function (3.3%). Among the DEPs, Chitinase, Bet protein and glutamate-glyoxylate aminotransferase were upregulated under drought, suggesting that these proteins have a role in providing stronger drought stress tolerance of "Ga da dou". Another DEP, namely 50S ribosomal protein, was also upregulated upon drought stress in faba plants, suggesting its role in protecting plants against stress by re-establishing normal protein conformations (Li et al. 2018a).

Abiotic stresses can adversely affect the yield of pea crops and are responsible for heavy economic losses. In order to develop pea cultivars, well adapted to drought conditions, it is necessary to unravel the proteomic profiles of pea plants having nutritional and economic importance. Chickpea has beneficial effects on some of the important human diseases such as CVD, type 2 diabetes, digestive diseases, and some cancers (Wallace et al. 2016). Chickpea is agronomically important because of its nutritional value, and it also enhances soil quality by fixing atmospheric nitrogen. It is the second most commonly cultivated legume all over the globe. Roots function as a first signal transducer of drought stress because they establish direct contact with water. In addition, roots transport nutrients from soil to the aerial plant parts for growth, thus roots play an essential role in maintaining homeostasis. As expected, drought or water-deficit conditions can severely affect this homeostasis, which results in the structural and functional modification in plants. A comparative roots proteomic analysis of drought-tolerant chickpea seedlings grown under hydroponic conditions identified 75 DEPs. Functional annotation analysis revealed that many of these DEPs (23%) are involved in carbon and energy metabolism (mainly glyceraldehyde-3-phosphate dehydrogenase, malate dehydrogenase, transaldolase, and isocitrate dehydrogenase). Other DEPs are enriched in stress response (heat-shock protein, CS domain protein, and chitinase 2-like), protein metabolism (adenosine kinase 2, and protein disulfide isomerase), ROS metabolism (glutathione S-transferase, ascorbate peroxidase, and thioredoxin), signal transduction (actin-101, and 14-3-3-like protein B), secondary metabolism (cinnamoyl-CoA reductase-1 and chalcone-flavonone isomerase 2) as well as nitrogen and amino acid metabolism (glutamine synthetase and homocysteine methyltransferase) (Gupta

Table 2 A list of proteomic studies conducted in medicinal plants in response to drought stress

Plant name	Part studied	Treatment	Method	References
Xanthiceras sorbifolium	Leaves	No irrigation	iTRAQ coupled nanoRPLC-MS/MS	Du et al. (2021)
Cicer arietinum	Roots	Used drought tolerant plant	2-DE coupled to MALDI-TOF/TOF	Gupta et al. (2020)
Cicer arietinum	Leaves of seedlings	Gradual, progressive dehydration by withdrawing water and allowing evapotranspiration	2DE coupled with LC-MS/MS	Vessal et al. (2020)
Solanum lycopersicum	Chloroplast	No irrigation	2D-DIGE coupled with nLC-ESI-LIT-MS/MS	Tamburino et al. (2017)
Glycine max	Leaf, hypocotyl, root	Drought and flooding	LC-MS/MSNano	Wang et al. (2017a)
Glycine max	Leaves	No watering for 7 days	2D-DIGE coupled with MALDI-TOF/TOF-MS/MS	Das et al. (2016)
Glycine max	Leaves	Drought treatment was exposed for three weeks	2DE coupled with nano-LC–MS/MS	Yahoueian et al. (2021)
Morus alba	Leaves and roots	Withdrawal of water	TMT-based coupled with high PH reverse phase (hpRP) fractionation and NanoLC-MS/MS analysis	Liu et al. (2019a)
Phoenix dactylifera	Seedlings	No water for 1 month	2D-DIGE coupled with nanoLC-MS/MS	El Rabey et al. (2015)
Vigna radiata	Roots	Withholding water and then re-watered for the next 6 days for recovery	2DE coupled with MALDI-TOF/TOF-MS/MS	Sengupta et al. (2011)
Medicago sativa	Pod walls	Watering to 4 kg while control watered 7 kg	nanoLC-MS	Wang et al. (2020a)
Medicago sativa	Seedlings	PEG-mimicked drought stress conditions 180 g L^{-1} of PEG4000	2DE coupled with TOF-MS/MS	Ma et al. (2017)
Helianthus annuus	Primary leaves	PEG 6000 mimicked drought stress conditions	2DE coupled with MALDI-TOF-MS	Fulda et al. (2011)
Camellia sinensis	Leaves	Withholding watering	TMT labeling with HPLC fractionation and LC-MS/MS analysis	Gu et al. (2020)
Camellia sinensis	Tea seedlings	Withholding watering	iTRAQ coupled with	Wang et al. (2017b)

(continued)

Table 2 (continued)

Plant name	Part studied	Treatment	Method	References
Arachis hypogaea	Leaves	Gradual water stress was implicated plants for 15 days by withdrawing water	MALDI-TOF/TOF mass	Katam et al. (2016)
Hordeum vulgare	Shoot	PEG mimicked drought stress	2DE coupled with MALDI-TOF-MS	Kausar et al. (2013)
Cucumis sativus	Chloroplasts	Moderate and severe drought stress by polyethylene glycol 6000)	iTRAQ coupled with nanoLC-MS/MS	Cui et al. (2019)
Cucumis sativus	Leaves	10% PEG mimicked	iTRAQ coupled with LC-ESI-MS/MS	Du et al. (2019)
Zingiber officinale	Root, stem and leaf	5% PEG-6000	iTRAQ coupled with LC-ESI-MS/MS	Lv et al. (2020)
Sorghum bicolor	Roots	PEG treatment	2DE coupled with (MALDI-TOF-TOF MS	Li et al. (2020a)
Cucumis melo	Leaves	Without irrigation	2DE coupled with MALDI–TOF–MS	Ansari et al. (2019)
Carthamus tinctorius	Leaves	Without irrigation for 7 days followed by 5 days of re-watering	2DE coupled with MALDI-TOF/TOF–MS	Çulha Erdal et al. (2021)
Brassica napus	Seedlings	Restricting water for 7 days	2De coupled with nanoLC-MS/MS	Mohammadi et al. (2012)
Eleusine coracana	Seedlings	Grown for 19 days without water	TNT based nLC UPLC-MS/MS	Li et al. (2021)
Hippophaë rhamnoides	Leaves		2D-DIGE coupled with MALDI-TOF MS/MS	He et al. (2016)
Glycin max	Root tip of soybean	Exposed to flooding or to drought by withholding watering	nanoLC-LTQ orbitrap MS	Wang et al. (2016b)
Glycin max	Roots	Exposed to flooding or to drought by withholding watering	Nano-liquid chromatography (LC) mass spectrometry (MS)/MS	Oh and Komatsu (2015)
Humulus lupulus	Leaves	Progressive soil water deficit	2D-DIGE-MALDI-MALDI-TOF/TOF	Kolenc et al. (2016)
Pinus halepensis	Root and needles	Withholding water	SDS-PAGE coupled with LC-MS/MS	Taïbi et al. (2017)
Hordeum vulgare	Leaves	Water content in the pots was gradually reduced to 33–35% soil water content	2DE coupled with MALDI-TOF/TOF	Gołębiowska-Pikania et al. (2017)
Hordeum vulgare	Leaves and roots	-NA-	2D-PAGE coupled with MALDI-TOF and MALDI-TOF/TOF	Chmielewska et al. (2016)

(continued)

Table 2 (continued)

Plant name	Part studied	Treatment	Method	References
Phaseolus vulgaris	Leaves	No watering	2D-DIGE coupled with LC-MS/MS	Zadražnik et al. (2013)
Vicia faba	Leaves	Withdrawing water for 7 days	2DE coupled with MALDI-TOF/TOF	Li et al. (2018a)
Helianthus annuus	Roots			Ghaffari et al. (2013)
Brassica napus	Leaves	No watering	iTRAQ coupled with LC-MS/MS	Koh et al. (2015)
Olea europaea	Leaves	Complete water depletion for 21 days	nanoLCESI-LIT-MS/MS	Ben Abdallah et al. (2018)
Quercus ilex	Leaves	No irrigation during 7 days followed by a watering period of 7 days	2DE coupled with LC–MS/MS	Echevarría-Zomeño et al. (2009)
Setaria italica	Seedlings	Drought-treated experimental units was controlled at 60–70% and 20–30% of field capacity respectively, and the treatments lasted for 7 days	TMT labeling and HPLC fractionation coupled with LC-MS/MS analysis	Pan et al. (2018)
Pisum sativum	Leaves	Withholding water	1D-nanoLC-MS/MS	Castillejo et al. (2016)
Selaginella tamariscina	Aerial parts	Water-deprived for up to seven days followed by 12 h of rewatering	2DE coupled with MALDI-TOF/TOF MS	Wang et al. (2010)
Arachis hypogea	Total pod	Full irrigation plots received 50 mm of water and deficit plots received 25 mm per week	1-DGE coupled with LC−MS/MS	Kottapalli et al. (2013)
Vitis vinifera	Leaves	Plants were grown for 2 weeks under drought stress	2DE coupled with MALDI TOF/TOF MS	Król and Weidner (2017)

et al. 2020). Likewise, in *Pisum sativum* (pea), a shotgun proteomic approach unraveled a total of 367 significantly expressed drought-stress-responsive proteins. More than half of these DEPs belong to primary metabolism and protein regulation, suggesting that maintenance of the primary metabolism and protein protection is a drought stress tolerance strategy in *P. sativum* (Castillejo et al. 2016).

The analysis of the dynamic regulation of root proteomes during water deficit is essential to further understand drought response mechanisms in food legumes. The Mung bean (*Vigna radiate*) is a leguminous plant that has a long history of usage as traditional medicine. It has been known to be an excellent source of protein, dietary fiber, minerals, vitamins, and significant amounts of bioactive compounds, including polyphenols, polysaccharides, and peptides, therefore, becoming a popular functional food in promoting good health (Sudhakaran and Bukkan 2021). Root proteomic approach identified 26 DEPs in mung bean during short-term and consecutive long-term water deficit as well as during recovery after re-watering. Cytoskeleton-related DEPs were down-regulated initially during short-term drought stress but regained their expression levels during the subsequent long-term water-deficit. However, enhanced expression levels of glycoprotein like lectins, which were primarily known to be involved in legume-rhizobia symbiosis, was reported during both short- and long-term drought treatment, indicating their possible role in the drought-stress response of legumes. Other DEPs related to oxidative stress-related proteins such as Cu/Zn superoxide dismutase, oxidoreductase, and aldehyde reductase, were also up-regulated in drought-stressed mung bean (Sengupta et al. 2011). Since root is the primary water and mineral sensing organ, researchers have focused on the drought-induced root protein profiles. Sorghum is the fifth most consumed cereal in the world and is a source of nutrients and bioactive compounds that confer health benefits for humans (Dykes 2019). Proteome map demonstrated that the levels of 43 DEPs spots were increased and 22 were decreased in roots of sorghum under drought condition. These DEPs were functionally annotated to a variety of cellular functions, including carbohydrate and energy metabolism, antioxidant and defense response, protein synthesis/processing/degradation, transcriptional regulation, amino acid biosynthesis, and nitrogen metabolism, which contribute jointly to the molecular mechanism of outstanding drought tolerance in *Sorghum bicolor* plants (Li et al. 2020a).

Sunflower RGK 21 and BGK 329 are the most sensitive and tolerant lines, respectively. Differential root proteomic approach identified a decrease in abundance of DEPs related to metabolism in both sensitive and tolerant lines. However, the abundance of disease/defense and energy-related proteins decreased in the sensitive line but increased in the tolerant line, suggesting that changes in energy usage, water transport, and ROS scavenging are important mechanisms for maintaining root growth under water-deficit or drought conditions (Ghaffari et al. 2013). The root tip is the most sensitive organ, vulnerable to flooding and drought stresses affecting the early stages of growth in plants. Moreover, under drought stress conditions the root tip tends to penetrate the soil in search of water, therefore it is necessary to unravel the underlying drought and flood stress-responsive mechanisms in the root tip. So, protein profiling was analyzed in the root tip of soybean plants under flooding and drought stresses. The Proteomic approach identified that UDP glucose: glycoprotein glucosyltransferase was decreased and increased in soybean root under flooding and drought, respectively. UGGT, essential for protein folding, is a key player in the quality control mechanism in the endoplasmic reticulum and serves as a molecular chaperone to the proper folding of glucoproteins. It was also suggested that fermentation is essential for root tip under flooding, whereas protein synthesis/degradation is essential for root tip under drought conditions. Furthermore, a decrease in biotin/lipoyl attachment domain-containing protein (BLAP), which is involved in the post-transcriptional modification of protein as well as Class II aminoacyl tRNA/biotin synthetases superfamily protein, was noted, indicating the crucial role of biotin and biotinylation in the energy management to cope with flooding and drought in the early stage of the soybean root tip (Wang et al. 2016b). Similarly, DEPs related to fermentation, stress, and cell wall-related proteins were increased in soybean roots in response to flooding stress, whereas cell organization and redox-related proteins were increased under drought stress (Oh and Komatsu 2015).

Chloroplasts are important cellular organelles in plants because they are involved in many vital cellular processes such as the biosynthesis of aromatic amino acids, fatty acids, and carotenoids, and most importantly photosynthesis (Iqbal et al. 2000a). Chloroplasts act as a sensor of environmental changes and help in optimizing different cell functions for triggering the adaptive response to stressful conditions. However, only partial information is available on stress-induced mechanisms within plastids. The Proteomics approach revealed that water deficit deeply affects the chloroplast protein profile. A total of 31 DEPs were identified which are mainly implicated in energy-related functions (Tamburino et al. 2017).

Proteomic study revealed that a majority of DEPs related to the tricarboxylic acid cycle followed by photosynthesis, RNA, DNA, and signaling, were predominately affected in leaf, hypocotyl, and root in response to drought stress in soybean. Moreover, other proteins such as beta-glucosidase 31 and beta-amylase 5 were increased in leaf and root, suggesting that beta-amylase 5 may be involved in carbohydrate mobilization to provide energy to the leaf of soybean seedlings exposed to drought (Wang et al. 2017a). Protein synthesis elongation factor (EF-Tu) is a highly conserved multifunctional protein involved in transporting the aminoacyl-tRNA complex to the A site of the ribosome during protein biosynthesis; chaperone activity in protecting other proteins from aggregation caused by environmental stresses, facilitating renaturation of proteins when conditions return to normal and participating in the degradation of N-terminally blocked proteins by the proteasome. EF-Tu upregulates the abundance of this protein in plants under abiotic stress. Proteomic analysis revealed a drought-induced higher abundance of EF-Tu protein in soybean plants. EF-Tu might activate heat-tolerance mechanisms in the soybean (Das et al. 2016).

Mulberry (*Morus alba*) is an economically important resource in traditional medicine. Its leaves, fruits and roots are the richest sources of a diverse range of flavonoids showing a variety of pharmacological properties (Chan et al. 2016). Proteomic profile of drought-stressed mulberry showed a total of 577 and 270 DEPs in leaves and roots, respectively. These DEPs were mostly enriched in the sucrose-related metabolic pathway in both stressed leaves and roots. Other proteins were assigned to multiple pathways, including carbon metabolism, photosynthesis, redox, secondary metabolism, and hormone metabolism (Liu et al. 2019a).

Date palm (*Phoenix dactylifera*) is the most popular and widely consumed fruit, which has also been used traditionally for medicinal purposes. Dates are enriched with numerous therapeutic bioactive phytochemicals with potent pharmacological properties (Younas et al. 2020). Drought conditions decrease date production. El Rabey et al. (2015) investigated the proteomic profile of date palms to identify DEPs involved in drought-stress tolerance. Drought stress or water deficit conditions could be mimicked by the application of polyethylene glycol to plants in tissue culture. Proteomic analysis identified 47 DEPs in leaves of drought-treated palm seedlings. Mass spectrometric analysis showed that levels of ATP synthase alpha and beta subunits and some of RubisCO fragments were significantly changed in dates upon drought stress treatment (El Rabey et al. 2015).

It is important to note that non-leaf green organs can make important contributions to photosynthate formation, especially under stress conditions. Pod wall proteomic studies of alfalfa (*Medicago sativa*) under drought stress conditions suggest that the pod wall is capable of conducting photosynthesis and reducing the photosynthetic loss from drought stress through the promotion of C4 pathway, ATP synthesis, and resistance ability (Wang et al. 2020a). Another study on PEG-mediated drought-stressed alfalfa identified 17 DEPs, which were classified into six functional categories such as defense response, energy metabolism, protein synthesis and degradation, oxidative stress, carbohydrate metabolism-associated proteins, and unknown proteins. An increased abundance of proteins related to carbohydrate metabolism and energy production was noted under PEG-mediated drought stress, suggesting a common mechanism of energy consumption in alfalfa during abiotic stresses (Ma et al. 2017). Muskmelon (*Cucumis melo*) is also commercially cultivated in arid and semi-arid regions that often suffer from limited rainfall. Peptide mass fingerprinting showed that drought increased the relative abundance of 38 DEPs while decreased10 DEPs in the leaves of drought-resistant muskmelon genotype SC-15. The identified DEPs were enriched in protein synthesis, photosynthesis, nucleotide biosynthesis, stress response, transcription regulation, metabolism, energy, and DNA binding, indicating that enhanced defense proteins and suppression of catabolic proteins are the drought stress-responsive strategies in muskmelon (Ansari et al. 2019).

Drought can also adversely affect the growth and development of oilseed crop plants such as *Helianthus annuus* (Sunflower). Flowers of this plant possess strong anti-inflammatory and antioxidant activity. Sunflower is a useful botanical source of protection against UVB-mediated skin aging (Hwang et al. 2019). Leaf proteomic approaches in sunflower plant identified significant upregulation of six general stress proteins, i.e. putative caffeoyl-CoA 3-O-methyltransferase, a fructokinase 3, a vegetative storage protein, a glycine-rich RNA binding protein, a CuZn-superoxide dismutase, and an unknown low molecular weight protein, indicating the induction of stress response proteins under drought conditions (Fulda et al. 2011). Researchers have also analyzed the peptide mass fingerprinting of another oil seed crop, namely, *Carthamus tinctorius* L. (safflower). Safflower seed oil contains significantly high levels of unsaturated fatty acids and phytochemicals and this oil has been traditionally used in China, Japan, and Korea as an anti-wrinkle agent to improve skin health (Jeong et al. 2020). Drought stress dramatically affects the yield of safflower. Protein profile indicated that a total of 72 DEPs upon drought stress and these proteins were mainly enriched in photosynthesis and carbohydrate, protein, defense, and energy metabolisms. Protein accumulation related to these metabolisms in drought-tolerant safflower cultivar (Remzibey-05) decreased under drought, while increased following re-watering. However, a sensitive cultivar (Linas) could not exhibit an effective performance and recovery, as compared with other safflower genotypes (Çulha Erdal et al. 2021). Rapeseed (*Brassica napus*) is the third leading source of vegetable oil which is sensitive to drought stress during early vegetative growth. Protein mass fingerprinting of a drought-sensitive and a drought-tolerant genotypes, and their F1 hybrid revealed that 35 DEPs related to metabolism, energy, disease/defense, and transport were decreased in the sensitive rapeseed line under drought stress. In the tolerant line, 32 DEPs related to metabolism, disease/defense, and transport were increased, while energy-related proteins were decreased. It was also found that tubulin beta-2 and heat shock protein 70 were decreased in the drought-sensitive line and F1 hybrid plants, while jasmonate-inducible protein and 20S proteasome subunit PAF1 were increased in the drought-tolerant line and F1 hybrids. Reduced levels of heat shock protein 70 and tubulin beta-2 in the drought-sensitive and hybrid F1 lines might be linked to the reduced growth of these lines under drought conditions (Mohammadi et al. 2012). In another study involving the rapeseed plant subjected to drought stress, leaf proteomic analyses indicated a significant change in the abundance of DEPs. Functional annotation of these DEPs identified that proteins associated with metabolism, protein folding and degradation, and signaling were decreased, while those related to energy (photosynthesis), protein synthesis, and stress and defense increased in response to drought stress (Koh et al. 2015).

Drought stress triggers a series of physiological and biochemical changes in tea plants (*Camellia sinensis*) and is a crucial limiting factor for tea yield and quality. It is speculated that flavonoids, lignin, and long-chain fatty acids play important roles in drought resistance. A study conducted to explore changes in proteins related to these three metabolic pathways in drought-stressed tea plants, revealed that 11 DEPs were upregulated and 100 DEPs were downregulated under drought stress. Functional annotation indicated that these DEPs were implicated in the biosynthesis of lignin, flavonoids, and long-chain fatty acids, thus supporting the fact that tea plants might improve drought resistance by inhibiting the accumulation of synthases related to lignin, flavonoids, and long-chain fatty acids (Gu et al. 2020). Another tea leaf proteomic study suggests that mild drought stress may promote polyphenol biosynthesis, while severe drought stress leads to inhibition. The expression of lipoxygenase and short-chain dehydrogenase increased during slight drought stress and some volatile metabolite pathways were enriched, indicating that drought stress might affect the tea aroma (Wang et al. 2017b). Proteomic mass fingerprinting of leaves of sea buckthorn (*Hippophae rhamnoides*) identified 51 DEPs in leaves with functions related to epigenetic modification, post-transcriptional modification as well as normal metabolism, photosynthesis, signal transduction, antioxidative systems, and responses to stimuli (He et al. 2016).

Peanut (*Arachis hypogaea*), a commercially important crop used mainly for oil production, is an excellent source of bioactive compounds like resveratrol, phenolic acids, flavonoids, and phytosterols that confer health benefits (Arya et al. 2016; Azad et al. 2020). Water stress predisposes peanut plants to fungal infection resulting in pre-harvest aflatoxin contamination. Leaf proteomics of two peanut cultivars with diverse drought tolerance characteristics showed that DEPs were involved in metabolism, defense, and cellular biogenesis and some DEPs were related to playing a role as cryoprotectants through signal transduction, which was induced in drought-tolerant cultivar following water stress (Katam et al. 2016).

Highland barley contains a wide range of bioactive carbohydrates and polyphenols, minerals, vitamins, phenolic, flavonoids, and β-glucan. The unique composition of highland barley contributes to its various health benefits (Obadi et al. 2021). Drought is a severe environmental constraint to barley yield. Barley seedlings proteomics revealed that DEPs related to metabolism and photosynthesis decreased in the sensitive genotype and increased in tolerant genotype when exposed to drought. Abundance of DEPs related to amino acid synthesis and degradation-related proteins increased in the tolerant genotypes, suggesting that metabolism and energy related proteins might play a significant role in drought-stress tolerance of barley seedlings (Kausar et al. 2013). A comparative leaf proteomic analysis in drought tolerant (GN-3074) and drought sensitive (GN-2032) genotypes of soybean identified 20 DEPs, which are mostly implicated in photosynthesis and oxidative stress defense system. The up-regulation of several photosynthetic proteins and also a high abundance of oxidative stress defense proteins in drought-tolerant genotype than in the sensitive genotype suggest that effective photosynthetic machinery and defense against oxidative stress are key for drought tolerance in drought-tolerant soybean (Yahoueian et al. 2021).

Drought is the primary limitation to plant growth and yield in agricultural systems. Cucumber (*Cucumis sativus*) is one of the most important vegetables that has little tolerance for water deficit. Cucumber seeds have been traditionally used for diabetes mellitus treatment (Minaiyan et al. 2011). Elevated carbon dioxide (CO_2) can alleviate the negative consequences of drought stress to cucumber seedlings. Proteomics analyses to unveil the underlying molecular mechanisms in CO_2-mediated alleviation of drought stress, indicated that in cucumber seedlings exposed to drought, elevated CO_2 increased the abundance of several DEPs, which are related to stress and defense response, redox homeostasis, and metabolic pathways (Cui et al. 2019). In another proteomic study, a total of 320 and 246 proteins exhibited significant abundance in response to drought and recovery, respectively, in cucumber plant. Drought and recovery regulated different DEPs, allowing plants to adapt to environmental stress or restore growth. However, 63 co-regulated proteins were shared between drought and rewatering conditions in cucumber. These DEPs are functionally categorized to regulatory processes including the biosynthesis of secondary metabolites and the glutathione metabolism pathway. This suggests that glutathione metabolism being an important antioxidant strategy, may be one of the main responses to drought in cucumber plants (Du et al. 2019).

Ginger (*Zingiber officinale*) rhizome is an important widely used kitchen spice with myriad of health benefits (Ali et al. 2008). Differential proteomics was used to study the drought stress response of ginger, especially with respect to photosynthetic electron transport. It brought out expression of proteins related to photosystem I and cytochrome b6f, suggesting an enhanced drought tolerance by enhancing the cyclic electron flow, which might improve photosynthetic efficiency under drought (Lv et al. 2020).

Finger millet (*Eleusine coracana*), an important staple food in India, is a minor cereal known for several health benefits, which are attributed to its polyphenol and dietary fiber contents (Devi et al. 2014; Divya et al. 2020). It exhibits high drought tolerance. The transcriptomic and proteomic approaches revealed that the drought tolerance-related DEPs were be enriched in hydrolase activity, glycosyl bond formation, oxidoreductase activity, carbohydrate-binding, and biosynthesis of unsaturated fatty acids (Li et al. 2021).

Humulus lupulus (Hops) contains numerous dietary phytochemicals potent biological and pharmacological properties (Kyrou et al. 2017; Lin et al. 2019). In hop plants subjected to drought stress, leaf proteomic mass fingerprinting identified 28 DEPs. Most of these DEPs, which are involved in photosynthesis (41%) and sugar metabolism (33%), were downregulated. Other DPEs were found to be related to nitrogen metabolism, ROS-related pathway and others (Kolenc et al. 2016).

Pinus halepensis (Aleppo pine) is a Mediterranean ethnomedicinal plant with numerous traditional applications including neuroprotective functions (Postu et al. 2019; El Omari et al. 2021). Drought is also one of the main stress factors that can affect the growth, survival, and productivity of forestry trees like Aleppo pine. Therefore, proteomic investigations might help to establish distinctive markers to predict drought tolerance in *Pinus halepensis* that could be reliably used in reforestation programs in drought-prone areas. The proteomic profile identified two heat shock proteins and an enzyme related to methionine biosynthesis in drought-tolerant seedlings but not in drought-sensitive seedlings (Taïbi et al. 2017). Olive fruit is the richest source of olive oil. The phenolic compounds present in olive leaves, especially the oleuropein, are associated with antioxidant, antihypertensive, hypoglycemic, hypocholesterolemic, and cardioprotective activity (Vogel et al. 2014; Gorzynik-Debicka et al. 2018). Olive is an economically important crop for the Mediterranean basin, where extended drought occurs. To cope with the adverse environmental conditions, the olive plant has developed a series of stress-tolerant mechanisms to grow better. A shotgun proteomic approach identified that the majority of DEPs (16 in number) are involved in photosynthesis in the drought-stressed leaves of olive. In addition, other DEPs identified were enriched in nitrogen metabolism, protein storage and energy (Ben Abdallah et al. 2018).

Photosynthesis and carbon metabolism are severely affected by drought stress. MS proteomic approach could identify 47 DEPs differentiating tolerant genotype from sensitive genotype of barley. The majority of the DEPs were involved in the dark phase of photosynthesis and antioxidant defense, pointing to the fact that high drought tolerance in barley is regulated by the rate of protein synthesis and antioxidant activity level (Gołębiowska-Pikania et al. 2017). Researchers have also revealed 121 and 182 drought-responsive proteins in barley leaves and roots, respectively. As expected, a majority of the identified drought-responsive DEPs were associated with photosynthesis, carbon metabolism and defense mechanisms in both roots and leaves. Besides, the accumulation of DEPs related to phenylpropanoid metabolism, a process that was formerly unassociated with drought response was also detected (Chmielewska et al. 2016). A similar proteomic profile, indicating the abundance of DEPs in photosynthesis and defense, was observed

Phaseolus vulgaris (common bean) cultivars cultivated under drought conditions. Although maintaining common bean plants under drought stress is challenging, proteomic approaches help to identify the drought stress responsive markers to improve bean production (Zadražnik et al. 2013).

Quercus ilex L. (Holm oak) is one of the most commonly used plants in folk medicine in the Mediterranean region to treat several human ailments such as gastrointestinal disorders as well as hemorrhages and skin and throat infections, among (Karioti et al. 2011; Castejón et al. 2019). A total of 14 differentially protein spots were observed when comparing the leaf protein signature profile of control to the drought-hit and recovered olive plants. These DEPs belong to the photosynthesis, carbohydrate and nitrogen metabolisms, and stress-related protein functional categories (Echevarría-Zomeño et al. 2009).

Setaria italica is commonly known as Foxtail milletIt has been used in folk medicine to treat inflammation, pain, arthralgia and many neurological disorders (Dong et al. 2019). It exhibits significant abiotic stress-tolerance characteristics. A quantitative proteomic analysis after subjecting foxtail millet seedlings to drought conditions identified a total of 321 DEPs. Of these, 252 DEPs were up-regulated and 69 were down-regulated proteins. Functional analysis indicates that a majority of these DEPs were implicated in stress and defense responses, photosynthesis, carbon metabolism, ROS scavenging, protein synthesis. DEPs implicated in fatty acid and amino acid metabolism, polyamine biosynthesis, hormone metabolism, and cell wall modifications were also identified, pointing to the fact that physiological and metabolic processes might function in coordination to maintain dynamic homeostasis, and hence the drought-stress tolerance in Foxtail millet (Pan et al. 2018).

Selaginella tamariscina is a traditional medicinal plant used to treat various human diseases, including cancer (Lee et al. 2017). It is one of the most primitive vascular resurrection plants that has evolved unique mechanisms of desiccation tolerance and can tolerate water deficit conditions. To better understand the mechanism of desiccation tolerance, a shotgun proteomics study was conducted and the protein signature identified 103 DEPs. Hierarchical clustering analysis revealed that a majority (83%) of these DEPs were down-regulated upon dehydration and are mainly involved in photosynthesis, carbohydrate and energy metabolism, stress and defense, protein metabolism, signaling, membrane/transport, cell structure, and cell division. This study provides strong evidence that cell structure modification, photosynthesis reduction, and antioxidant system activation are essential for desiccation-stress tolerance in *S. tamariscina* (Wang et al. 2010).

A comprehensive understanding of seed metabolism and the effects of drought stress on seeds will enhance our ability to improve seed quality and yield through molecular breeding programs. In a quantitative proteomics approach, a pod-specific protein-signature profile was generated from pea plants subjected to water-stress conditions. Researchers identified 93 significantly and differentially expressed proteins between well-watered and drought-stressed seeds. These DEPs were mapped mostly to three pathways such as glycolysis, sucrose and starch metabolism, and fatty acid metabolism, suggesting that alterations in the abundance of DEPs related to these three pathways are possibly linked to the decline in pod yield and biomass, and the reduced germination, vigor, and seed-membrane integrity under drought stress conditions (Kottapalli et al. 2013).

A shotgun proteomic analysis of the proteome of grapevine leaves subjected to prolonged drought stress led to the detection of many proteins associated with carbohydrate and energy metabolism, mostly connected to the pathways of glycolysis and photosystem II protein components. It was found that drought decreased the accumulation of key enzymes of the glycolysis pathway and increased the accumulation of many proteins associated with photosynthesis and photorespiration. In addition, drought stress caused increased accumulation of kynurenine formamidase, the only protein assigned to the amino acid metabolism. DEPs, especially those with increased accumulation during drought stress, may be responsible for the adaptation of grapevine to drought (Król and Weidner 2017).

4 Medicinal Plant Proteomics in Response to Light/UV Stress

Light is a well-known physical factor that can affect metabolite production in plants. There are three types of Ultraviolet (UV) radiation: UVA, UVB, and UVC. Of these, UVC does not affect life on the Earth much because its rays are blocked by the ozone layer. UV-A is less harmful than UV-B. Plants are inevitably exposed to UV-B radiation, which modifies the architecture and structure of plants. UV-B radiation acts as an elicitor to enhance the production of secondary metabolites in medicinal plants (Fischbach et al. 1999; Binder et al. 2009). The UV acclimation is the result of biochemical and physiological processes, such as enhancement of the antioxidant enzymatic system and accumulation of UV-absorbing phenolic compounds (e.g. flavonoids). Plants respond to ultraviolet stress by inducing self-defense through the regulation of specific gene family members. The light/UV-induced proteomic changes have been studied in several medicinal plants including *Prunus persica* (Peach) (Zhou et al. 2020), *Lonicera japonica Thunb* (Zhang et al. 2013; Zhu et al. 2017), *Euphorbia kansui* (Zhao et al. 2019), *Hordeum vulgare* (Kaspar et al. 2010), *Chrysanthemum* (Yao et al. 2015; Hong et al. 2019), *Artichoke (Cynara scolymus)* (Falvo et al. 2012), *Ginkgo biloba* (Zheng et al. 2015), *Catharanthus roseus* (Zhong et al. 2019), *Mirabilis himalaica* (Gu et al. 2018), *Taxus chinensis* (Zheng et al. 2016), *Mahonia bealei* (Zhang et al. 2014a; Zhu et al. 2021), *Glycine max* (Xu et al. 2008; Jiao and Gu 2019), *Mangifera indica* (George et al. 2016), *Pinus radiate* (Pascual et al. 2016, 2017), *Clematis terniflora* (Gao et al. 2016; Yang et al. 2016, 2017) (Table 3).

As UV stress negatively affects the plant productivity in forest species, there is a need to understand the UV-induced stress-response mechanism in forest plants. This would help in designing new strategies to maintain future forest species with increased productivity. Proteomic studies have revealed that UV-induced phototoxicity is prevented by reducing photosystem activity and the electron transfer chain,

Table 3 A list of proteomic studies conducted in medicinal plants in response to Light stress

Plant name	Part studied	Stress treatment	Proteomics approach	References
Prunus persica	Peach fruit	UV-C light (254 nm)	Nano-LC-MS/MS	Zhou et al. (2020)
Lonicera japonica	Flower buds	UV-B light (306 nm)	2DE coupled with MALDI-TOF/TOF-MS/MS	Zhu et al. (2017)
Lonicera japonica	Flower buds	UV-A light (365 nm) or UV-B light (306 nm)	2DE in combination with MALDI-TOF/TOF MS	Zhang et al. (2013)
Euphorbia kansui	Latex	UV-B	iTRAQ coupled 2D-LC-MS/MS	Zhao et al. (2019)
Hordeum vulgare	Leaves	UV-B light	2DE coupled with MALDI-TOF-MS and nano LCESI-Q-TOF MS/MS	Kaspar et al. (2010)
Chrysanthemum	Flowers	UV-B radiation	2DE coupled with MALDI-TOF-MS	Yao et al. (2015)
Chrysanthemum morifolium	Capitulum of chrysanthemum	Light stress using fluorescent lamp	iTRAQ based LC–MS/MS analysis	Hong et al. (2019)
Cynara scolymus	Globe artichoke	UV-C light (254 nm)	2D-DIGE coupled with nano LC-nanospray MS/MS	Falvo et al. (2012)
Ginkgo biloba	Whole leaf and chloroplasts	UV-B	2DE coupled with MALDI TOF/TOF-MS	Zheng et al. (2015)
Catharanthus roseus	Leaves	UVB	nanoLC-MS/MS	Zhong et al. (2019)
Mirabilis himalaica	Radix samples	UV-B radiation	iTRAQ/TMT labeling coupled with strong cation exchange (SCX) fractionation and LC-MS/MS	Gu et al. (2018)
Taxus chinensis	Leaves	UV-A radiation	NanoLC–MS/MS	Zheng et al. (2016)
Mahonia bealei	Leaves	UV-B radiation and incubation under darkness (UV-D) (binary stress)	2DE coupled with MALDI-TOF/TOF	Zhang et al. (2014a)
Mahonia bealei	Leaves	UV-B irradiation and induction under dark (UV-D) (binary stress)	Nano LC-MS/MS	Zhu et al. (2021)

(continued)

Table 3 (continued)

Plant name	Part studied	Stress treatment	Proteomics approach	References
Pinus radiata	Isolated nuclei from needles	UV light (250–350 nm lamp peaking at 300 nm)	1-D SDS-PAGE coupled with 1D nano-flow LC-MS/MS	Pascual et al. (2016)
Pinus radiata	Needles	UV light (250–350 nm; peaking at 300 nm)	1-D SDS-PAGE coupled with nano-flow LC-MS/MS	Pascual et al. (2017)
Clematis terniflora	Leaves	High levels UV-B (HUV-B) irradiation followed by incubation in dark (UV-D) (binary stress-UV-B+D)	Nano LC-MS/MS	Yang et al. (2017)
Clematis terniflora	Leaves	After being irradiated by UV-B the plants were incubated in the dark	2DE coupled with MALDI-TOF-MS/MS	Gao et al. (2016)
Clematis terniflora	Leaves	After UV-B irradiation, plants were incubated in dark	Nano LC-MS/MS	Yang et al. (2016)
Glycine max	Soybean sprouts	UV-B light	ITRAQ labeling and fractionation by high pH RP chromatography and nano LC-MS/MS	Jiao and Gu (2019)
Glycine max	First trifoliate leaves	Solar VU-B	2D PAGE coupled with MALDI-TOF	Xu et al. (2008)
Mangifera indica	Mango fruit pulp	UV light (254 nm)	2DE coupled with triple TOF-MS	George et al. (2016)

together with the accumulation of photoprotectors and photorespiration. In addition, the primary metabolism was modulated to alleviate ROS-mediated oxidative stress under UV exposure. Furthermore, protein kinases and proteases related to signaling, coordination, and regulation of UV stress responses were also identified in response to UV stress, suggesting that the role of a complex molecular network is to improve the UV-stress tolerance in pine trees (Pascual et al. 2017). Understanding the nuclear proteome dynamics is essential for understanding the gene expression and regulation of plant physiology. Researchers have characterized dynamics of nuclear proteome triggered by UV irradiation and suggested that the nuclear events are involved in stress response and adaption to UV. The upregulated DEPs were related to protein, RNA and amino acids metabolisms and the downregulated DEPs were related to

DNA metabolism, possibly linked to active protein re-modeling and DNA damage, respectively. Besides, 33 transcription factors were identified, which were correlated to stress-responsive mechanisms like an increased accumulation of photoprotective pigments and reduced photosynthesis, pointing them as strong candidate biomarkers for breeding programs aimed to improve UV resistance of pine trees (Pascual et al. 2016).

Peach (*Prunus persica*) fruit is edible with a high nutritional value and benefits human health due to its phenolic content (Elshamy et al. 2019; Mihaylova et al. 2021). Ultraviolet-C (UV-C) irradiation is known for prolonging the shelf life of many fruits by regulating different pathways. Proteomics analysis could provide a better understanding of the role of UV-C treatment in peach fruit during cold storage. DEPs identified were largely related to carbohydrates and secondary metabolites. Proteomics and transcriptomics together indicated that changes of sugars and acids were associated with the expressions of invertase, sucrose synthase, fructokinase, malate dehydrogenase and citrate synthase. UV-C irradiation promoted the synthesis of phenols, flavonoids and anthocyanins by up-regulating expressions of phenylalanine ammonia-lyase, 4-coumarate-CoA ligase, chalcone synthase, dihydroflavonol 4-reductase and UDP-glucose:flavonoid glucosyltransferase (Zhou et al. 2020).

Lonicera japonica Thunb. (Caprifoliaceae) is a widely used traditional Chinese herbal medicine. It is suggested to promote healthy aging and have a potency to design therapeutics for age-related diseases (Yang et al. 2018). A comparative quantitative proteomic approach was used to investigate the molecular processes involved in *L. japon*ica development from buds to flowers exposed to UV radiation. A total of 54 DEPs were identified, of which 42 DEPs were upregulated and 12 downregulated. The upregulated DEPs were related to glycolysis, TCA/organic acid transformation, major carbohydrate metabolism, oxidative pentose phosphate, stress, secondary metabolism, hormone, and mitochondrial electron transport and are upregulated during the flower-opening process after UV exposure, suggesting that the *L. japonica* bud stage may be a more optimal time to harvest than the flowering stage for using it as medicine (Zhu et al. 2017). UV exposure can enhance the levels of major bioactive compounds (caffeoylquinic acids and iridoids) in *L. japonica*. This was confirmed by the protein mass fingerprinting approach, which identified a total of 75 DEPs that were enhanced in response to UV stress and these DEPs were functionally annotated to involve in a wide range of molecular processes including the secondary metabolites (caffeoylquinic acids and iridoids) biosynthetic-related proteins, photosynthesis, carbohydrate and energy metabolism, stress, DNA, transport-related proteins, lipid metabolism, amino acid metabolism, and cell wall synthesis (Zhang et al. 2013).

The root of *Euphorbia kansui* is a well-known traditional Chinese herbal medicine, known to possess multiple pharmacological activities, including diuretic, purgation, and antitumor effects (Shen et al. 2016). All parts of *E. kansui* contain white latex comprising of many proteins with various biological functions. A shotgun proteomic approach identified proteins related to terpenoid biosynthesis-related proteins, 14-3-3 protein, V-ATPase and lysosomal enzymes, which increased under UV-B treatment, indicating that partial cytoplasmic degradation is positively correlated to secondary metabolite synthesis in the development of *E. kansui* laticifers. Besides, UV-B radiation can increase plant resistance by promoting laticifer development in *E. kansui* (Zhao et al. 2019).

A label-free MS-based approach detected 15 (11 up-regulated and four down-regulated) DEPs in the epidermis of leaves of *Hordeum vulgare L* exposed to UV-B. Functional annotation of these DEPs revealed that a majority of the DEPs were implicated in oxidative stress and others were related to primary metabolism proteins involved in the supply of precursors for secondary metabolites (Kaspar et al. 2010).

Chrysanthemum, famous as the Queen of the East, has multiple health benefits (Shahrajabian et al. 2019). A shotgun proteomic approach in flowers exposed to UV-B identified 19 DEPs that are assigned to photosynthesis, respiration, protein biosynthesis and degradation and defense. An overall assessment using biochemical and differential proteomic data revealed that UV-B radiation could affect biochemical reactions and promote secondary metabolism processes in postharvest flowers (Yao et al. 2015). In another study, researchers studied the relationship between light intensity and accumulation of anthocyanin pigments in chrysanthemum. The flower color of chrysanthemum is mainly attributed to the accumulation of anthocyanins. Even though light can affect anthocyanin biosynthesis, the deep molecular mechanism remains elusive. Therefore, researchers used a quantitative proteomic approach and identified 160 DEPs in the chrysanthemum flower. Pathway enrichment and the gene expression patterns indicated that most of the proteins involved in the anthocyanin biosynthetic pathway were downregulated after shading, while five light-harvesting chlorophyll a/b-binding proteins were initially downregulated after shading, and their expressions were enhanced with the capitulum development thereafter. This suggests that light induced the anthocyanin biosynthesis in chrysanthemum (Hong et al. 2019).

Cynara scolymus (Artichoke) leaf extract is one of the few herbal remedies complemented with clinical and experimental trials. It is well known for its antioxidant, choleretic, hepatoprotective, and lipid-lowering effects (Ben Salem et al. 2015). Proteomic fingerprinting of globe artichoke exposed to UV-C identified 119 DEPs, which are mostly chloroplast located and the majority of them are implicated in photosynthesis, sugar metabolisms, protein folding, and abiotic stress. The identification of UV-C-responsive proteins may provide insight about the molecular mechanisms underlying the plant responses to UV stress (Falvo et al. 2012).

Ginkgo biloba is a traditional medicinal plant that has been widely used as a phytomedicine in the prevention and treatment of various human diseases. Antioxidant flavonoids and terpene lactones are the major bioactive components, e.g. ginkgolic acids (GAs) with strong allergenic properties. In a comparative proteomic approach analysis, upregulation of DEPs related to antioxidants and stress-responsive proteins in leaves exposed to UV-B radiation, was observed. However, UV-B radiation reduced the rate of photosynthesis. With high UV absorption properties and antioxidant activities, the flavonoids were likely highly induced as protective molecules, following the UV-B irradiation (Zheng et al. 2015).

Catharanthus roseus (or *Vinca rosea*) is a source of anti-cancer compounds (Loh 2008). A phosphoproteomic technique was used to explore the mechanisms involved in the biosynthesis of secondary metabolites in *C. roseus* exposed to UVB radiation. Phosphoproteins such as calmodulin, calcium-dependent kinase, and heat shock proteins increased. Phosphoproteins related to protein synthesis/modification/degradation and signaling were intensively changed. Phosphoproteomic and immunoblot analyses indicated that proteins related to glycolysis and the ROS-scavenging system were activated upon UV-B exposure. These results suggest that UVB radiation activates the calcium-related pathway and ROS-scavenging system in *C. roseus*, leading to the upregulation of proteins that are responsible for redox reactions in secondary metabolism and are important for the accumulation of secondary metabolites in *C. roseus* under UVB radiation (Zhong et al. 2019).

Mirabilis himalaica (Edgew.) Heimerl (Nyctaginaceae) is an important medicinal plant, widely used in traditional Tibetan folk medicine for the treatment of kidney diseases, edema, periorbital puffiness, low back pain, arthralgia, inflammation, and anti-cancer activities (Pan-pan 2012; Zhou et al. 2017; Li et al. 2018b). MS-based proteomic approaches are useful for the identification of molecular mechanisms by which environmental factors affect the biosynthesis of secondary metabolic components. A comparative proteomic approach identified 116 DEPs in *M. himalaica* between UV-B treatment and control plants. Most of the DEPs were enriched in plant hormone signal transduction and phosphatidylinositol signaling system pathways. Most importantly, UV-B radiation caused a significant increase in the expression of two chalcone synthase enzymes that are involved in the biosynthesis of rotenoids, the representative medicinal component of *M. himalaica* (Gu et al. 2018).

Taxus chinensis, commonly called the Chinese yew, serves as one of the commercial sources for the anti-cancer medicine paclitaxel which is well known for its effective clinical activity (Li et al. 2008). Proteomics analyses in leaves and chloroplasts showed that DEPs mainly related to photosynthesis, glycolysis, secondary metabolism, stress, and protein synthesis, degradation, and activation-related systems were altered under UV-A radiation. Four glycolysis-related key enzymes increased and thereby enhanced glycolysis, which provided precursors for secondary metabolism. UV-A treatment also enhanced the expression of two key enzymes of paclitaxel biosynthesis, pointing to the fact that a short-term high dose of UV-A radiation could stimulate the plant stress defense system and paclitaxel production as a stress-response strategy in *Taxus chinensis* (Zheng et al. 2016).

γ-aminobutiric acid (GABA), a non-protein amino acid, accumulates in plants especially in legumes and is beneficial for health when consumed in diet. The inositol 1,4,5-trisphosphate (IP3) is involved in the signaling pathways and is considered to be functional component in human health. UV-B radiation can induce accumulation of γ-aminobutiric acid (GABA), inositol 1,4,5-trisphosphate (IP3), and abscisic acid (ABA) in soybean. Findings from the quantitative proteomic approaches suggest that UV-B treatment effectively regulated proteins involved in GABA biosynthesis, inositol phosphate metabolism, and ABA biosynthesis and signal transduction pathways. DEPs related to GABA biosynthesis are glutamate synthase, glutamate decarboxylase, methionine synthetase, 5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase, and aminoaldehyde dehydrogenase. DEPs associated with the inositol phosphate metabolism pathway are phosphoinositide phospholipase C, purple acid phosphatase, and inositol polyphosphate 5-phosphatase. DEPs related to ABA biosynthesis and signal transduction are 9-*cis*-epoxycarotenoid dioxygenase, abscisic-aldehyde oxidase, SNF1-related protein kinase, protein phosphatase 2C, guanine nucleotide-binding protein, and calreticulin-3 (Jiao and Gu 2019). Combined UV-B radiation and dark treatment could also increase the GABA content in *Clemantis terniflora*, a chinese medicinal plant (Yang et al. 2016). Researchers also showed that UV-B significantly affects the proteome of two isoloines of soybean one with moderate levels of flavonoids (Clark) and the other with reduced levels of flavonoids (magneta). Leaf proteomic mass fingerprinting analysis identified 67 DEPs between two lines and these DEPs were involved in metabolism, energy, protein destination/storage, protein synthesis, disease/defense, transcription, and secondary metabolism. A majority of DEPs were altered by UV-B in the magenta line than in the Clark line, indicating that high levels of flavonoids lead to a reduction in UV-B sensitivity at the proteomic level (Xu et al. 2008).

Mangifera indica L. is a fruit tree of tropical regions, also used as medicine by indigenous people. Its active constituents are present in stem bark, leaves, heartwood, roots and fruit (Batool et al. 2018). Postharvest treatment of mango fruits could increase the fruit quality and shelf-life. Proteomic profiles of UV-C exposed post-harvested mango fruits identified 20 DEPs, many of which were related to stress response (40%), energy and metabolism (45%), and ripening and senescence (15%), suggesting the potential effects of the UV-C on the extension of shelf-life of mangoes (George et al. 2016).

Mahonia bealei is a Chinese medicinal plant rich in alkaloids exerting several pharmacological properties. However, plants generally have trace amounts of alkaloids, therefore it is essential to unravel the mechanism of biosynthetic pathways involved in the alkaloid induction under UV-B radiation. Comparative proteomics approach strongly helps to address the underlying proteome variations that accounted for the alkaloid induction under binary stress (UV-B irradiation and dark incubation). In total, 13 DEPs were identified by shotgun proteomic approaches, of these 4 DEPs were found to be upregulated and 9 DEPs downregulated. These DEPs were functionally related to photosynthetic system, tricarboxylic acid/organic acid transformation, nitrogen metabolism, amino acid metabolism, redox, and stress. Besides, combinatorial peptide ligand libraries (CPLL) technology enriched the abundance of the secondary metabolism related proteins such as sanguinarine reductase, isoflavone reductase homolog and phenylalanine ammonia lyase. Of these, sanguinarine reductase is implicated in the benzylisoquinoline alkaloid metabolism, indicating that beanzylisoquinoline alkaloids levels were increased in response to UV-B exposure (Zhang et al. 2014a). The combined UV-B and dark treatment (UV+D) increased the alkaloid levels in the leaves of *M. bealei*. Proteomic analysis revealed that DEPs related to tricarboxylic acid cycle, transport and signaling varied greatly only under the UV + D treatment. Notably, the levels of calmodulin and ATP-binding cassette transporter increased in response to binary stress. Calmodulin is involved in calcium signaling and the ATP-binding cassette transporter is involved in the transport of endogenous secondary metabolites including alkaloids, suggesting active calcium signaling crosstalk with ROS-induced biosynthesis of protoberberine alkaloids in *M. bealei*, and then transported to leaves to protect the plant from stresses. It can also be suggested that the biosynthesis of flavonoids and protoberberine alkaloids is a stress-responsive strategy to alleviate the oxidative stress caused by the UV + D treatment (Zhu et al. 2021). The combined UV-B and dark treatment was found to increase the secondary metabolism pathways in *Clematis ternifolia* (Yang et al. 2017). Proteomic approaches have also showed that, in *Clematis ternifolia*, a high-level UV-B radiation upregulated the aminoacid metabolism, especially the shikimated pathway, which is involved in indole alkaloid biosynthesis, strongly suggesting the induction of alkaloid biosynthesis as a tolerance strategy against UV-B stress (Gao et al. 2016).

5 Medicinal Plant Proteomics in Response to Salt Stress

Salinity is one of the major threats to sustainable agriculture that globally decreases plant production by impairing germination, growth, photosynthesis, transpiration, and stomatal conductance. Salt environment leads to cellular dehydration, which causes osmotic stress and removal of water from the cytoplasm, resulting in a reduction of the cytosolic and vacuolar volumes (Ramakrishna and Ravishankar 2011). Salt stress often creates both ionic as well as osmotic stress in plants, resulting in increase or decrease of specific secondary metabolites in plants (Parida and Das 2005; Daneshmand et al. 2009). Many salt-tolerant species accumulate methylated metabolites, which play crucial dual roles as osmoprotectants and as radical scavengers (Parida and Das 2005). Plants respond to salinity by modulating various morpho-physiological, anatomical and biochemical traits by regulating ion homeostasis and compartmentalization, antioxidant machinery, and biosynthesis of osmoprotectants and phytohormones (Arif et al. 2020). Proteomics technologies provide valuable information toward understanding the complex plant salt-tolerance mechanisms in photosynthesis, ROS scavenging, ion homeostasis, osmotic modulation, signaling transduction, transcription, protein synthesis/turnover, cytoskeleton dynamics, and cross-tolerance to different stress conditions (Zhang et al. 2012). We briefly discuss here the proteomics changes caused by salt stress in medicinal plants (Table 4).

Both drought and salt stress can simultaneously occur in the field and severely affect plant physiology. A shotgun quantitative proteomic approach was performed in mulberry plants that were subjected to a combination of both salt and drought stress. In leaves and roots, DEPs identified were greatly altered and were functionally annotated to multiple pathways, including carbon metabolism, photosynthesis, redox, secondary metabolism, and hormone metabolism. Among these pathways, the sucrose-related metabolic pathway was distinctly enriched in the stressed leaves

Table 4 A list of proteomic studies conducted in medicinal plants in response to salt stress

Plant name	Part used	Treatment	Proteomic approach	References
Morus alba	Seedlings	200 mM NaCl Combined with drought stress	TMT-based labeling and high PH reverse phase (hpRP) fractionation and NanoLC-MS/MS analysis	Liu et al. (2019a)
Brassica juncea	Chloroplasts isolated from leaves	150 mM sodium chloride	2DE coupled with MALDI-TOF-MS	Yousuf et al. (2016a)
Brassica juncea	Leaves	150 mM sodium chloride	2DE coupled with MALDI-TOF MS	Yousuf et al. (2016a, b)
Robinia pseudoacacia	Leaves	250 mM (moderate salt stress) or 500 mM NaCl (high salt stress)	2DE coupled with *MALDI-TOF-MS*	Meng et al. (2016)
Robinia pseudoacacia	Leaves	500 mM NaCl	2DE coupled with *MALDI-TOF-MS*	Wang et al. (2013b)
Brassica napus	Seedlings leaves	200 mM NaCl	2DE coupled with MALDI-TOF/TOF-MS	Jia et al. (2015)
Brassica napus	Leaves	175, and 350 mM NaCl	2DE coupled with MALDI TOF/TOF MS	Bandehagh et al. (2011)
Brassica napus	Roots	150 and 300 mM NaCl	MALDI)-time-of-flight (TOF)/MS analysis	Kholghi et al. (2019)
Beta vulgaris	Leaf and roots	NaCL 300 mM	iTRAQ labeling nano-LC-ESI-MS/MS analysis	Li et al. (2020b)
Beta vulgaris	Roots or leaves of seedlings	280 mM NaCl	iTRAQ labeling, and high pH RPLC Separation and LC-MS/MS	Wang et al. (2019)
Beta vulgaris	Leaves	200 mM and 400 mM NaCl	LC-MS/MS	Yu et al. (2016)
Beta vulgaris	Roots and leaves	900 mM NaCl	2DE coupled with nanoESI MS/MS	Yang et al. (2012a)
Citrus reshni	Leaves	50 mM NaCl	2DE coupled with LC-MS	Podda et al. (2013)
Vigna unguiculata	Leaves	75 mM NaCl	2DE coupled with LC-ESI-MS/MS	de Abreu et al. (2014)
Bruguiera gymnorrhiza	Leaves	200 mM NaCl and 500 mM NaCl	2DE coupled with LC-MS/MS	Zhu et al. (2012)
Helianthus tuberosus	Roots	50, 150 or 250 mM NaCl	iTRAQ labeling and LC–ESI–MS/MS	Zhang et al. (2018a)
Sorghum bicolor	Leaves	100 mM NaCl	2D PAGE in combination with MALDI-TOF-TOF MS	Ngara et al. (2012)
Saccharum	Leaves	100 mM of NaCl	2DE coupled with MALDI-TOF/TOF MS	Murad et al. (2014)

(continued)

Table 4 (continued)

Plant name	Part used	Treatment	Proteomic approach	References
Saccharum spp.	Roots	200 mM NaCl	2DE coupled with UPLC-ESI-Q-ToF-MS	Pacheco et al. (2013)
Saccharum spp.	Shoots	180 mM NaCl	ESI-LC-MS/MS	Passamani et al. (2017)
Glycine max	Leaves, hypocotyls and roots	20, 40 or 80 mM NaCl	2DE coupled with MALDI-TOF-MS	Sobhanian et al. (2010b)
Glycine max	Leaves and roots	200 mM NaCl	iTRAQ coupled with reverse phase NanoLC-MS/MS	Ji et al. (2016)
Glycine max	Hypocotyls and roots	100 mM NaCl	2D PAGE coupled with ESI-Q/TOF-MS/MS	Aghaei et al. (2009)
Glycine max	Germinated seeds	100 mmol/L NaCl	2D PAGE coupled with MALDI-TOF-MS	Xu et al. (2011)
Solanum lycopersicum	Roots	100 mM NaCl	2DE coupled with MALDI-TOF/MS	Manaa et al. (2011)
Solanum lycopersicum	Fruits	40.8 mM NaCl	2D PAGE-nano LC-MS/MS	Manaa et al. (2013)
Hordeum vulgare	Roots	100 mM NaCl	2DE coupled with MALDI-TOF/TOF	Mostek et al. (2015)
Cajanus cajan	Root and shoot	250 mM NaCl	2DE coupled with MALDI-TOF/TOF-MS	Awana et al. (2020)
Medicago sativa	Leaves	100 mM NaCl	iTRAQ labeling and high pH reversed-phase (HpRP) chromatography fractionation and LC-MS/MS	Gao et al. (2019a)
Medicago sativa	Seedlings	200 mmol L^{-1} NaCl	2D PAGE coupled with MALDI-TOF-MS/MS	Gao et al. (2019b)
Medicago sativa	Roots	300 mM NaCl	2DE coupled with MALDI-TOF/TOF-MS	Long et al. (2016)
Cucumis sativus	Roots	75 mM NaCl and 0.8 mM Put	2DE coupled with MALDI-TOF/TOF-MS	Yuan et al. (2016b)
Cucumis sativus	Seedling roots	50 mM NaCl	MALDI-TOF MS and LC–ESI-MS/MS	Du et al. (2010)
Carthamus tinctorius	Leaves	200 mM NaCl with or without SA (1 mM) or PEN (15 mg L^{-1})	2DE and then the protein spots were excised from gels, and were analyzed with progenesis Samespots and Melanie (8.0) software	Shaki et al. (2020)
Aeluropus lagopoides	Shoots	450 mM NaCl	2DE coupled with Nano-LC-MS/MS	Sobhanian et al. (2010a)
Vitis vinifera	Leaves	150 mM NaCl	NanoAcquity ultra-performance liquid chromatography system	Patil et al. (2020)

(continued)

Table 4 (continued)

Plant name	Part used	Treatment	Proteomic approach	References
			Coupled to an SYNAPT-G1 high definition mass spectrometer	
Raphanus sativus	Roots	100 and 200 mM NaCl	iTRAQ labeling and SCX Fractionation-LC-ESI-MS/MS analysis	Sun et al. (2017)
Abelmoschus esculentus	Seedlings	300 mmol L^{-1} NaCl	Tandem mass tag (TMT) labeling and high-performance liquid chromatography¯tandem mass spectrometry analysis	Yu et al. (2019)
Abelmoschus esculentus	Seedlings	300 mmol L^{-1} NaCl t	Tandem mass tag labeling and LC-MS/MS	Zhan et al. (2019)
Opuntia ficus-indica	Young plant	250 mM NaCl	Gel free methods and nanoLC–MS/MS	Hashiguchi et al. (2021)
Musa paradisiaca	Leaflets	60 mmol/L NaCl	iTRAQ labeling and LC–MS/MS	Ji et al. (2019)
Spica prunellae	Fruit spikes	50, 100 or 150 mM NaCl	iTRAQ labeling and hpRP chromatography for fractionation and Nano-LC-MS/MS	Liu et al. (2019b)
Avicennia marina	Leaves	400 mM NaCl. 100 μM of Sodium Nitro Prusside as exogenous NO donor	2DE coupled with MALDI-TOF/TOF MS	Shen et al. (2018)
Andrographis paniculata	Leaves and roots	12 dS m ($^{-1}$) of NaCl	2DE coupled with MALDI TOF MS	Talei et al. (2014)

and roots, indicating an important adaptive mechanism of mulberry in response to salt and drought stress (Liu et al. 2019a).

Proper functioning of chloroplasts under salinity conditions is highly advantageous to maintain crop productivity. Therefore, a comprehensive understanding of the mechanisms underlying the salt tolerance at sub-cellular levels (i.e., at chloroplast levels) serves to develop the salt-tolerant plants. Production of Indian mustard (*Brassica juncea*) plant is significantly affected by soil salinity; therefore, a chlorophyll proteomic profile was analyzed in two *B. juncea* genotypes, one of which was salt-sensitive and the other was salt-tolerant. A total of 12 DEPs that differed in expression in the salt-tolerant and salt-sensitive genotypes were identified. These DEPs were related to a variety of chloroplast-associated molecular processes including the oxygen-evolving process, PS I and PS II functioning, Calvin cycle, and redox homeostasis, suggesting that modulating the expression of chloroplast proteins associated with stabilization of photosystems and oxidative defense plays imperative roles in adaptation to salt stress (Yousuf et al. 2016a). Another study focusing the shoot proteomics of salt-tolerant and salt-sensitive genotypes of this species revealed that on exposure to salinity stress, the salt-tolerant genotype showed a relatively higher number of DEPs enriched in the cellular processes such as turgor regulation, stabilization of photosystems and proteins, and salt compartmentalization, as compared to the salt-sensitive genotype, thus pointing to the fact that modulating the expression of salt-responsive DEPs might serve to develop salt-tolerant mustard plants (Yousuf et al. 2016b). It was further observed in a study of 14 genotypes of this species that their productivity was markedly reduced by salt stress, as it inhibited biomass accumulation, and the protein and chlorophyll contents, but simultaneously triggered overproduction of reactive-oxygen species and upregulation of the expression of different antioxidants. The genotypes exhibiting the minimum and maximum damage, were further investigated through gel-based proteomic approach. In consequence, 42 salinity-responsive proteins related to different metabolic modifications were identified and quantified. Molecular processes, including photosynthesis, redox homeostasis, nitrogen metabolism, ATP synthesis, protein synthesis and degradation, signal transduction and respiratory pathways, underwent significant changes. It was suggested that the identified stress-responsive proteins could prove helpful in strategies to develop salt tolerance in *B. juncea* genotypes (Yousuf et al. 2017). The use of peptide mass fingerprinting (PMF) by Bagheri et al. (2015) enabled identification of maturase K and PPD4 in Spinach (*Spinacia oleracea* L.) exposed to salinity and cadmium stresses. Salinity stress silenced the presence of polycomb protein EZ2, while the combined ($NaCl+CdCl_2$) stress silenced two proteins, *viz.* cellulose synthase-like protein and ubiquitin conjugation factor E4 (Bagheri et al. 2015). The identified proteins were functionally associated with signal transduction (15%), protein synthesis (16%), stress response and defence (33%), photosynthesis (13%), plant growth/cell division (9%), energy generation (4%), transport (4%), secondary metabolism (3%) and cell death (3%), thus clearly maintaining a high ratio of defence and disease-responsive proteins. The study suggested that plants have differential mechanisms, with unique sets of proteins, to respond to stresses caused by Cd, salinity, and their combination (Bagheri et al. 2015).

Polyploidy, present in a majority of angiosperms may contribute to their superior environmental stress tolerance capacity, which may be attributable to duplicate gene expression or simply to evolutionary time. It must be interesting to explore the exact mechanism responsible for stress tolerance in polyploid woody plants at the proteome level. A shotgun quantitative chloroplast proteomic profile of black locust (*Robinia pseudoacacia*) revealed that most of the DEPs were implicated in the photosynthetic carbon reduction pathway (Calvin-Benson Cycle) that occurs in the chloroplast stroma. Ribulose bisphosphate carboxylase/oxygenase is an enzyme complex involved in the Calvin cycle that is comprised of 8 large catalytic subunits (LSU) and 8 small subunits (SSU). DEPs related to Rubisco larger subunits were induced at low salt stress, but repressed at high salt stress in diploid plants, indicating their moderate salt stress tolerance. of diploid plants. In contrast, DEPs related to Rubisco larger subunits were increased in tetraploid plants under high salt stress, suggesting a higher salt stress tolerance of tetraploid plants (Meng et al. 2016). Leaf proteomic analysis further confirms the superior environmental stress tolerance of tetraploid black locust plants, compared to their corresponding diploid plants (Wang et al. 2013b).

A comparative proteomic analysis of *Brassica napus* seedling leaves exposed to salt stress (200 mM NaCl) identified 42 DEPs. The majority of the upregulated DEPs were found to be associated with protein metabolism, damage repair, and defense response, which might contribute to the mitigation of the harmful effect of salt stress on chlorophyll biosynthesis, photosynthesis, energy synthesis, and respiration in *B. napus* leaves, thus providing a basis for genetic engineering of *B. napus* plants with improved salt stress tolerance (Jia et al. 2015). Despite the fact that *B. napus* are a moderately salt-tolerant species, salt stress can significantly reduce its growth, seed yield, and oil production. Therefore, a quantitative proteomic study was conducted to identify the salt stress-responsive protein in the second and third newly developed leaves of salt-tolerant (Hyola 308) and salt-sensitive (Sarigol) cultivars of *B. napus* exposed to NaCl stress. MS analysis identified 44 and 31 DEPs in the tolerant and susceptible genotypes, respectively, and these DEPs were functionally annotated to oxidative stress, energy production, electron transport, translation, and photosynthesis. A greater number of salt responsive proteins (44) in the salt-tolerant *B. napus* genotype, which maintains a high growth rate during salt stress than the salt sensitive cultivar (31). In particular, second newly developed leaves from the salt tolerant cultivar expressed more salt-responsive proteins than the corresponding second day leaves from the salt-sensitive cultivar, indicating a high salt tolerance capacity of Hyola 308 as well as the crucial roles of the younger leaf in salt tolerance (Bandehagh et al. 2011). As roots are the primary sites of salinity perception, a root proteomic profile was investigated in two *B. napus* salt tolerant (Safi 7) and sensitive (Zafar) cultivars. 36 DEPs identified in salt tolerant ((Safi 7) genotype were functionally assigned to 14 functional categories, while 25 DEPs identified in sensitive cultivars (Zafar) cultivars could be assigned to 9 functional categories. Enhanced levels of salt responsive DEPs were reported only in salt tolerant genotype and those DEPs were functionally assigned to glycolysis (glyceraldehyde-3-phosphate dehydrogenase, fructose-bisphosphate aldolase, phosphoglycerate kinase 3), stress (heat shock proteins), redox regulation (Glutathione *S*-transferase DHAR1, L-ascorbate peroxidase), energy metabolism (ATP synthase subunit B), and transport (V-type proton ATPase subunit B1), further unraveling the salinity tolerance molecular mechanisms in *B.napus* cultivars (Kholghi et al. 2019).

Beetroot (*Beeta vulgaris*) also offers a promising therapeutic treatment in a range of clinical pathologies associated with oxidative stress and inflammation (Clifford et al. 2015). Proteomic data revealed that DEPs identified in roots and leaves were related to metabolism and cellular, environmental and genetic information processing, whereas some DEPs were closely related to salt resistance, such as choline monooxygenase, betaine aldehyde dehydrogenase, glutathione *S*-transferase (GST) and F-type H^+-transporting ATPase, were closely related to salt resistance, pointing to the fact that during sugar beet adaptation to salt stress, leaves and roots cope to maintain cellular homeostasis (Li et al. 2020b). Using the comparative proteomics method, Wang et al. (2019) identified 47 and 56 DEPs in roots and leaves, respectively, of beetroot salt-sensitive (S210). In beetroot salt-tolerant (T510) cultivar, 56 and 50 DEPs were changed in the roots and leaves, respectively. The proteins that were differentially expressed between salt-tolerant and susceptible cultivars were mapped to protein modification, amino acid metabolism, tricarboxylic acid cycle, cell wall synthesis, and ROS scavenging, suggesting that these pathways may encourage salt tolerance in the salt tolerant (T510) cultivar (Wang et al. 2019). Proteomic methods could also identify significantly changed proteins and phosphoproteins in the beetroot monosomic addition line M14 (a hybrid from the intercross between *Beta vulgaris* L. and salt tolerant *Beta corolliflora Zoss*) and most interestingly, 189 phosphoproteins exhibited significant changes at the phosphorylation level under salt stress. Several signaling components, for instance 14-3-3 and mitogen-activated protein kinases (MAPK) associated with salt stress, was found in beetroot M14 line, suggesting the potential relevance of phosphoproteins to salt stress response (Yu et al. 2016). Furthermore, DEPs were enriched in metabolism, protein folding, photosynthesis, and protein degradation in beetroot M14 line (Yang et al. 2012a).

It is important to note that genotype factors have a predominant effect on the salt response. Genotype factors affect the salt-responsive pattern, especially that of carbon metabolism. For instance, a proteomic study in two mandarin genotypes (‘Cleopatra’ and ‘Willow leaf’) revealed that the no ion accumulator ‘Cleopatra’ mandarin genotype showed the highest number of salt-responsive proteins, and up-regulation of Calvin cycle-related proteins; conversely, the ion accumulator ‘Willow leaf’ mandarin showed high levels of several photorespiration-related enzymes. This suggests that genetic factors have a predominant effect on the salt stress response (Podda et al. 2013).

Cowpea (*Vigna unguiculata*) is a legume consumed as a high-quality plant protein source, and possesses anti-diabetic, anti-cancer, anti-hyperlipidemic, anti-inflammatory and anti-hypertensive properties (Jayathilake et al. 2018). Cowpea cultivars differing in salt tolerance show differences in protein profiles and different strategies against salt stress. Proteomic data revealed that salt-tolerant cultivar shows increased abundance of DEPs (such as rubisco activase, ribulose-5-phosphate kinase, glycine decarboxylase and oxygen-evolving enhancer (OEE) protein 2) related to photosynthesis and energy metabolism, whereas salt-sensitive cultivar (TVu) shows the down-regulation of Rubisco, OEE protein 1, Mn-stabilizing protein-II, and carbonic anhydrase leading to energy reduction and a decline in plant growth of TVu (de Abreu et al. 2014). The productivity of *Cajanus cajan* (pigeonpea) is also negatively affected by salt stress. The root and shoot proteomic profiles of *C cajan* under salt stress shows the highest abundance of DEPs related to carbohydrate metabolism, followed by protein folding/degradation, amino acids and lipids, suggesting the vital role of these genes/proteins in providing salt-stress tolerance in pigeon pea (Awana et al. 2020).

Bruguiera gymnorrhiza is a medicinal mangrove and its fruit is edible and has traditionally been used to treat diarrhea (also known as ulcerative colitis) (Chen et al. 2020). *B. gymnorrhiza* is a halophyte and one of the most salt-tolerant plant species. However, proteomic data showed that salt-responsive mechanism was different under a low (200 mM NaCl) and a high (500 mM NaCl) dose of salinity. DEPs were found to be involved in photosynthesis, antioxidation, protein folding, cell organization and metabolism. Low salt-stress tolerance of *B. gymnorrhiza* was suggested to be due to effective osmotic adjustment, accumulation of inorganic ions (especially Na^+ and Cl^-) as well as increased expression of photosynthesis-related proteins and antioxidant enzymes, which further promoted plant growth. In contrast, high salt-stress (500 mM NaCl) caused downregulation of photosynthesis-related proteins and antioxidant enzymes, leading to the reduced growth of *B. gymnorrhiza*, but protein folding and degradation-related proteins and cell organization-related protein were up-regulated, which in turn were responsible for the high salt-stress tolerance of *B. gymnorrhiza* (Zhu et al. 2012).

Drought and salinity are inevitable abiotic stress factors which can simultaneously affect the plant growth and productivity. To unveil the salt-tolerance mechanism in *Sorghum bicolor*, a drought-tolerant cereal crop, a proteomic approach was applied and DEPs were assigned to both known and novel/putative stress responsive proteins (Ngara et al. 2012).

Soil salinity is a limiting factor to sugarcane crop development. Leaf proteomic profiles of sugarcane salt-tolerant and sensitive cultivars revealed that four proteins were differentially expressed between control and salt-treated plants. Expression of Fructose 1,6-bisphosphate aldolase was down-regulated, while that of germin-like protein and glyceraldehyde 3-phosphate dehydrogenase increased under salt stress, and heat-shock protein 70 was expressed only in salt-treated plants, suggesting that proteins associated with energy metabolism and defense-related responses may be involved in salt stress protection mechanisms in sugarcane (Murad et al. 2014). Shoot proteomic profile of sugarcane salt-tolerant and salt-sensitive cultivars also demonstrated that abundance of DEPs in salt-tolerant sugarcane under salt stress and those DEPs were implicated in non-enzymatic antioxidant mechanisms, ion transport, and photosynthesis. Some DEPs such as calcium-dependent protein kinase, photosystem I, phospholipase D, and glyceraldehyde-3-phosphate dehydrogenase, were more abundant in the salt-tolerant cultivar exposed to salt stress (Passamani et al. 2017). Root proteomic data also identified enormous accumulation of DEPs in the salt-tolerant cultivar 2 days after salt-stress induction and these DEPs were involved in growth, development, carbohydrate and energy metabolism, reactive oxygen species metabolization, protein protection, and membrane stabilization. On the other hand, presence of these proteins in the sensitive variety was verified only in stress treatment after 72 h, indicating that the effectiveness of these stress responses pathways for phenotypical tolerance depends on early stress detection (Pacheco et al. 2013).

In a study of protein profile of soybean plants exposed to salt stress, about 19, 22 and 14 proteins were identified by mass spectroscopy in the leaves, hypocotyls and roots, respectively. Glyceraldehyde-3-phosphate dehydrogenase was down-regulated in the leaf/hypocotyls, and fructokinase 2 was down-regulated in the hypocotyls/root. Stem 31 kDa glycoprotein precursor was up-regulated in all the three organs under salt treatment. Glyceraldehyde-3-phosphate dehydrogenase was specifically down-regulated at the RNA and protein levels by salt stress. Altogether, proteomic results suggest that the metabolism-related proteins play a role in each organ in order to adapt to salinity stress (Sobhanian et al. 2010b). A shotgun proteomic studies in leaves and roots of soyabean under short-term (12 h) salt stress also identified DEPs related to metabolism, carbohydrate and energy metabolism, protein synthesis and redox homeostasis, which could be assigned to four high salt stress response networks, indicating that effective regulatory protein expression related to signaling, membrane and transport, stress defense and metabolism plays important roles in the short-term salt response of soybean seedlings (Ji et al. 2016). The root and hypocotyl protein profiles also showed upregulation of some special proteins such as late embryogenesis-abundant protein, beta-conglycinin, elicitor peptide three precursor, and basic/helix-loop-helix protein, while protease inhibitor, lectin, and stem 31-kDa glycoprotein precursor were downregulated in response to salt stress, indicating that salinity can modulate the expression level of some special proteins that endue adaptation to saline conditions (Aghaei et al. 2009). Protein mass fingerprinting of germinated seeds of soyabean salt-tolerant cultivar 'Lee68' and salt-sensitive cultivar 'N2899' grown under salt stress revealed that proteins such as Ferritin and 20S proteasome subunit β-6 were up-regulated in both cultivars. Glyceraldehyde 3-phosphate dehydrogenase, glutathione S-transferase 9, glutathione *S*-transferase 10, and seed maturation protein PM36 were down-regulated in Lee68 by salt stress. However, these proteins were present in lower levels in control N2899 and were up-regulated under salt stress, indicating that these might play a key role in defense mechanisms against salt stress during soybean seed germination (Xu et al. 2011).

In tomato, root proteomic profile showed that DEPs are known to be implicated in a wide range of physiological processes including energy metabolism, ROS detoxification, protein translation, processing and degradation, signal transduction, hormone and amino acid metabolism, and cell wall modifications, suggesting that the complex protein network might function cooperatively to maintain the cellular homeostasis under salt stress (Manaa et al. 2011). Proteomic studies have also revealed the protective effect of calcium on salt stress induced alterations in the tomato fruit proteome. Most of the identified DEPs were involved in carbon and energy metabolism, salt stress, oxidative stress, and proteins associated with ripening process, indicating a protective effect of calcium that limited the impact of salinization on metabolism, ripening process, and enhanced the salt stress tolerance in tomato plants (Manaa et al. 2013).

In *Hordeum vulgare* (barley) **r**oot proteome of salt-sensitive (DH14) and salt-tolerant (DH187) lines exposed to salt-stress, most of DEPs are involved in signal transduction (annexin, translationally-controlled tumor protein homolog, lipoxygenases), detoxification (osmotin, vacuolar ATP-ase), protein folding processes (protein disulfide isomerase) and cell wall metabolism (UDP-glucuronic acid decarboxylase, β-d-glucan exohydrolase, UDP-glucose pyrophosphorylase), pointing to the fact that increased activity of signal-transduction mechanisms eventually lead to the accumulation of stress protective proteins and cell wall structure changes that are responsible for increase in salt-stress tolerance of barley tolerant (DH187) lines (Mostek et al. 2015).

A comparative leaf proteomic profile of salt-tolerant (ZM1) and salt-sensitive (A17) genotypes of alfalfa (*Medicago sativa*) showed 120 and 282 DEPs, respectively. Salt stress dramatically inhibited photosynthesis of salt-sensitive A17 due to the downregulation of the light-harvesting complex and photosystem II related protein, whereas in salt-tolerant ZM1, key DEPs were primarily involved in antioxidant system, starch and sucrose metabolism, and secondary metabolism, indicating the superior ability of ZM1 to remove ROS and methylglyoxal under salt stress, as demonstrated by enhancement of the antioxidant system and the secondary metabolism. It can also be suggested that induction of starch and sucrose metabolism in ZM1 under salt stress led to accumulate various soluble sugars (sucrose, maltose, glucose, and trehalose), which in turn facilitate osmotic homeostasis (Gao et al. 2019a). Likewise, proteomic profile of seedlings of alfalfa under salt stress showed that DEPs were associated with antioxidants/detoxifying enzymes, molecular chaperones, energy metabolic enzymes, a secondary metabolic enzyme, and pathogenesis-related proteins which may be the primary contributors to salt tolerance in salt-tolerant (Zhongmu 3) cultivar than the salt-sensitive (Daxiyang) cultivar (Gao et al. 2019b). Functional analysis of the protein mass fingerprint of roots under salt stress showed that DEPs were related to function in various molecular processes, including defense against oxidative stress, metabolism, photosynthesis, protein synthesis and processing, and signal transduction (Long et al. 2016).

MS spectroscopic analysis of seedling root proteomic data showed that most of the DEPs had functions related to metabolism, energy and transport, and were involved in regulating reactions and defending against stress in *Cucumis sativus* under salt stress (Du et al. 2010). Studies also unraveled the protective molecular mechanisms of exogenous application of putrascine against salt stress in *Cucumis sativus*. It was suggested that putrascine might alleviate the salt-stress-induced growth inhibition through degradation of misfolded/damaged proteins, activation of stress defense, and the promotion of carbohydrate metabolism to generate more energy (Yuan et al. 2016b). Likewise, proteomic approach in safflower (*Carthamus tinctorius* L.) could help in understanding the protective molecular mechanisms of exogenously applied growth regulators such as salicylic acid and penconazole against salt stress. The salt-responsive DEPs were mainly involved in photosynthesis, ion homeostasis, and oxidative stress response, as well as nitrogen, protein, and carbohydrate metabolism, thus the identified stress-responsive molecular pathways might help to develop salt tolerance in safflower (Shaki et al. 2020).

Aeluropus lagopoides is a halophyte medicinal plant used for healing wounds and killing pain and has also been reported to possess anti-cancer effects (Phondani et al. 2016; Saleh et al. 2019). A shotgun shoot proteomic profile revealed that 39 DEPs related to metabolism were up-regulated and 44 DEPs related photosynthesis were down-regulated by treatment with NaCl, suggesting that up-regulation of energy formation, amino acid biosynthesis, C(4) photosynthesis, and detoxification are the main salt tolerance strategies in *A. lagopoides* (Sobhanian et al. 2010a). *Helianthus tuberosus L* (Jerusalem artichoke) is an important halophyte that can survive in saline-alkali soil and its tubers and aerial parts can be used as antidiabetic, anti-carcinogenic, anti-fungistatic, anti-constipation, body mass-reducing, metabolism-improving agents (Sawicka et al. 2020). A comparative proteomic approach identified 43 DEPs in H. tuberosus under salt stress, which were enriched in carbohydrate metabolism, ribosome activation and translation, oxidation-reduction and ion binding. Zhang et al. suggested that the induced ribosomal activation and sugar signaling might provide salt-stress tolerance to the plant (Zhang et al. 2018a).

Thompson seedless grape (*Vitis vinifera*) variety is sensitive to salinity when grown on its own roots. The accumulation of salts near the root zone severely hampers plant growth, causing a heavy grapevine yield losses to the farmer. However, grafting onto salinity-tolerant wild grapevine rootstocks (TS110R) can have profound preventive effects on the salt-sensitive Thompson seedless grapevine. A label-free shotgun proteomics approach identified 246 DEPs between own-rooted Thompson Seedless (TSOR) and grafted Thompson seedless (TS110R) grapevines. Functional analysis revealed that identified DEPs were involved in several biological processes such as photosynthesis, amino acid metabolism, translation, chlorophyll biosynthesis, and generation of precursor metabolites. It was also reported that that TSOR vines responded fervently to salt stress, while 110R-grafted vines (TS110R) adopted a preventive approach. Altogether, knowledge on the proteomic changes in grafted and non-grafted plants in response to salt stress opens the scope for future studies on salt stress-specific differences induced by grafting (Patil et al. 2020).

Functional annotation analysis revealed that salt stress elicited complex proteomic alterations in radish (*Raphanus sativus*) roots involved in carbohydrate and energy metabolism, protein metabolism, signal transduction, transcription regulation, stress and defense and transport (Sun et al. 2017).

Abelmoschus esculentus has fruit popularly known as okra and is commonly used in cooking but also in traditional medicine in the treatment of worms, dysentery, inflammation, and irritation of stomach, intestines and kidneys (Dantas et al. 2021). A shotgun proteomic approach and phosphoproteome analysis revealed that 91 phosphorylation sites were upregulated and 307 phosphorylation sites were downregulated in the seedlings of NaCl-treated or control group of this species. Subsequent bioinformatics analysis revealed that the DEPs were most strongly associated with photosynthesis antenna proteins and RNA degradation, suggesting that these proteins are possibly linked to salt stress responses in Okra (Yu et al. 2019). In addition, it has also been shown that DEPs were most strongly associated with response to stress and protein processing in endoplasmic reticulum (Zhan et al. 2019).

Opuntia ficus-indica, commonly referred to as prickly pear or nopal cactus, is characterized by its remarkable adaptation to arid and semi-arid climates and it exhibits biologically activities including anti-inflammatory, antioxidant, hypoglycemic, antimicrobial and neuroprotective properties (El-Mostafa et al. 2014). *Opuntia* spp. exhibit high stress-tolerance capacity. A gel-free quantitative proteomic approach revealed that salt stress increased the abundance of phosphoenolpyruvate carboxylase and decreased the abundance of ribulose-bisphosphate carboxylase in *O. ficus indica* plants, indicating that Crassulacean acid metabolism is promoted under salinity (Hashiguchi et al. 2021).

Salinity affects the yield and quality of banana (*Musa paradisiaca*). Leaflet Proteomic data showed that DEPs were mostly implicated in protein synthesis and degradation, photosynthesis, defense response, and energy and carbohydrate metabolism, suggesting that these pathways act as salt stress responsive strategies in banana (Ji et al. 2019).

Spica prunellae is an important Chinese herbal medicine which is consumed in large quantities in clinical applications (Zhu et al. 2018; Lei et al. 2021). Salt stress can affect the concentration of metabolites in *S. prunellae*. A quantitative proteomics approach was applied to identify DEPs in *S. prunellae* under salt stress and the researchers identified 35 significantly differentially expressed proteins which are functionally assigned to cellular protein and carbohydrate metabolism, calcium ion transport, photosynthesis, and protein synthesis (Liu et al. 2019b).

Avicennia marina is a well-known traditional and folk use medicine (ElDohaji et al. 2020) and it is one of the most salt-tolerant mangrove species. Proteomic analysis identified 49 DEPs involved in various biological processes such as photosynthesis, energy metabolism, primary metabolism, RNA transcription, protein translation and stress response proteins. Nitric oxide might have protective effects on improving salt tolerance in plants. Under high salt stress, it caused a significant decrease in the abundances of DEPs related to photosynthesis, such as ribulose-phosphate 3-epimerase, RuBisCO large subunit, RuBisCO activase A, and quinine oxidoreductase-like protein isoform 1. However, the abundance of proteins such as RBCL and QOR1 increased by sodium nitroprusside (as a nitric oxide donor) treatment. In addition, exogenous NO supply alleviated salt tolerance by increasing the accumulation of some proteins involved in energy metabolism, primary metabolism, RNA transcription and stress response proteins, therefore it can be strongly suggested that NO positively improves salt tolerance in *A. marina* by regulating the protein abundance involved in photosynthesis, energy metabolism, primary metabolism and stress response (Shen et al. 2018).

Andrographis paniculata is a multifunctional medicinal plant and a potent source of bioactive compounds (Talei et al. 2015). Proteomic analysis led to the detection of 32 induced proteins (19 proteins in leaf and 13 proteins in the root) as well as 12 upregulated proteins (four proteins in leaf and eight proteins in the root) in the salt-treated plants. Three of the DEPs were related to superoxide dismutase, ascorbate peroxidase and ribulose-1, 5-bisphosphate oxygenase which are associated with plants response to environmental stresses and could represent the common stress responsive proteins in response to salt stress in *A. paniculata* too (Talei et al. 2014).

6 Medicinal Plant Proteomics in Response to Heavy Metal Stress

In recent times there is a growing interest in the exploitation of high-throughput OMICS techniques to unravel the underlying molecular mechanisms of plant stress responses to diverse toxic chemicals including heavy metals (HMs). Contamination of soils with accumulated heavy metals is a constraint for the growth of the plants which subsequently affects the yield of the plants. By definition, elements having specific gravity above five are considered as HMs. Nevertheless, the term heavy metal commonly refers to toxic metals, e.g., cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), zinc (Zn) as well as hazardous metalloids, *viz*., arsenic (As), boron (B), which exert negative effects on plant growth and development (Hossain et al. 2012). Most of the HMs get their entry into plant root system via specific/generic ion carriers or channels (Bubb and Lester 1991). Efficiency of transporter system in a species determines the rate of metal-ion uptake by its roots (Ahmad et al. 2005). Once HM ions enter the cell, cellular functions are affected by a wide range of actions. The negative impact of HM includes binding of HM ions to sulfhydryl groups of proteins, replacement of essential cations from specific binding sites, leading to enzyme inactivation and production of reactive oxygen species (ROS), which causes oxidative damages to lipids, proteins and nucleic acids (Sharma and Dietz 2009). Metal toxicity can cause changes at both cellular and molecular level. Plants growing in HM-contaminated environment have evolved coordinated homeostatic mechanisms to regulate the uptake, mobilization, and intracellular concentration of toxic metal ions to alleviate stress damages. Proteins, the functional translated portion of the genome play a crucial role in plant stress response. Therefore, proteomic studies provide us a better idea regarding the metabolic pathways and protein networks involved in cellular detoxification and stress-tolerance mechanism against the metal-induced stress. Despite the significant contribution of genomics in addressing plant responses to abiotic stress, changes in gene expression at transcript level have not always been reflected at protein level. Therefore, a thorough proteomic analysis could significantly identify the metal-stress responsive proteins that actively participate in the detoxification mechanisms (Hossain and Komatsu 2013). Moreover, quantitative proteomics can provide accurate information about molecular changes, allowing the identification of specific stress-response mechanisms, and facilitate an effective management of metal contamination in crops by genetic manipulation (Ahmad et al. 2022). A list of proteomic studies conducted on medicinal plants in response to metal stress is given in Table 5.

6.1 Lead (Pb) Stress

Ionic lead (Pb) in the environment has accumulated due to anthropogenic activities, causing a potential threat to plants and plant consumers. Soybean is a leguminous

Table 5 Proteomic studies conducted in medicinal plants response to metal stress

Plant name	Part studied	Treatment	Method	References
Lead (Pb) stress				
Allium cepa	Onion roots	5.0 and 15.0 μM Pb solutions of $Pb(NO_3)^2$	2DE-coupled with TOF/TOF II-MS	Lyu et al. (2020)
Cannabis sativa	Leaves	Pb stress, in which Pb $(NO_3)^2$ was applied at 3 g/kg soil dry	RPLC separation with ESI-MS/MS	Xia et al. (2019)
Glycine max	Root nodule	30 ppm $PbCl_2$ and 0.5 ppm $HgCl_2$	2DE coupled MALDI-TOF MS/MS	Baig et al. (2018)
Raphanus sativus	Radish roots	$Pb(NO_3)_2$ at 500 mg L^{-1} (Pb500) and 1000 mg L^{-1} (Pb1000)	iTRAQ coupled LC-MS/MS	Wang et al. (2016c)
Talinum triangulare	Plant roots	1.25 mM Pb	2DE-coupled MALDI-TOF MS	Kumar and Majeti (2014)
Theobroma cacao	Roots and leaves of seedlings	Pb (0.8 g L^{-1}) in the form lead nitrate	2DE coupled to Q-TOF-M/MS	Reis et al. (2015)
Rhoeo discolor	Leaves	1000-ppm Pb^{2+}	2DE coupled with LC-MS/MS	Enot et al. (2020)
Camellia sinensis	Roots	Pb (13.65 mg/L) in the form of $Pb(NO_3)^2$ solution	iTRAQ coupled to RPLC with Triple TOF—MS/MS	Duan et al. (2020)
Copper stress				
Ocimum basilicum	Leaves	Cu 200, 500, 1000 ppm from the solutions of $CuCl_2$	2DE coupled with LC-MS-MS/MS	Georgiadou et al. (2018)
Elsholtzia splendens	Roots and leaves	100 μM $CuSO_4$	2DE coupled to MALDI-TOF MS and LTQ-ESI-MS/MS	Li et al. (2009)
Sorghum bicolor	Roots of seedlings	50 and 100 μM $CuSO_4$	2DE coupled to MALDI-TOF-TOF MS	Roy et al. (2017)
Cadmium stress				
Brasssica napus	Leaves	$CdCl_2$ 100 and 500 μM	2DE-MALDI-TOF-TOF	Ali et al. (2015)
Brasssica napus	Xylem sap	10 μM Cd	nLC-quadrupole MS	Luo and Zhang (2019)
Spinacea oleracea	Leaves	40 μg $CdCl_2$/g soil	SDS PAGE and MALDI-TOF MS/MS	Bagheri et al. (2017)
Vigna radiata	Leaves	50 μM $CdCl_2$	2DE coupled MALDI-TOF MS/MS	Muneer et al. (2012)
Vigna radiata	Root nodules	50 μM $CdCl_2$	2DE coupled MALDI-TOF MS/MS	Muneer et al. (2013)

(continued)

Table 5 (continued)

Plant name	Part studied	Treatment	Method	References
Spinacea oleracea	Leaves	100 μM $CdCl_2$	Two-dimensional gel electrophoresis (2 DGE)	Bagheri et al. (2013)
Spinacea oleracea	Leaves	50 μg $CdCl_2$ g^{-1} soil	Two-dimensional gel electrophoresis (2 DGE)	Bagheri et al. (2015)
Brassica juncea	Thylakoids	125 μM $CdCl_2$	2DGE/ESI-MS/MS	Qureshi et al. (2010a)
Populous nigra	Leaves	50 μM $CdSO_4$	2D LC coupled with MALDI-TOF-MS	Visioli et al. (2010)
Sorghum bicolor	Seedlings	100, 150 μM$CdCl_2$	2DE coupled to MALDI-TOF/TOF MS	Roy et al. (2016)
Amaranthus gangeticus	Root, stem, and leaf	Low Cd (1.82 mg kg^{-1}), and high Cd (11.79 mg kg^{-1})	2DE coupled to MALDI-TOF-TOF mass spectrometer	He et al. (2018)
Iris lactea var. *chinensis*	Seedlings	25 mg/L $CdCl_2$ for	LC-TOF-MS/MS	Liu et al. (2020)
Medicago sativa	Stem	10 mg Cd kg^{-1} soil as $CdSO_4$	2D DIGE coupled with MALDI-TOF/TOF-MS/MS	Gutsch et al. (2018)
Raphanus sativus	Root	10 or 50 μM $CdCl_2$	iTRAQ coupled LC-ESI-MS/MS	Xu et al. (2017)
Glycine max	Leaves	100 μM $CdCl_2$	2De coupled with nanoLC-MS/MS	Hossain et al. (2012)
Cucumis sativus	Leaves	100 μM $CdCl_2$	iTRAQ coupled to LC-ESI-MS/MS	Gong et al. (2017)
Cucumis sativus	Leaves and roots	50 mM Cd	2DE coupled with MALDI-TOF-MS-MS	Sun et al. (2016)
Brassica juncea	Leaves	Cd stress at low (25 μM) and high (100 μM) doses	2DE coupled to nanoLC-MS/MS	D'Alessandro et al. (2013)
Chromium stress				
Brassica napus	Leaves	100 μM Cr(VI)	2DE coupled to MALDI-TOF/TOF mass spectrometry	Yildiz and Terzi (2016)
Arsenic stress				
Brassica napus	Leaves	200 μM arsenic (As)	iTRAQ coupled to LC-MS/MS	Farooq et al. (2018)
Spinacea oleracea	Leaves	31 ppm Na_3AsO_4 in soil	2DE coupled MALDI-TOF MS/MS	Amna et al. (2020)
Brassica juncea	Leaves	100 μM Na_3AsO_4	SDS PAGE and MALDI-TOF MS/MS	Ahmad et al. (2020)

plant having N_2-fixing root nodules, which harbour symbiotic bacteria and fix atmospheric nitrogen to available nitrogenous forms. Thus, root nodules are the primary sites at the risk of metal toxicity (Qureshi et al. 2010a, b). Soybean growing soils in India are often contaminated with Pb and Hg. Proteomic mass fingerprinting approach identified the abundance of 76 DEPs by Pb and Hg treatment of root nodules. Lead treatment led to the upregulation of 17 DEPs and downregulation of 16 DEPs, while Hg treatment caused upregulation of 33 DEPs and downregulation of 10 DEPs in soyabean root nodule. Most of these DEPs (catalase, allene oxide synthase, glutathione S-transferase, calcineurin B like, calmodulin like and rapid alkalinization factor) were upregulated. These are functionally associated with numerous cellular functions especially in defense and development, and repair related proteins, which confer stress response in plants to both Pb and Hg stress (Baig et al. 2018). *Talinum triangulare* Willd, an erect, succulent, perennial herb, has been traditionally used in the management of cardiovascular diseases in Nigeria and is known to possess different pharamacological properties (Olorunnisola et al. 2016). A shotgun proteomic approach in the Pb-stressed roots revealed an increase in relative abundance of 20 major proteins and 3 new proteins. A majority of these DEPs are involved in root architecture, energy metabolism, ROS detoxification, cell signaling, primary and secondary metabolisms, and molecular transport systems (Kumar and Majeti 2014).

Cocoa is a dry, powdered, non-fat component product prepared from seeds of the *Theobroma cacao* L. tree and is known to contain biologically active substances that are beneficial for human health (Ellam and Williamson 2013; Camandola et al. 2019). Both *T. cacao* and its products are highly contaminated with Pb, therefore it is essential to unveil the stress-responsive mechanisms in these plants against Pb stress. Protein signatures from MS-based proteomic strategy revealed that Pb induced the expression of oxidative stress related proteins including peroxidase, glutathione S transferase, and ascorbate peroxidase, suggesting that Pb stress can cause free radicals mediated cellular damage. This further indicates that plants have used antioxidant defense mechanisms against Pb stress (Reis et al. 2015).

Radish is a widely consumed root vegetable, known for its nutritional and several health benefits (Manivannan et al. 2019). Heavy metals can penetrate the roots and then enter the food chain, causing potential health risks for humans. A shotgun proteomic approach revealed a total of 135 differentially expressed proteins under Pb-stressed conditions. Pathway-enrichment analysis indicated that most of these DEPs were related to translation, processing and degradation of proteins, ROS scavenging, photosynthesis, and respiration and carbon metabolism. In particular, key enzymes of the glycolysis and TCA cycles were negatively affected. In contrast, defense responses including ascorbate -glutathione cycle for ROS scavenging and defense proteins such as glutaredoxin, aldose 1-epimerase malate dehydrogenase and thioredoxin were triggered as a Pb stress tolerance strategy (Wang et al. 2016c).

Hempseeds are the edible fruits of the *Cannabis sativa* L. plant which are known to offer multiple health benefits to humans (Farinon et al. 2020). It can accumulate Pb and tolerate Pb stress. Therefore, proteomics approaches focusing on its Pb stress response mechanism would be useful to for breeding of hemp with enhanced Pb tolerance and accumulation. Towards this end, Xia et al. (2019) have investigated the leaf proteomics and found a total of 63 and 372 DEPs in a Pb-tolerant (Bamahuoma) and a Pb-sensitive (Yunma 1) hemp variety, respectively, under Pb stress. These DEPs were functionally involved in metabolism, respiration and energy, defense, and transport. Their studies provided reference protein information and suggested that Hemp adapted to Pb stress by accelerating adenosine triphosphate (ATP) metabolism; enhancing respiration, light absorption and light energy transfer; promoting assimilation of intercellular nitrogen and carbon; detoxification of ROS; improving exchange of water and CO_2 in leaves; promoting intercellular transport; preventing aggregation of unfolded proteins; degrading misfolded proteins; and increasing the transmembrane transport of ATP in chloroplasts (Xia et al. 2019).

There is a growing interest in the development of phytoremediation technologies involving the plant-based cleanup of metal-contaminated soils. Proteomic approaches help to identify the underlying molecular information on the mechanism of plants' metal-stress response, which can be applied to further improve the phytoremediation efficiency, using the breeding techniques. *Rhoeo discolor* is a medical plant with antioxidant and chemoprotective properties (González-Avila et al. 2003; García-Varela et al. 2016). *R. discolor* is known as a hyper accumulator of Pb because of its ability to intake Pb from the contaminated soils, and this is of relevance to phytoremediation applications. Proteomic studies in this plant revealed a significant decrease in the DEPs related to photosynthesis, carbon fixation and metabolism in the leaves upon treatment with Pb (Enot et al. 2020).

A shotgun proteomic approach in roots identified a total of 17 DEPs between control and Pb-treated garlic plants. Downregulated DEPs were related to defense response (Anx D1), respiration (SHMT1), and DNA repair (POLD1), while upregulated DEPs were related to transcription and protein synthesis (NDPK), pathogen invasion (PR1 and CHI1), and DNA replicating activity (ORC5 and MPK5), suggesting that Pb stress could slow down the cellular processes such as defensive response, respiration, DNA repair activity, DNA replicating activity and increased pathogen invasion, which are the possible reasons for Pb toxicity in plants (Lyu et al. 2020). Despite the fact that humic acid offers protection against Pb-induced stress in plants, its molecular mechanism is still unknown. However, differential proteomic analysis could provide the underlying mechanism of protection offered by the humic acid and suggest that humic acid strengthened the antioxidant system, promoted the synthesis of cell wall, and stabilized the protein and sulfur-containing substance metabolism, and thus alleviated the Pb toxicity (Duan et al. 2020).

6.2 Cadmium (Cd) Stress

Cadmium (Cd) is a non-essential, toxic heavy metal that poses serious threats to ecosystems and human health. Plants employ various cellular and molecular mechanisms to reduce the impact of Cd toxicity and cell walls function as a defensive barrier during Cd exposure. Cadmium stress may cause serious morphological and physiological abnormalities in addition to altering the proteome in plants. In leaves of *Brassica napus* exposed to cadmium, quantitative proteomic approach identified up-regulation of DEPs that are implicated in carbohydrate metabolism, CO_2 assimilation/photosynthesis, defense to stress, and protein synthesis/regulation. The DEPs were identified as epithiospecifier protein and ribulose bisphosphate carboxylase, which are involved in protein metabolism and assimilation. Other upregulated DEPs were found to be iron superoxide dismutase, disease resistance protein and regulator of ribonuclease, which are involved in defense, stress and regulation of proteins, respectively (Ali et al. 2015). DEPs related to carbohydrate and energy metabolism, stress and defense and signal transduction processes were also observed in the roots of *Raphanus sativus* under cadmium stress (Xu et al. 2017).

As the xylem sap of vascular plants primarily transports water and mineral nutrients including metals from roots to the aerial parts, proteomic changes in xylem sap provide an important mechanism for detoxifying Cd by plants. Proteomic approaches identified 28 DEPs, most of them being involved in cell wall modifications, stress/oxidoreductases, and lipid and protein metabolism. Researchers found that a plant defensin-like protein, BnPDFL, which belongs to the stress/oxidoreductase category, was unique to the Cd-treated plants, suggesting that plant defensin plays a positive role in Cd tolerance (Luo and Zhang 2019). *Populus nigra* L. buds are known to possess various bioactive compounds such phenolic compounds, terpenoids, flavones, flavanones, caffeic/ferulic acids and their derivates (Kis et al. 2020). Leaf proteomic profile of Cd-treated plants showed that proteins found in greater abundance in the treated samples, were located in chloroplast and mitochondrion, suggesting the importance of these organelles in the response and adaptation to metal stress (Visioli et al. 2010). Proteomic studies in *Sorghum bicolor* revealed that Cd stress can also have inhibitory effect on carbon fixation, ATP production and the regulation of protein synthesis (Roy et al. 2016).

Red amaranth, distributed in eastern Asia, is rich in a variety of nutraceuticals that can have health-benefits. In red amaranthus, DEPs accumulated in response to Cd stress and these DEPs were related to metabolism, energy, protein synthesis, protein degradation and storage, transport, cell construction, disease defense, and second metabolism. However, the abundance of these DEPs varies from tissues to tissue. In leaf, DEPs between cultivars mainly function in metabolism (30.77%), energy (26.92%), and protein degradation and storage (23.08%). In stem, the DEPs were related mainly to metabolism (24.39%), energy (17.07%), disease defense (17.07%), and protein degradation and storage (14.63%), while in root, they were mainly related to energy (26.32%), protein degradation and storage (21.05%), metabolism (15.79%), and disease defense (15.79%) (He et al. 2018). In *Medicago sativa*, a shotgun-MS-based quantitative proteomics revealed abundance for 179 cell wall proteins and 30 proteins in the soluble fraction under Cd stress. Functional annotation indicated that these DEPs are involved in cell wall remodeling, defence response, carbohydrate metabolism and promotion of the lignification process, pointing to the function of the cell wall as an effective barrier against Cd stress (Gutsch et al. 2018). Proteomic studies also provided an insight on the protective effects of beta amino butyric acid or nitric oxide (NO) in alleviating the Cd stress in soybean (Hossain et al. 2012) and cucumber (Gong et al. 2017). Beta amino butyric acid significantly enhanced the defense-related protein peroxiredoxin and glycolytic enzymes in response to Cd exposure (Hossain et al. 2012). On the other hand, nitric oxide (NO) reversed several Cd-disruptive pathways including Cd transport and localization, photosynthesis, chlorophyll metabolism, redox homeostasis, glutathione-mediated Cd detoxification and Ca^{2+} signaling transduction (Gong et al. 2017). Similarly, Cd-disruptive pathways were mitigated by Selenium in cucumber plants (Sun et al. 2016).

Brassica juncea (Indian mustard) is an oilseed plant used in many types of food and gained special attention due to its potential beneficial effects on human health (Oulad El Majdoub et al. 2020). Protein profiles indicated that Cd treatment could alter photosystem components and the alteration of metabolic enzymes, including ATP synthase subunits, carbonic anhydrase, and enzymes involved in antioxidant responses (especially glutathione and phytochelatin homeostasis) and the Calvin cycle (D'Alessandro et al. 2013). A membrane proteomic study on multi-protein complexes of *B. juncea* showed a Cd-mediated alteration in the photosynthetic pigments, more during Fe-deficiency (Qureshi et al. 2010a). Similarly, in the root nodules of *Vigna radiata,* Cd stress significantly changed the abundance of 20 proteins, associated mainly with RNA metabolism and storage (Muneer et al. 2012), whereas in leaves the stress caused a significant change in the abundance of 40 proteins, which were associated mainly with defence, secondary metabolism, and photosynthesis (Muneer et al. 2013).

Another plant, *Iris lactea* var. *chinens*, exhibits strong Cd tolerance and accumulation ability, therefore can also be used for phytoremediation of Cd-contaminated soil. Besides, this plant consists of a broad range of secondary metabolites with numerous biological activities such as antibacterial, antioxidant, anti-inflammatory, and anti-cancer activities (Fang et al. 2008; Ibrahim et al. 2012; Kostić et al. 2019). A total of 163 and 196 DEPs identified in the shoots and roots, respectively, following treatment with Cadmium. These DEPs are involved mainly in signal transduction, ion transport, redox, amino acid biosynthesis, lignin biosynthesis, glycerolipid metabolism and glutathione metabolism (Liu et al. 2020).

Cd-exposed spinach (*Spinacia oleracea*) showed changes in the leaf proteomic profile; RubisCO smaller subunit was particularly sensitive. However, the content of S-rich proteins increased in the presence of Cd, particularly when S was in abundance (Bagheri et al. 2017). With a lower dose of Cd, 117 proteins changed their abundance to generate stress tolerance (Bagheri et al. 2013). Spinach plants exposed to Cd (50 μg $CdCl_2$ g^{-1} soil), salinity (10 mg NaCl g^{-1} soil) and their combination (NaCl + Cd) showed significant increase and decrease in the relative abundance of 14 and 39 proteins by Cd; 11 and 46 proteins by salinity, whereas 22 and 37 proteins by the combined stress, respectively. Proteins PPD4 and maturase K increased with all stresses. Salinity silenced the presence of one protein (polycomb protein EZ2) whereas combination stress (salinity+Cd) silenced two proteins viz. ubiquitin conjugation factor E4 and cellulose synthase-like protein. In total, 33% of proteins with altered abundance had a role in stress response and defence (Bagheri et al. 2015).

6.3 Copper (Cu) Stress

Copper (Cu) is an important micronutrient for plants, however at higher concentrations it is extremely toxic to plants and can inactivate and disturb protein structures. Protein mass fingerprinting approach identified a significant induction of proteins in basil (*Ocimum basilicum*) plants treated with Cu when compared to untreated control plants. The DEPs that were upregulated include ribosomal protein L14, ATP synthase and ribulose-1,5-bisphosphate carboxylase/oxygenase which are assigned to primary metabolic pathways such as respiration, protein synthesis and photosynthesis respectively. This upregulation of primary metabolic pathways is likely an adaptation of the plants to stimulate defense mechanisms in response to metal-induced stress (Georgiadou et al. 2018). Likewise, proteomic analysis revealed that DEPs in response to copper stress were related to defense, metabolism and transcription and regulation in the roots of *Sorghum bicolor* (Roy et al. 2017) and *Elsholtzia splendens* (Li et al. 2009).

6.4 Chromium (Cr) and Arsenic (As) Stress

Leaf proteomic analysis revealed that Chromium (Cr) stress significantly enhanced the expression of defense-related proteins including 2-Cys peroxiredoxin BAS1, glutathione *S*-transferase, ferritin-1, l-ascorbate peroxidase, thiazole biosynthetic enzyme and myrosinase-binding protein-like At3g16470 in a Cr tolerant *Brassica napus* cultivar (NK Petrol), indicating that stress-related proteins play an important role in the detoxification of Cr and in maintaining cellular homeostasis (Yildiz and Terzi 2016). Proteomic approach could also elucidate the molecular mechanisms involved in methyl jasmonate-induced arsenic tolerance in *B. napus* leaves and suggests a significant role of methyl jasmonate in plant physiological processes (Farooq et al. 2018). Exposure of spinach to arsenic changed the abundance of 57 proteins (21 down-regulated and 36 up-regulated). *S*-deficiency altered the abundance of 55 proteins (36 down-regulated and 19 up-regulated), whereas the combined stress of arsenic and S-deficiency changed the abundance of 69 proteins (31 down-regulated and 38 up-regulated) (Amna et al. 2020).

A study on Indian mustard exposed to As, Cr and Cu stress showed changes in protein abundance of leaf tissues. HSP (15.7 kDa) and autophagy protein 5–like were in higher abundance under As and combined stress. RuBisCO (large subunit), mediator of RNA polymerase II transcription subunit 1 isoform X1, and ribosomal protein S3 were more abundant under Cr and Cu stress. A protein, resembling the zinc-finger A20/AN1 domain-containing stress-associated protein 5, was more abundant under Cu stress (Ahmad et al. 2020).

7 Conclusion

Dynamic environmental factors can severely affect the proteome of plants and, in turn, the growth of plants and quality of plant productivity. Proteins are common effectors of biological response to various abiotic stress factors. Proteomic approaches have been widely used to unravel the proteomic changes, and proved to be a promising tool to identify stress-responsive proteins against different abiotic stresses, allowing the discovery of protein biomarkers of a given abiotic stress. Proteomics provided important clues to our understating at the molecular level of how medicinal plants respond to changing abiotic factors. Comparative proteomic studies allow monitoring of protein expression during different times, contributing significantly to the understanding of the mechanisms of adaptation of plants under stressful conditions. Therefore, a comprehensive proteomic analysis of plant stress responses will be crucial for future crop improvements using plant breeding strategies. Moreover, quantitative proteomic studies with the development of new mass-spectrometry-based methodologies might solve limitations associated with the analytical variability of the technique, allowing a greater reproducibility of proteomic results, and allow us to understand better the processes of plant stress acclimation and stress tolerance acquisition.

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Medicinal Plants Metabolomics in Response to Abiotic Stresses

Phalguni Bhattacharyya and Amitava Ghosh

Abstract Medicinal plants are the life source of human civilization. Nevertheless, these plants face great danger with the advancement of urbanization encroaching on natural fertile lands. Plants have been used as a medicine on this planet from ancient times for remedial purposes against different diseases by experience, and in India, we have ancient documents like Charak and Sushrut Samhita (Indian Ayurveda 1st millennium BC). Plants synthesize specialized secondary metabolites to tolerate biotic and abiotic stresses. Metabolites are the voucher of correlation among different genetic architecture and the impact of stress. Metabolomics has been instrumental to unveil the functional components of the physiological system of plants using sophisticated bioanalytical technologies and softwares for the interception of target analysis, metabolite profiling and metabolic fingerprinting. Various equipments like LC-MS/MS, GC-MS, and NMR are used to trace out and identify many known and unknown metabolites. Since, metabolites and the phenotype of an organism are interlinked, metabolomics can monitor responses to biotic and abiotic stress with simultaneous gene functioning. Metabolomics plays a role in filling up the gap between genotype and phenotype, reviewing cell function, and identifying the stress-related alteration in metabolites and expression of low molecular weight peptides in medicinal plants.

Keywords Metabolomics · Medicinal plants · Abiotic and biotic stresses

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A. Husen, M. Iqbal (eds.), *Medicinal Plants*,
https://doi.org/10.1007/978-981-19-5611-9_3

1 Introduction

Metabolomics is the power of mankind to tackle the climatic or environmental stress conditions that adversely affect the global demand for food and nutrition. This instrument-based advanced methodology helps scan the unexplored wild specimens to find new molecular or genetic traits vouchers. The successful screening, purification and selective use of metabolic derivatives can be used to develop improved new cultivars. The fate of *Homo sapiens* is at stake for their rapidly increasing population and then demand for basic requirements like food, shelter, quality of life, and disease management. Recently in 2018, the Food and Agriculture Organization (FAO) has expressed doubt about the doubling of crop productivity by 2050, because the increasing climatic and environmental stress, reduction in the agricultural land area, chemical pollution and the inevitable urbanization may reduce the crop productivity up to 50% by the year 2080. The economic crops rapidly eliminate the diversity of medicinal plants with a simultaneous extinction of wild shrubs and herbs, exaggerating the loss of future hopes of natural molecular antidotes to sustain the existence of mankind (Beigh et al. 2002b, 2003). The condition of pollution of air, water and soil is often serious, especially in thickly populated countries with underdeveloped socio-economics and corrupt political system. Wild vegetation is the worst sufferer of this contaminated atmosphere (Iqbal et al. 2000a, b, c; Iqbal and Khudsar 2000). There is a need for protecting the threatened and endangered species on priority basis (Beigh et al. 2002a, 2005, 2011) and exploring the useful biological traits from medicinally important plants. Though the task is difficult, and daily urgencies of life of the vast human population form the barriers, yet the newer scientific approaches, such as the metabolomics, offer a ray of hope (Chakraborty 2018). Here we concentrate on the generation of such molecules in response to some important abiotic stresses, such as drought, waterlogging, temperature extremes, radiations, salinity, acidity, ozone, and toxic metals, hovering around the structural features (leaf anatomy, root structure), physiological processes (growth, photosynthesis, transpiration, water regime, stomatal functioning), and biochemical parameters (reactive oxygen species, photosynthetic pigments, activities of antioxidant enzymes) of these plants. However, the most significant effect of abiotic stress factors on the metabolomics of medicinal plants is the alteration in secondary metabolites (Fig. 1). In the present chapter, this problem will be highlighted by evaluating the abiotic stress effect on the metabolomics of medicinal plants.

Medicinal Plants

Since the inception of the earliest human civilization, plant metabolites have been used and practiced, based on observations, for health care management. The effect of these metabolites is related to the growth of the plant, environment, period of collection, drying process and methods of extraction. The ancient Indian systems of medicine, like Ayurveda and Unani, are based primarily on the practicing knowledge of traditional herbal medicine (Lad 1987; Hankey 2005; Parveen et al. 2020, 2022). The World Health Organization has listed 21,000 medicinal plants, among which 2500 species are in India and now India is known as the largest

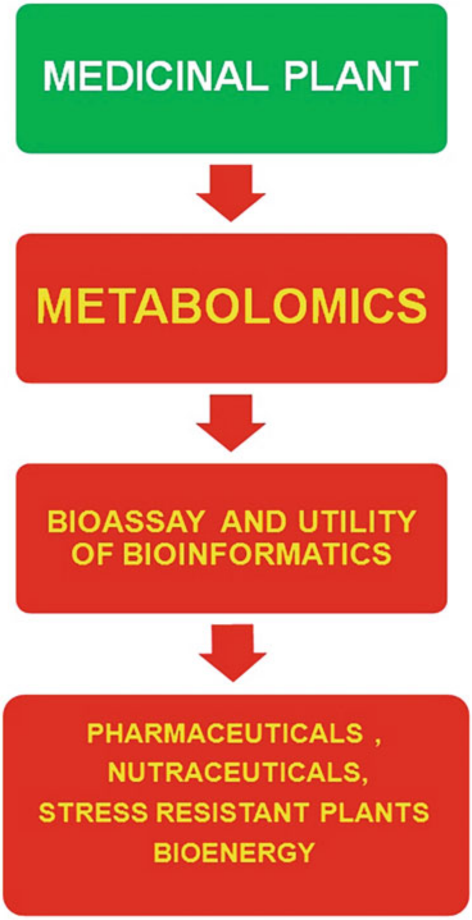


Fig. 1 Metabolomics and medicinal plants

producer of medicinal herbs (Modak et al. 2007). Description of medicinal plants has been found in the Rig Veda, Sushruta Samhita and Charaka Samhita for their therapeutic uses. Record says despite the exploration of medicinal values of more than 20,000 plants in the world, only 4478 plants are used regularly for manufacturing the herbal medicinal products. Some of the angiosperm families like Moraceae, Apiaceae, Lamiaceae, Solanaceae, Euphorbiaceae, Apocynaceae, and Fabaceae contribute the maximum number of medicinal plant species (Fig. 2).

Modern analytical techniques are now available and must be used in quality evaluation and scientific validation of medicinal plants and their formulations to fulfil market demands (Chaudhary et al. 2014). The isolation and characterisation of these bioactive molecules utilise their chemistry, molecular structure, molecular orientation or geometrical arrangement against the foreign particles. The newer molecule can achieve a newer route of activation through alteration in the metabolic

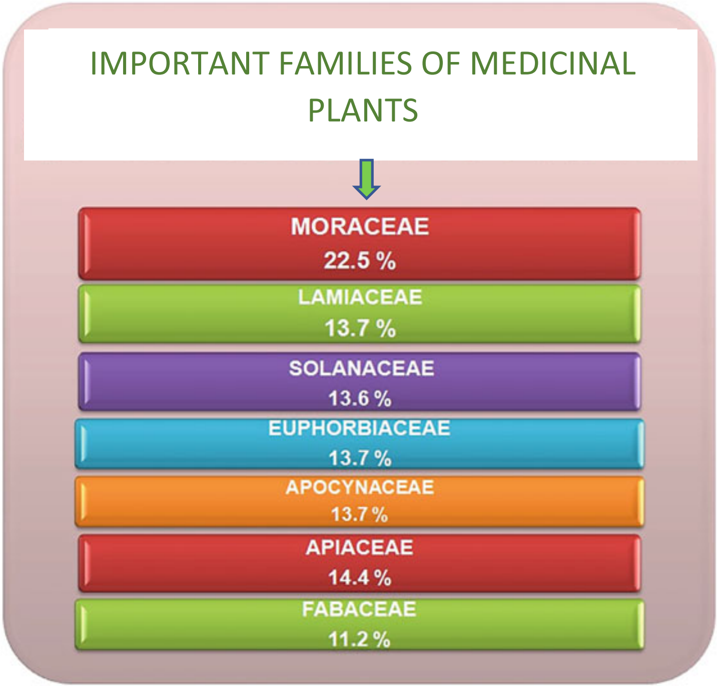


Fig. 2 Different families known for having abundance of medicinal plants

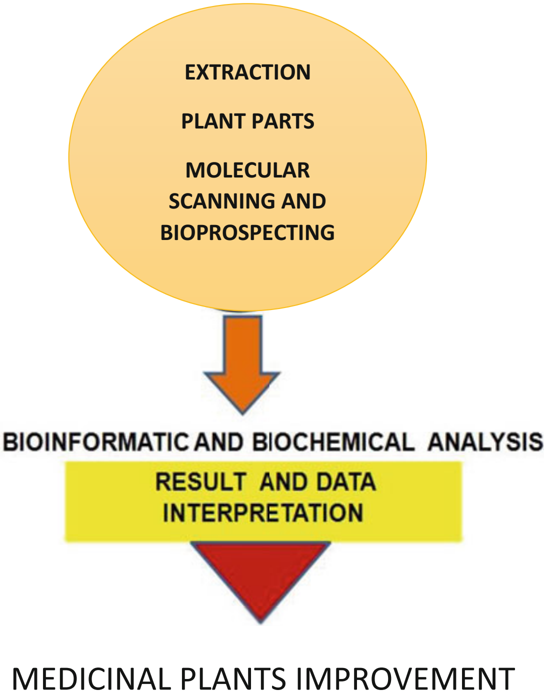
pathway or by any impressive secondary metabolites following the conventional biosynthetic pathways. This level of research needs an orchestral way of experimentation involving different disciplines of science.

2 Metabolomics

Metabolomics is the critical documentation and identification of metabolites in a biological system. The network with metabolites in a plant system may often alter or modulate due to stress effects. Clear-cut ideas about the regulation often relate to the future sustenance of the plant physiological system and bring answers to the machinery of stress protection (Figs. 3 and 4). As a subjective and investigative tool, metabolomics estimates and characterizes the inevitable chemical signals due to stress effects. Genomic expression is not always responsible for those instant vouchers.

The plant biological system is maintained by the production of various metabolites either as a reaction outcome of primary metabolism, i.e., protein, carbohydrate and lipid, which are signature units of plant growth and developmental process, or so

Fig. 3 The route of metabolomics analysis



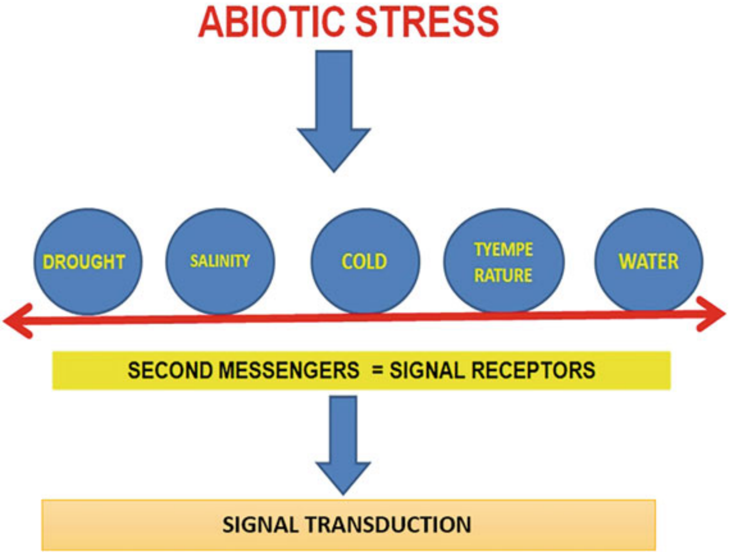


Fig. 4 Different abiotic stresses and the mechanism of action

many instant novel reactants of secondary origin. These metabolites vary with plant species and work for a large variety of plant responses to stress. Those secondary metabolites are alkaloids, polyphenol terpenoids, and many others. Metabolomic instruments and methodologies have so far identified at least 14,000–20,000 active molecules in the plant system. Thus, metabolomics can be used as a diagnostic tool to observe the environmental expression of plant morphological, phenotypic and physiological responses. Metabolomes, the derivative of the metabolic system, constitute a large package of primary and secondary metabolites (molecular weight less than 1500 Da). They originate following a definite biosynthetic pathway of the physiological system of plants. These molecular derivatives are indicators of the symbiosis between existing environmental status and the responses of the biological organism.

Extreme hypersensitivity helps a plant to sense the stress through the diversity of metabolomes and here the metabolomics provides us tools to visualize the comprehensive fingerprints (Chakraborty 2018) to screen the bioactive metabolites used in agriculture, medicine, and pharmaceutical sectors, and to analyse the network pathways in plant systems (Temitayo and Olubukola 2021). The metabolomic study includes professional and fundamental knowledge of chemistry, biochemistry, bioinformatics and statistics with suitable and specific experimental design and sample preparation, solvent selection, metabolite extraction, derivation, separation and detection, bioassay and quantification, followed by data analysis. It also employs a minimum number of experimental errors. The modelling and design should be based on a concrete procedural work line supported by critical analysis (Mushtaq et al. 2014). The possibility of huge deviation in a vast area of assimilated data always needs application of well-devised statistical and multi–variant data-analysis softwares. They include cluster analysis, pathway mapping, comparative overlays, and heat maps like exciting tools. The employment of these tools reduces and separates the ingenuity in the data collected from sample analysis (Roessner and Bowne 2009).

(a) Biotechniques and Instrumentations involved in Metabolomics

The modern instruments used in metabolomics research are direct spectroscopy, chromatography (fingerprinting), high-performance liquid chromatography (HPLC) combined with spectroscopy (IR, NIR, MS), nuclear magnetic resonance spectroscopy (NMR), liquid and gas chromatography with mass spectrometry (LC and GC-MS) and capillary electrophoresis (CE), and direct infusion electrospray mass spectrometry (Di–MS) (CE-MS) (Parida and Das 2018). These instruments analyze the abiotic stress-induced metabolic changes along with other metabolites in plants. These methodologies utilize bioassay as the proof of stress induction. The LC-MS or LC–NMR, or Fourier transformed (FT) MS are the highest precision techniques for metabolite analyses. Fingerprinting of metabolites in medicinal plants can be detected at a very minute concentration. Effective analysis is always based on scientific extraction methods like classical solvent extraction, steam extraction, supercritical fluids extraction, microwave-assisted extraction, subcritical water extraction or high hydrostatic pressure extraction (Wishart 2016). To achieve precise accuracy in quantification and characterization, chemistry based knowledge is used in target analysis (Khoomrung et al. 2014). Chawla and Ranjan (2016) showed experimental procedure where a combination of UPLC (Ultra performance liquid chromatography) with QTOF-MS (Quadrupole time of flight Mass spectrometer) rapidly identified more than 100 metabolites, increasing peak resolution with mass accuracy. Charged, neutral, polar, and hydrophobic metabolites are separated in metabolomics study through CE–MS, FT–ICR–MS. (Lopes et al. 2017). The CE-MS has been used to analyse plant primary metabolic pathway intermediates including glycolysis, TCA cycle, and pentose phosphate pathway (Jorge et al. 2016). When chromatograms of GC-MS and LC-MS trace a vast number of metabolites with their fingerprint database in response to several abiotic stress, statistical support is required to prove their fitness. Proper bioactive metabolites from induced metabolic pathways are to be compared using databases. Students t-test, ANOVA, Mann-Witney *U*-test, multiple testing corrections with false discovery rate or FDR, principal component analysis (PCA) and partial least square analysis (PLS), PLS-DA (partial least square discriminant analysis), and OPLS-DA (orthogonal partial least square discriminate analysis) have been used to analyze plant metabolomics. Commercial softwares, such as Agilent Technologies, Thermo Fisher, Waters Corporation, the National Institute of Standards and Technology (NIST), MS libraries using Metlin, Lipid maps, HMDB and mass bank, MET-COFEA, Met-Align, Chroma TOF, MET-XAlign, etc. also provide support for the analysis process (Johnson and Lange 2015; de Souza et al. 2017).

3 Metabolomics in Relation to Abiotic Stress

Green plants suffer from strains of different abiotic stresses, like salinity, water scarcity (drought), cold, heat, light intensity/UV-B, heavy metal pollution, air and water pollutions, and nutritional deficiency (Verma et al. 2006; Anjum et al. 2011; Bagheri et al. 2015; Yousuf et al. 2016a, b). Plants have identical signal perception and subsequent transcriptional expression ultimately responsible for generation of proteins that help the plant function in the metabolic system (Hirayama and Shinozaki 2010). This exercise directly influences the synthesis of different metabolites. Plant growth pattern, photoperiod, sensitivity, time of flowering and fruiting, and every change in morphological features due to the impact of abiotic factors determine the quality and quantity of secondary metabolites (Singh et al. 2000; Iqbal et al. 2004; Ali et al. 2008; Verma and Shukla 2015; Husen 2021a, b, 2022; Bachheti et al. 2021). The active constituents with medicinal properties are extracted from various plant parts like leaf, stem, flower and inflorescence, root, fruits and seeds (Ahmad et al. 2004, 2007; Siddiqi et al. 2003; Trag et al. 2005a, b). Specific molecules in response to abiotic stress or novel biomolecules with new structure are generated with new signals essential for growth and development of plants. The vast profiling of metabolites, and also their alteration under abiotic stress, signify

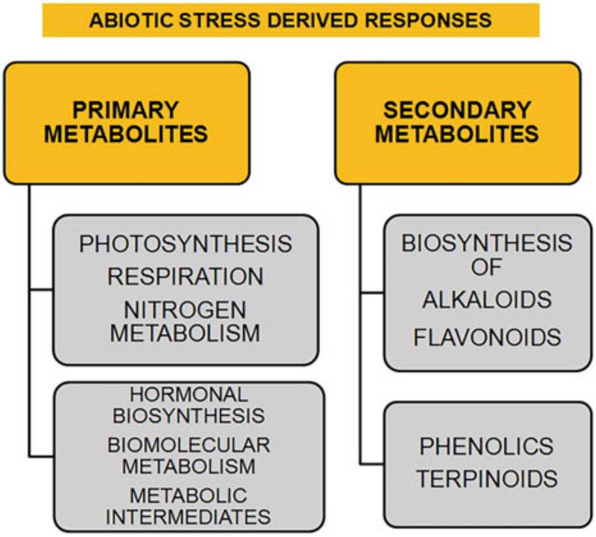


Fig. 5 Abiotic stress derived responses of plants

their definite role in cellular processes. Establishing the chemical nature of these new metabolites is an essential task in metabolomics (Li et al. 2020).

The abiotic stress-induced secondary metabolites are influenced by many such factors as competition between plants, temperature, light, humidity, and nature of soil (Das et al. 2016; Mishra 2016) (Figs. 4 and 5). The reproductive phase, grain yield, and fruit production may suffer from soil salinity and temperature rise (Lopez Delacalle et al. 2021; Nakabayashi and Saito 2015). Metabolomics is useful in analysing the secondary metabolites for drug development or preparation of herbal products with medicinal properties and in evaluating the system biology of the medicinal plants (Tweeddale et al. 1998; Ulrich-Merzenich et al. 2007; Fukusaki and Kobayashi 2005). Green plants are flexible enough against environmental stress factors in the expression of inhibited enzymatic activities, increased synthesis of reactive oxygen species (ROS), rigorous synthesis of metabolites, altered or decreased photosynthetic capacity, perceived development, low fertility with reduced reproduction with a direct effect on crop production or yield (Krasensky and Jonak 2012; Gururani et al. 2015; Mohanapriya et al. 2019).

The adverse effects of stress and subsequent metabolomic course are observed mostly in leaf and aerial parts of the plant. For instance, induction of ABA followed by the attenuated level of amino acids, raffinose, flavonols and anthocyanin in *Arabidopsis thaliana*, or proline, tryptophan, phenylalanine and histidine in maize leaves, are the common features during drought stress (Kang et al. 2019; Nakabayashi et al. 2014; Witt et al. 2012; Urano et al. 2009). The comparison of susceptible and tolerant cultivars is often associated with definite signature molecules, which differentiate the severity of the stress. Thymine, L-cysteinyl glycine and fructoselysine, phenolic compounds are traced in tolerant cultivars, whereas proline remains as a marker of susceptible variety. The TCA cycle residues citric, fumaric, malic acid, valine, and tryptophan are synthesized more in the intolerant wheat cultivars. Some other metabolites like reducing sugars, nigrose, seduheptose and galactose, pentitol, phytol, xylonic acid, d-xylopyranose, stearic acid and D-ribose have been noted in leaves and roots of peanut susceptible variety. Glucose, fructose, galactose, agmatine, cadaverine, and polyphenols such as syringic acid, vanillic acid, and catechin are recorded in tolerant cultivars (Kang et al. 2019; Gundaraniya et al. 2020 Guo et al. 2020, Hosseini et al. 2018).

There are reports of promoting intermediates of TCA cycle in wild soybeans drought-tolerant cultivars. Production of *g*-aminobutyric acid (GABA), asparagine, methionine, organic acids like galactonic acid, glucoheptonic, malonic and glycolic acids, unsaturated fatty acids including linolenic and linoleic acids, secondary antioxidant metabolites including 5-methoxytryptamine and fluorine, has been reported to increase in tolerant species. Other metabolites such as glucose-1-phosphate, D-fructose 1, 6-bisphosphate, pyruvic acid, D-glyceric acid, oxalic acid and 2-methyl fumarate have shown a significant increase in susceptible and tolerant cultivars of many crop cultivars. Some amino acids, including proline, glycine, serine, valine, beta-alanine, threonine and isoleucine, accumulate significantly in both of the drought-tolerant and drought-sensitive cultivars (Wang et al. 2019). Similar reports are available on metabolite production in barley, *Populus deltoides*, *Eucalyptus globulus*, wheat cultivars, *Hypericum polyanthemum*, *Oryza sativa*, *Salvia officinalis* and *Hordeum vulgare* (Tschaplinski et al. 2019; Yuan et al. 2018; Correia et al. 2018; Wu et al. 2017; Xiong et al. 2018; Michaletti et al. 2018; Lawas et al. 2019). Similar metabolomics reports are available on *Arabidopsis* seedling, tobacco, tomato, cucumber, aerial parts of *Lotus japonicus* seedlings, barley roots, *Lonicerae japonica*, banana, roots of *Salicornia brachiata, S. maritima and S. portulacastrum*, five genotypes of *Triticum durum* and other plants grown under salinity stress. Saponins, proline and total antioxidant capacity, sugars, flavonoids and other phenolic compounds, GABA, threonine, leucine, glutamic acid, glycine, mannose and fructose with the intermediates of TCA cycles in association cause tolerance against salinity in different genotypes.

Salinity stress increased the serotonin and gentisic acid levels in the leaves of salinity-tolerant varieties of rice (Borrelli et al. 2018; Gupta and De 2017). Metabolites synthesized in response to abiotic stress mostly exhibit antioxidative activity. They reduce the overactivity of reactive oxygen species (ROS) and resist against the destruction of biomolecules like lipid, protein, DNA. It may be mentioned that low molecular weight peptides (0.5–3.0 kDa) of mulberry leaf with high antioxidative properties are helpful in rearing of silkworm by contributing to different attributes of the process. In wheat plant, in response to chlorinated compounds, high synthesis of antioxidants has been observed, whereas in cucumber, nanoparticle exposure induced stress, which ultimately expressed with high antioxidative property (Liu et al. 2020; Guo et al. 2020). More than two lakh secondary metabolites have been traced in medicinal plants by metabolomics investigation. Above 5000 secondary metabolites have been detected by metabolomics study in *Arabidopsis thaliana* alone. Harvey (2007) reported a group of anticancer metabolites, namely paclitaxel (taxol), camptothecin (irinotecan, topotecan) and podophyllotoxins (etoposide, teniposide). Metabolic fingerprinting is of common occurrence now for new chemical entities (NCEs) in drug discovery and development. Aspirin has been derivatized from *Salix alba* using NMR–MS techniques and about 200 metabolites have been tested in *Triphala*, using HPLC (Newman and Cragg 2007; Dudley et al. 2010). Kooy et al. (2008) undertook the metabolite screening of *Artemisia* and determined the artemisinin by NMR–PCA analysis and LC-MS method. Mazzei et al. (2016) applied NMR to study the metabolic profile of tomato leaves, where seeds were treated with secondary metabolites, *viz.* harzianic acid and 6-pentyl-2H-pyran-2-one, released by the fungus *Trichoderma*. (Ouakhssase et al. 2019)

The metabolic network consists of all chemical reactions that involve metabolism of small molecules known as metabolites with catalytic proteins called enzymes. Bacteria, fungi and plants produce various specialised metabolites (also known as natural products) that allow them to flourish in their respective habitats. These molecules play important roles in the microbiome, which include competition and collaboration, using signals, nutrient scavenging agents, weapons, and stress protectants (Medema 2018). Medicinal Plant Metabolomics Resource (MPMR) provides a framework for generating experimentally testable hypotheses about the metabolic networks that lead to the generation of specialised compounds, identifying genes that control their biosynthesis and establishing a basis for modelling metabolism in less-studied species. The database is publicly available and can be used by researchers in medicine and plant biology. It started with a joint venture of the illustrative field of medicinal plant chemistry, biochemistry, metabolic modelling and drug development. At present, 13 research units in 7 different institutions are working worldwide on the metabolomics of 14 important medicinal plants. Markley et al. (2017) prescribed NMR for detailed studies of a complex mixture of small biomolecules, their network in cells and their interactive participation with other macromolecules. On the other hand, GC–MS system separates a particular compound from a mixture through gas chromatography, ionization followed by mass analysis, detection of ions, and analysis of the collected material through a computer database (Mani et al. 2017). The LC–MS is also sensitive with high-resolution technique to separate and identify the charge of the metabolite (Pérez-Victoria et al. 2016; Cattaneo et al. 2016). Clear-cut extraction and identification of metabolic networks and metabolites often help isolate the specialised metabolites that may act as stress protectants (Medema 2018). To investigate g-aminobutyric acid in tomato and vindoline in *Catharanthus roseus*, new metabolomics technique, *viz.* virus-induced gene silencing (VIGS), and in potato transcription activator-like effector nuclease (TALEN) technology have been utilized (Besseau et al. 2013; Zhang et al. 2018). It is known that being a treasure house of secondary metabolites, medicinal plants may supply natural drugs to 80% of the world population, as the dependency on natural drugs is increasing in the advanced nations also.

Flavonoids synthesis to initiate antioxidative function under abiotic stress has been observed in *Arabidopsis*; likewise, *Hordeum vulgare* synthesizes chlorogenic acid, flavonol glycosides and anthocyanins against H_2O_2 and $^*OH$ (as antioxidative units). Over 259 metabolites have been identified through electrospray ionization-based MS/MS spectral analysis in metabolomics study of different medicinal plants (Tohge et al. 2013; Sueishi et al. 2014; Nakabayashi et al. 2014; Premai et al. 2018). Elevated stress tolerance is achieved by metabolic alterations (Banerjee and Roychoudhury 2019). Drought condition alters the metabolic sequence of reactions, which elevates the expression of flavonoids and phenolics and associated enzymes in *Scutellaria baicalensis* (Ghatak et al. 2018; Chen et al. 2019), *Hypericum brasiliense*, *Artemisia* spp. (Verma and Shukla 2015), and *Glycyrrhiza glabra* (Hussain et al. 2018). At least 100,000 secondary metabolites are reported from different plant families. Cyanogenic glycosides, alkaloids, and glucosinolates, flavonoids and phenylpropanoids, isoprenoids are the major secondary metabolites with medicinal potency, which are modulated by abiotic stress factors that have foot prints in metabolic pathways (Borges et al. 2017). Metabolite fingerprinting of *Emblica officinalis* Gaertn. (Euphorbiaceae), *Terminalia bellerica* Roxb. (Combretaceae) and *Terminalia chebula* Retz. (Combretaceae), the famous Triphala, has been made possible through HPLC–PDA analysis (Ponnusankar et al. 2011).

It has been reported that production of secondary metabolites in many medicinal plants such as *Betula spp., Salix myrsinifolia, Arabidopsis thaliana, Secale cereale, Artemisia annua, Sambucus nigra, Cornus sanguinea, Prunus serotina, Frangula alnus, Corylus avellana, Pteridium arachnoideum, Solanum tuberosum, Arnica montana, Hypericum perforatum, Achnatherum inebrians, Quercus* spp., suffers from extreme light condition (Das et al. 2016). Abiotic stresses disturb the synthesis and expression of phytohormones (Khan et al. 2017). The effect of UV light on the synthesis of secondary metabolites often appears promotive in certain medicinal plants (Katerova et al. 2017). The biosynthesis of secondary metabolites, their quality as active ingredients, their storage capacity and yield depend on photoperiod and light intensity (Verma and Shukla 2015; Li et al. 2020). In *Ocimum tenuiflorum*, salt stress affects the active ingredients like phenylpropanoids and terpenoids. In *Lonicerae japonicae*, salt stress has been found to be responsible for the expression of 47 different metabolites (Rastogi et al. 2019). Metabolomics analysis (NMR and GC-MS) of *Aegle marmelos*, *Andrographis paniculata*, *Bacopa monnieri*, and *Commiphora wightii* has been conducted with leaves, stem, roots, latex and fruits. Recently, metabolomics analysis was done on leaves of *Emblica officinalis* (Amla) and *Ocimum sanctum* (Tulsi), using LC–MS. Metabolomics in *Withania somnifera* roots has also been carried out (Liu et al. 2020). Similar studies have been done in fruits, leaves and roots of *Piper longum*, in seeds of *Rauwolfia serpentina*, and in the stem of *Santalum album* for essential oils. (Cai et al. 2020)

4 Conclusion

Plants with properties that can heal human diseases suffer from abiotic stress factors. Sometimes the resultant effect may lead to their extinction. However, our discussion on the subject clarifies that plant metabolic system is prepared with a machinery that uses the stress period for its own sustenance. The induction of tolerance brings changes through the metabolic system in the molecular world of the plant. The responsive molecules, in many instances, express medicinal properties of the plant on several fronts. The effect of abiotic stress factors on plants and the expression of newly synthesized metabolites are detected by sophisticated ultrasensitive instruments and methods related to different science disciplines. Plants are huge in number, so the informative data or analytics coming to date only count the handful of sands standing in a vast desert. The wealth of information using the metabolomics study may be a future storehouse of miracle molecules that may greatly contribute to human civilization's development, progress, and sustenance.

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Secondary Metabolite Production in Medicinal Plants under Abiotic Stress

Ritu Mahajan, Tania Sagar, Shajaat Hussain, Nipunta, and Nisha Kapoor

Abstract Like other plants, medicinal and aromatic plants have evolved several mechanisms to accommodate themselves under changing environmental conditions, so as to carry various functions necessary for their physiological processes. Several abiotic factors have hindered their growth and development. This has resulted in the production of repertoire of secondary metabolites in order to cope with stress and develop defensive means. Numerous environmental factors, like drought, temperature, light and salinity, trigger various signaling pathways that result in secondary metabolite production. These metabolites also serve as exceptional sources for flavours, food additives and industrially important pharmaceuticals. The secondary metabolite production also depends upon the genotype and developmental stage of the plant and upon various environmental factors during its period of growth. *In vitro* medicinal plant cultures are widely used for studying and enhancing the production of plant secondary metabolites. Also, the use of various molecular biology tools has facilitated the understanding of different pathways involved in the secondary metabolite production. This chapter summarizes the effect of various abiotic factors on the production of secondary metabolites in medicinal plants.

Keywords Abiotic stress · Environmental factors · *In vitro* · Medicinal plants · Secondary metabolites

1 Introduction

Plants produce many non-nutritive compounds that have no fundamental role in their life processes but have a role in their interaction with environment, and prove useful in adaptation and defense. Plants do not require these compounds, called secondary metabolites, for survival, but often these are important for human health (Aslam et al. 2020; Husen 2021a; Rahman and Husen 2021). They are the low molecular weight chemical molecules that are distinct from the primary metabolites and help plants to

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A. Husen, M. Iqbal (eds.), *Medicinal Plants*,
https://doi.org/10.1007/978-981-19-5611-9_4

interact with their biotic and abiotic environments, as well as establish defense mechanisms (Vardhan and Shukla 2017; Kaur et al. 2021). These metabolites represent a subset of the metabolome of the plant species. The accumulation of such metabolites is common in plants that are exposed to various microbes or harsh environmental conditions. These metabolites are useful for humans as they are an important source of food additives, flavours, medicines, cosmetics, nutraceuticals, and other industrially important drugs (Kumar and Sharma 2018). Plants are constantly exposed to a variety of environmental stresses, which is the primary cause of increased metabolite concentration in plants (Iqbal et al. 2018). Also, abiotic stresses can influence the phenological events; structural patterns, physiological phenomena, and biochemical status of plants (Iqbal et al. 2000a, 2005, 2010; Gharibi et al. 2016; Husen 2021b, c, d, e; Zhang et al. 2022; Deepti et al. 2022a, b). The biological ability of plants to produce metabolites is regarded as an adaptive capacity for coping with stressful constraints in challenging and changing environmental conditions, which include the production of complex chemicals and interactions in structural and functional stabilization through signaling processes and pathways (Ali and Baek 2020). The concentration of secondary plant products is highly influenced by the factors involved in growth, and they have an impact on the metabolic pathways that lead to the production and accumulation of naturally related products. When plants detect stress at the cellular level due to high and low temperatures, drought, alkalinity, salinity, UV exposure, or pathogen infection, a stress response is triggered and the level of secondary metabolites increases (Ramakrishna and Ravishankar 2011) (Table 1). Certain extreme stressful conditions like heat or severe temperature, photosynthesis and respiration also have a significant and detrimental effect on the whole plant (Aref et al. 2013a, 2016; Bashir et al. 2015; Zandalinas et al. 2020). However, synthesis of these secondary metabolites is often limited and is highly dependent on the developmental and physiological stage of plants. So, it has become vital to create new ways for designing new plant varieties that can resist and sustain the maximum production potential. In response to various stimuli, plants activate a number of physiological and biochemical pathways that are either adopted or common as sometimes excessive formation of reactive oxygen species is a frequent and rather general response to plant stress (Bashir et al. 2007; Aref et al. 2016; Sewelam et al. 2016) (Fig. 1). Nowadays, individual cells are cultured in bioreactors to produce important substances especially secondary metabolites for the chemical-pharmaceutical industries hence heterologous expression of foreign genes *in vitro* cells is also achievable with metabolic engineering technologies (Wawrosch and Zotchev 2021). Application of molecular biology approaches has facilitated in deciphering various molecular mechanisms that aid in the biosynthesis and accumulation of many secondary metabolites in medicinal plant species. This chapter summarizes role of various abiotic stresses on the production of secondary metabolites in medicinal plants and various genes involved in their synthesis.

Table 1 Abiotic stress and their effect on secondary metabolite production in medicinal plants

S. no	Plant Species	Type of Stress	Secondary Metabolite produced	Reference
1	*Catharanthus roseus*	UV radiation	Vinblastine	Liu et al. (2011)
2	*Centella asiatica*	Low temperature and water dehydration	Asiaticoside	Plengmuankhae and Tantitadapitak (2015)
3	*Dysoxylum binectariferum*	Drought	Rohitukine	Kumara et al. (2016)
4	*Artemesia annua*	Drought	Artemisinin	Vashisth et al. (2018)
5	*Nicotiana tabacum*	Chilling	Glucosinolates	Zhou et al. (2018)
6	*Solanum lycopersicum*	Salt stress	Sesquiterpenes	Kang et al. (2019)
7	*Mentha piperata*	Heat and drought stress	Terpenoids and alkaloids	Alhaithloul et al. (2019)
8	*Vitex agnus-castus*	Drought and shading	Caryophyllene	Rezaei et al. (2019)
9	*Ocimum basilicum*	Cold stress	Phenylpropanoid contents	Rezaie et al. (2020)
10	*Andrographis paniculata*	Drought stress	Andrographolides	Chen et al. (2020)
11	*Astragalus compactus*	UV radiation	Phenolic acids	Naghiloo et al. (2021)
12	*Salvia fruticosa*	Water deficit	D-limonene	Ait Elallem et al. (2021)
13	*Eclipta prostrata*	Saline Conditions	Phenolic compounds	Duc et al. (2021)
14	*Pelargonium graveolens*	Metal stress	Flavonoids	Chrysargyris et al. (2021)
15	*Rhodiola semenowii*	Cold stress	γ-Tocopherol	Terletskaya et al. (2021)
16	*Panax ginseng*	Cold stress	Saponins	Zhang et al. (2021)
17	*Codonopsis pilosula*	Drought stress	Obetyolin and Syringin	Liang et al. (2021)
18	*Glycyrrhiza uralensis*	Metal stress	Carbon metabolites	Cui et al. (2021)

2 Drought Stress

In plants, drought stress is a prevalent and multifaceted abiotic stress that affects the phenomena of growth and development (Husen 2010; Husen et al. 2014; Getnet et al. 2015; Embiale et al. 2016). Various drought incidents have been noted in the previous few decades, which make the farming very challenging (Yadav et al. 2020). Drought stress occurs due to deficiency of water usually escorted by elevated solar

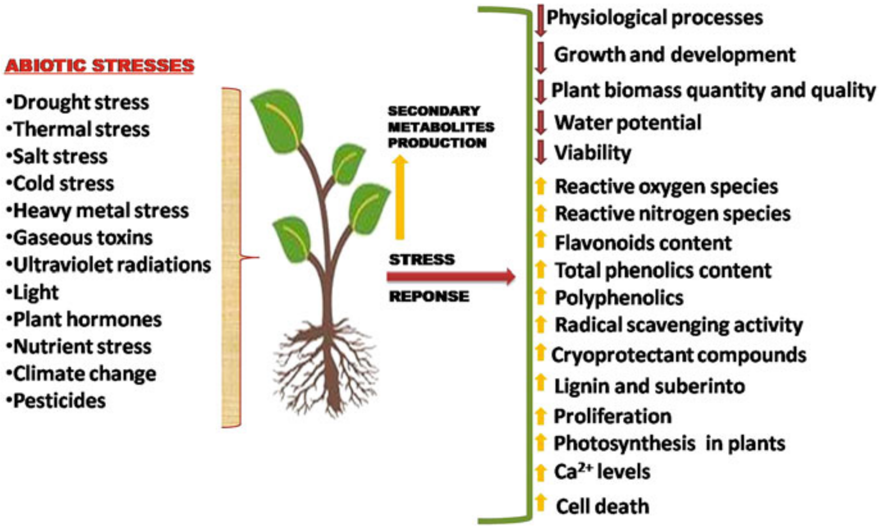

Fig. 1 Response of medicinal plants to various abiotic stresses

radiations as well as temperature, which causes reduction in water potential along with turgor pressure. Consequently, it leads to several changes in plants including physiological, morphological, molecular, ecological as well as biochemical, which in turn affect the plant biomass quantity and quality (Aref et al. 2013a, b; Mashilo et al. 2017). Eventually, plants have acclimated to drought environment by the production of secondary metabolites through the induction of ionic or osmotic stress (Isah 2019). Application of growth stimulants, such as indole acetic acid, may alleviate the damage caused by water stress (Husen et al. 2017). Secondary metabolites play a crucial role in the interaction of cells with the environment and to survive in stressful conditions via a signaling pathway (Erb and Kliebenstein 2020; Hatcher et al. 2020). Water scarcity results in enhanced creation of reactive oxygen species as well as reactive nitrogen species, and this disturbs the redox regulatory functioning of the cell (Aref et al. 2016; Laxa et al. 2019; Sharma et al. 2020a).

In several plant species, an enhanced level of secondary metabolite production has been observed in response to drought conditions. Wang et al. (2019) observed strong resistance to drought in *Matteuccia struthiopteris* and *Athyrium multidentatum* that caused as enhancement in flavonoids, total phenolics and proanthocyanidins, enzyme activity, osmotic substances along with reduction in the leaf water content. In *Achillea pachycephala*, the phenolic content improved simultaneously, whereas the flavonoids content declined under drought stress (Gharibi et al. 2019). In *Ophior rhizamungos*, manipulation in media impacts the camptothecin and biomass production (Deepthi and Satheeshkumar 2017). Several researchers have also induced drought stress conditions *in vitro* for secondary metabolite accumulation, by manipulating the culture medium (Ghosh et al. 2018).

Ahmad et al. (2020) induced drought stress using various concentrations of PEG in Stevia shoot cultures and observed enhancement in steviol glycosides and phenolics.

Jafari and Shahsavar (2021) demonstrated that foliar application of melatonin enhanced the flavonoid and total phenolic concentration in two citrus species including *Citrus latifolia* Tanaka (Persian lime) and *Citrus aurantifolia* (Mexican or Key lime) in response to drought stress. Furthermore, hesperidin was the foremost polyphenol and limonene was the main essential oil component observed in both the varieties. While, Naghizadeh et al. (2019) reported that melatonin foliar application enhanced secondary metabolites in *Dracocephalum moldavica* in response to drought stress via regulating the process of secondary metabolism and the activity of enzymes including phenylalanine ammonia-lyase and polyphenol oxidase.

In drought stress tolerance, metabolic genes are also key factors via their involvement in different metabolic pathways (Kumar et al. 2021). Moreover, transgenic *Arabidopsis thaliana* overexpressing the CeWR14 gene from *Cyperus esculentus*, showed increased drought stress tolerance and enhanced biosynthesis as well as deposition of cuticular wax (Cheng et al. 2020).

3 Thermal Stress

Thermal stress is a major abiotic stress that affects plants' performance and in turn substantially impact the production of secondary metabolites, due to alteration in their metabolic process during such unfavourable conditions (He et al. 2018). Several studies have reported the influence of higher temperatures on physiology, defence mechanism and metabolite production in plants (Aref et al. 2016). In a recent study, it is reported that the accumulation of anthocyanins, proline and furanocoumarins has been significantly increased in *Heracleum sosnowskyi* in response to high temperature, but at the same time it also causes serious human health hazards, especially severe dermatological effects when comes in contact with skin (Rysiak et al. 2021). Alhaithloul et al. (2021) observed variation in phenolics and flavonoids content in seedlings of *Solanum lycopersicum* under thermal stress. In contrast, In *Helicteres isora* callus cultures, thermal and light stress presented synergistic action on secondary metabolites production (Joshi 2015). In *Stevia rebaudiana*, enhancement in incubation temperature affected the growth of hairy root cultures, which further enhanced the stevioside production up to certain extent (Kumari and Chandra 2016). Sharma et al. (2020b) reported reduction in concentration of hyperforin, hypericin as well as pseudo hypericin due to the down-regulation of temperature in medicinal plant, *Hypericum perforatum* with hypericin concentration being 13.76% higher in Free Air Temperature Increase than in the ambient temperature.

Jespersen et al. (2017) studied expression patterns of some selected genes involved in regulating heat tolerance in *Agrostis capillaris* and *Agrostis stolonifera.* They reported PCR-based markers which were associated with candidate genes related to heat tolerance so as to develop new cultivars with increased tolerance, using marker-assisted selection. In transcriptional regulation of stress responses certain transcription factors play essential roles. Mohanta et al. (2020) reported the role of NAC transcription factors against heat stress tolerance.

4 Salt Stress

Salt stress is a major abiotic stress that reduces the growth and causes change in physiological as well as metabolic processes in plants (Hussein et al. 2017; Manuka et al. 2019). Salinity leads to cellular dehydration, which in turn creates ionic and osmotic stress that reduces or enhances the augmentation of specific secondary metabolites in plants (Qureshi et al. 2013; Gupta and Huang 2014) Prolonged exposure to high NaCl concentration activates the biosynthesis of proline so as to decrease the cellular damage and maintain the ROS homeostasis along with enhanced malondialdehyde concentration, thus enhancing the tolerance of plants to lower level of salinity stress (Ali et al. 1999; Arshi et al. 2010; Hnilickova et al. 2021). Sarri et al. (2021) demonstrated alteration in the metabolic profile of *Medicago arborea* and *Medicago sativa* along with their hybrid *Alborea* where overexpression of saponins was observed in the roots of *Medicago arborea*, while it was in shoot part of *Medicago sativa.* However, in hybrid *Alborea* most of the secondary metabolites were not upregulated when subjected to salt stress.

The secondary metabolite production in *Sesuvium portulacastrum* decreases physiological processes in response to salt stress, while their strong antioxidant properties enhance the survival of plant under saline conditions (Xu et al. 2016). Abdel-Farid et al. (2020) reported enhancement in the content of phenolics, flavonoids, saponins, proline as well as total antioxidant capacity in *Cucumis sativus* in response to saline stress. Also, the application of sodium chloride in embryogenic tissue culture of *Catharanthus roseus*, resulted in enhancement in the synthesis of vincristine and vinblastine (Fatima et al. 2015).

Ni et al. (2015) showed increase in sitosterol content in cell suspension culture of *Nitraria tangutorum* in response to NaCl treatment at a concentration of 250 mM. In another study, KCl and $CaCl_2$ enhanced the production of bacosideA content in *in vitro* cultures of *Bacopa monnieri* (Ahire et al. 2014), while the exogenous application of melatonin in *Malus hupehensis* modulated the content of flavonoids when subjected to salt stress (Wei et al. 2019a, b). The damage caused to plants by salinity could be reduced by application of certain growth-promoting substances like indole-acetic acid and salicylic acid (Husen et al. 2016, 2018, 2019). Bistgani et al. (2019) reported that under salt stress, melatonin foliar application enhanced the total phenolic contents in *Thymus daenensis* while in *Ocimum basilicum,* Bahcesular et al. (2020) reported the effects of melatonin priming on growth and development along with phenolic acids, essential oil as well as antioxidant activities in response to salt stress.

In plants, the genetic regulation mechanism in response to salinity stress involves the activation of various signaling transduction pathways (Qari and Tarbiyyah

2021). The well-known signaling molecules those gets active at the initial steps in response to abiotic stresses including Ca^{2+} ions and reactive oxygen species (Feno et al. 2019). It was found that salt overly sensitive-3 gene (SOS3) codes a protein that functions as a receptor for Ca^{2+} ions. Moreover, the enhanced Ca^{2+} levels activate the cascade of events which in turn causes the activation of genes that help in protecting the plant from salinity stress (Koster et al. 2019). Rastogi et al. (2019) reported *de novo* transcriptome sequencing in *O. tenuiflorum* against drought and salinity stress response where they observed decrease in eugenol which is the main secondary metabolite.

5 Cold Stress

Low temperature or severe cold conditions affects the temperate plants (Amiri et al. 2020). The temperate plant species adapt to such unfavourable conditions of low temperature via their metabolism and by enhancing the content of their cryoprotectant compounds. In plants, the mechanism of cold tolerance has been divided into chilling tolerance and freezing tolerance (Sun et al. 2020). Under chilling tolerance, plants respond to temperatures of 0–15 °C, while in freezing tolerance plants respond to subzero temperatures (Zhang et al. 2020). At present, metabolomics is a powerful tool for the detection of metabolites in plants when subjected to cold stress (Clemente-Moreno et al. 2020). Plants produce lignin and suberin to deal with cold stress in order to reduce the adverse damage from low temperature (Jian et al. 2020). Similarly, plants accumulate proline, raffinose, betaine, inositol to adjust them to low temperature stress, as these metabolites show protective functions toward adverse effects from freezing (Zhao et al. 2019). Rezaie et al. (2020) revealed a significant positive correlation between total phenolics, phenylalanine ammonia-lyase activity as well as total flavonoid content in *Ocimum basilicum* in response to cold stress. The seedlings of *Solanum lycopersicum* under cold stress showed more desirable profile of phytochemicals and exhibited higher antioxidant activity than those of heat stressed seedlings (Alhaithloul et al. 2021). Cold stress in combination with acidic pH affected the production of flavolignans in *Silybum marianum* hairy root cultures (Rahimi and Hasanloo 2016).

Xu et al. (2020) found many differentially expressed genes (DEG), which were upregulated in tobacco plant during the treatment of cold acclimation. These genes are essential for intracellular Ca^{2+} levels sensing and for starting calcium-dependent signaling processes. In *Broussonetia papyrifera*, LBD transcription factors play important roles in response to cold stress (Peng et al. 2015). Mala et al. (2021) reported number of genes associated with signaling pathway and secondary metabolites with cellular protection that helps in the survival of *Rheum australe* at high altitude alpine region of Himalayas.

6 Heavy Metal Stress

Heavy metal stress has become one of the most significant abiotic stresses that influence the production of secondary metabolites in plants (Hurmat and Bansal 2020). The enhanced use of chemicals in industries and agro-techniques has resulted in higher bioaccumulation and toxicity of heavy metals (Ansari et al. 2012; Diwan et al. 2012; Rasool et al. 2013). Heavy metals can alter the metabolic activity of plants, leading to alterations in their form and function (Umar et al. 2005; Ashraf et al. 2018; Haider et al. 2021). Saba et al. (2000b) noted that the yield of lepidine in *Lepidium sativum* was variable depending upon the source and type of the explant used, the maximum coming from mature *in vivo* plants at vegetative stage. They increased it further by adding $ZnSO_4$ or $CuSO_4$ to the culture medium. Application of $CuSO_4$ in shoot cultures of *Artemisia annua* resulted in noticeable enhancement in the content of artemisinin (Darki et al. 2019). Rao et al. (2021) demonstrated that the exposure of cadmium at a concentration of 0.5–4 mM to cell suspension cultures of *Abutilon indicum*, increased the production of stigmasterol. Similarly, addition of cadmium in *in vitro* grown tissues of *Allium sativum* resulted in allicin enrichment (Malik et al. 2020). Farrokhzad and Rezaei (2020) reported that the application of aluminium chloride at 50 and 100 μM concentration enhanced the production of Taxol in cell suspension culture of *Corylus avellana.* In *Ocimum basilicum* cell suspension cultures, $AgNO_3$ induced the Linalod Estragole production (Açıkgöz 2020). Recent investigations are also using nanoparticles to promote plant metabolic activities (Husen and Iqbal 2019). For example, Shakya et al. (2019) reported that the addition of iron and zinc nano-oxides to cell suspension cultures of *Hypericum perforatum* increased the production of hypericin and hyperforin. In *in vitro* cultures of *Camellia sinensis*, implication of Cobalt ions enhanced the cinnamic acid production (Sutini et al. 2019).

Exposure of *Belamcanda chinensis* to $CuCl_2$ enhanced the production of total flavonoids (Iristectorigenin A, tectorigenin and tectoridin), proline as well as activity of GPX and SOD (Zhu et al. 2020). Dobrikova et al. (2021) reported that cadmium application in *Salvia sclarea* increased the accumulation of phenolic compounds along with anthocyanins. Application of $CuSO_4$ in *Imperata cylindrica* enhanced the antioxidant capacity including accumulation of hydroxycinnamic acid, flavons and cyanidins (Vidal et al. 2020).

Tolerance to heavy metal stress has been studied in plants by determining the function of different genes involved in biosynthetic pathways of secondary metabolities (Anjitha et al. 2021). Yu et al. (2021) studied the overexpression of NtMT genes (MT2C, MT2F and MT2G) in tobacco seedlings, when they were administered with higher concentrations of Cu, Zn and Mn, which in turn provided tolerance to heavy metal stresses.

7 Gaseous Toxins

Diverse human activities cause addition of hazardous substances in the environment (Iqbal et al. 2000b). Air pollutants like hydrogen sulfide have an increased ability to enter into the plants through stomatal openings or through roots (Pandey and Gautam 2020). Liu et al. (2021) demonstrated the physiological functions of hydrogen sulfide in biotic as well as abiotic stress responses. Various studies have reported that exogenous treatment of hydrogen sulfide can increase oxidative stress due to the expression of some enzymes (Guo et al. 2018; Wei et al. 2019a, b). Chen et al. (2016b) reported that hydrogen sulfide mediate the biosynthesis of nicotine in *Nicotiana tabacum* under thermal stress.

Many studies have revealed that sulphur dioxide (SO_2) present in the coal-smoke emitted mainly from chimneys of thermal power plants, or chemically produced SO_2 for laboratory or field experiments, has tremendous impacts on the form and function of exposed plants, leading to alteration in their metabolic activities (Husen et al. 1999; Singh et al. 2000; Husen and Iqbal 2004; Verma et al. 2006; Ali et al. 2008; Nighat et al. 2008; Iqbal et al. 2011b; Husen 2021b; Rahman and Husen 2022). Giraud et al. (2012) studied the effect of sulfur dioxide fumigation and observed alteration in the synthesis of anthocyanin. However, flavon-3-ol transcript small abundance immediate after fumigation showed no anthocyanin reduction. The safflower plants pretreated with sodium hydrosulfide at 0.5 mM concentration, enhanced the production of secondary metabolites and braced up the antioxidant capacity by abating the oxidative damage in response to drought conditions (Amir et al. 2020).

Khan et al. (2018) reported that the exogenous application of hydrogen sulfide enhanced the expression of GSH-associated genes including GST Tau, MAAI, APX, GR and MDHAR on exposure to chilling stress. Moreover, sodium hydrosulfide exogenous application enhanced the activities of enzymes including D/L-CDes, CAS, OAS-TL and CA along with elevation of endogenous cysteine as well as hydrogen sulfide content (Khan et al. 2018; Li et al. 2019).

8 Ultraviolet Radiations

Ultraviolet radiations (UV) show negative impact on the proliferation and photosynthesis in plants that leads to over synthesis of reactive oxygen specie. These radiations also affect the macromolecules, oxidative stress, decrease cell viability and cause cell death (Sharma et al. 2019; Deepti et al. 2022b). UV radiations of wavelength 290–320 nm causes several changes in plant metabolites including alkaloids and flavonoids which are involved in the plant defence system (Schreiner et al. 2012). UV radiations have different effect on the phenolic acids, and these radiations also decreased the concentration of caffeic, chlorogenic, gallic and p-coumaric acids. Exposure of UV light to *Scenedesmus quadricauda* were not found to be sufficient for the stimulation of phenolic metabolites (Kovacik et al. 2010). Bian et al. (2018) reported B type UV radiation to be a potential elicitor agent for the synthesis of secondary metabolites in plants where significant increased in flavonoid levels were observed in *A. sessilis* and *A. brasiliana.* El-Garhy et al. (2016) showed that gamma radiation influences the flavonoid content with the up-regulation of chalcone synthase (CHS) genes expression. Vardhan and Shukla (2017) reported increased in the psoralen content and capsaicinoids in paprika when whole plant was exposed to the gamma radiations. Through exposure of ultraviolet (UV) light, chilling, heat shock in *Artemisia annua*, the transcriptional levels of cytochrome P450 monooxygenase (CYP71AV1) genes and amorpha-4,11-diene synthase (ADS) were upregulated with comparison to others plants (Yin et al. 2008; Sharma 2018).

9 Light

Light is one the main physical factors responsible for influencing the plant secondary metabolite production. Plant secondary metabolite biosynthesis and accumulation is highly affected by the light. Secondary metabolites such as carotenoids, flavonols, anthocyanins occur in low concentration and are essential for various metabolic functions, even survival of the plant (Pagare et al. 2015; Mehta et al. 2017). Pérez-López et al. (2018) reported that high light intensity and low CO_2 level results in ambient conditions needed for the accumulation of anthocyanins in red leaf lettuce. *Peucedanum japonicum* callus cultures when exposed to red and blue spectra of light, showed proliferation of calli, differentiation of somatic embryos as well as secondary metabolite production (Chen et al. 2016a). *Artemisia absinthium* cell suspension cultures with light and dark conditions showed substantial effect on biomass and secondary metabolites production (Ali and Abbasi 2014). In *Zingiber officinale* callus cultures, light stimulated the zingiberene and gingerol production while in *C. acuminata* seedlings it influenced the CPT biosynthesis (Anasori and Asghari 2009). Hairy root culture of *Artemisia annua* affected by the light radiations produced arteminsin while white light on cell cultures of *Taxus cuspidate* resulted in taxol and baccatin production (Liu et al. 2003). During growth and physiological state of tissues or organs, apparently light has influenced on them, while secondary metabolite metabolism has multifaceted and depended on species consideration (Ghosh et al. 2018). In *Xanthum* species, duration of light affects the phenolic, phenylpropane derivatives and also results in reduction of flavonoid content (Hu et al. 2019). Weremczuk-Jeżyna et al. (2021) demonstrated influence of different light sources on growth and phytochemical profile of *Dracocephalum forrestii* and consequently, white LED resulted in benefical shoot growth while blue light increased apigenin p-coumaryl rhamnoside biosynthesis. Hence, effect of light stress may turn out to be a simpler option of metabolic engineering for improving the production of secondary metabolites of therapeutic value (Weremczuk-Jeżyna et al. 2021). The exposure of sunlight on grapes enhanced the structural and regulatory expression of anthocyanins, and phenolics producing genes (Abeysinghe et al.

2019). Wang et al. (2020) found that high intensity of blue light immensely regulates various physiological processes and secondary metabolite production in tea plant. The transcriptomic and metabolomic analyses have shown the effect of transcription factor basic helix-loop-helix (bHLH) and MYB that are involved in the regulation of flavonoid synthesis (Kaur et al. 2019, 2020).

10 Plant Hormones

Plant hormones, also referred to as phytohormones, are a group of biochemical molecules that influences plant growth and production under various environmental conditions (Siddiqi and Husen 2017, 2019, 2021). Primary metabolites are mainly characterized with physiological processes that may regulate plant growth and development, whereas secondary metabolites are mostly related to the plant defense mechanism (Davies 2010). Plant hormones play a significant role as signaling molecules that induce stress on the secondary metabolite production (Farooq et al. 2011). Production of silybin content in the cultured tissues of *Silybum marianum* varied with age and the medium composition. Saba et al. (2000a) obtained the maximum yield of silybin after 8 weeks on media supplemented with zeatin, a cytokinin derived from adenine. Amoo and Van Staden (2013) reported the role of topolins either singly or with combination of auxins in *Huernia hystrix* that resulted in effective shoot proliferation and secondary metabolite production. Ramakrishna et al. (2011) investigated the role of calcium on *in vitro* cultures of *C. canephora* that resulted in improved somatic embrogenesis and indirectly enhance phenolic phytoalexins. Some growth regulators like Jasmonic acid and methyl jasmonates prompted the production of various organic compounds such as terpenoids, alkaloids, coumarins, taxanes and phenolic phytoalexins (Ruan et al. 2019; Wang et al. 2021). Grsic et al. (1999) reported the effect of methyljasmonates on biosynthesis of endogenous IAA that increased the content of shikonin and red naphthaquinonone in cultured cells of *Onosma paniculatum*. Cabanas-Garcia et al. (2021) reported efficient method for friable callus induction of medicinal cactus *Coryphantha macromeris*, studied its phytochemical profile, kinetic behaviour and also assessed maximum biomass production. Murashige and Skoog medium supplemented with 6-benzylaminopurine and picloram induced callus with maximum biomass accumulation. Even, using Ultra-High-Performance Liquid Chromatography-tandem Mass Spectrometry analysis showed phytochemical profile of *Coryphantha macromeris* and obtain 11 organic acids, 16 phenolic acids, 8 flavonoids, and 17 metabolites. Shoja and Shishavani (2021) in their study reported that with various types of cytokinins such as kinetin, benzyl adenine and thidiauron increased growth parameters and secondary metabolite production along with photosynthetic pigments in *Hissopus officinalis*. Karakas (2020) observed that phenylalanine ammonia-lyase enzyme triggered the production of phenolic molecules. Qari and Tarbiyyah (2021) reported that stress responsive genes produce protein products such as ethylene, Jasmonic acids which are regulated by the signaling cascade of mitogen activated protein kinases and calcium dependent protein kinases that contribute to the gene expression against plant hormonal stress.

11 Nutrient Stress

Due to nutrient stress, plants cells, tissues and organs undergo various physiological and molecular changes for the stabilization of photosystem II complex, accumulation of osmo-protectants, maintain membrane integrity, protein structure and ROS-scavenging activity (Rejeb et al. 2014). Carbon and nitrogen have significant effect on the biosynthetic pathways of bioactive compounds, most particularly on the secondary metabolites and biomass (Hansch and Mendel 2009). Ganie et al. (2017) observed that N application effectively altered the level of cysteine, methionine, asparagine, arginine, phenylalanine, glycine, glutamine, aspartate and glutamate in maize genotypes. The asparagine and glutamine contents increased, while those of glutamate, phenylalanine and aspartate decreased with low N (0.05 mM) supply. Serine content increased in low-N-tolerant genotype but decreased in low-N-sensitive genotype. Resupply of N to both genotypes restored the amino acids level to that in the control. Further, Ganai et al. (2020) noted that low-N condition reduced the levels of N-containing metabolites, organic acids and amino acids, but increased the soluble sugars. Hakeem et al. (2011) pointed out that high-affinity transporter systems, high NR, and glutamine synthetase activity, and distribution of soluble protein content have a key role in the N-use-efficiency of rice genotypes. Mineral nutrients may cause negative or positive impact on plant growth and biomass production, and modulate the secondary metabolite production depending upon the environmental factors (Hassan 2012; Savvas and Gruda 2018). Saba et al. (2000b) were able to increase the yield of lepidine in *Lepidium sativum* explants by adding 900 μM Zn^{2-} or 100 μM Cu^{2-} to the culture medium. Ramakrishna and Ravishankar (2011) reported that the copper ions present in the medium simulate the oxygenases and oxidases enzymes that have a significant role in cadaverine and putrescine biosynthesis. Sulphur (S) deficiency hampers plant productivity, as it affects the uptake and assimilation of nitrate. Sulfur application to plants grown on S-deficient soils has a positive impact on plant performance. In a field trial, Ahmad et al. (2005b) noted that application of S fertilizer in split doses during various growth stages (planting, pre-flowering, flowering and post-flowering stages) is better than applying the entire amount of S at any one stage, as it resulted in increased seed S (sulfate and organic S), better plant growth (leaf-area index, leaf photosynthetic rate, biomass accumulation) and higher yield in rapeseed (*Brassica rapa*). Sulphur transporter systems in plants help in maintaining sufficient S in plants (Ahmad et al. 2005a) and sulphur metabolites regulate the uptake of sulphate (Wadhwa et al. 2012). Bhattacharya et al. (2010) observed that the biosynthesis of phenylpropanoids and phenolics is influenced by phosphate, potassium, nitrogen and sulfur. This is due to the nutrition-based stress associated with the production of free radicals resulting in damage of plant tissues and can further be prevented by secondary metabolite synthesis. Hence the balanced combination of carbon and nitrogen nutrients has a positive influence on the yield of secondary metabolites. Various minerals like phosphate and sucrose induce synthesis of anthocyanins in plant cell cultures (Tuteja and Mahajan 2007). Nitrogen concentration in soil promotes total phenolic content in *Satureja hortensis* (Alizadeh et al. 2010), *Stevia rebaudiana* (Tavarini et al. 2015). Sadre et al. (2016) reported two key genes namely, TDC1 and TDC2 were essential for camptothecin biosynthesis and CYCLASE1 (CYC1) is co expressed with TDC1 gene. This study suggested that camptothecin biosynthesis can be regulated by the nutrients influence. Sahraroo et al. (2016) recorded enhancement of total nitrogen level in plants which accumulate more dry matter in the cells.

12 Influence of Climate Change

Climate is a continuous dynamic phenomenon affecting and being affected by the interactions of various organisms with their ecosystem. Studies have suggested adverse effects of climatic conditions such as solar radiations, temperature, excess carbon dioxide on the physiological process of plants including the formation of biologically active ingredients (Iqbal et al. 2011a; Raza et al. 2019). Ahuja et al. (2010) reported that changes in carbon dioxide levels, rainfall and temperature have greatly influenced the plant metabolism. Carbon dioxide being the main driver of climate change, directly affects photosynthesis, i.e. the plant process that harvests solar energy and converts it into chemical energy. Many studies revealed that plants grown in dry climate resulted in higher quality of aromatic and medicinal plants, compared with plants grown in mild climatic conditions (Sofowora et al. 2013). Presence of too high carbon dioxide concentrations result in the reduction of monoterpenes in plants (Selmar and Kleinwächter 2013). Increased carbon dioxide concentration generally inhibits the isoprene synthesis in plants (Rasulov et al. 2018). Ozone being phytotoxic for plants, reduces the photosynthesis and growth since it induces changes in gene expression, protein activity, and signaling pathways (Büker et al. 2015). Also, secondary metabolite production in highlands is more, as compared to lowlands (Setyawati et al. 2021). Elevated CO_2 has significant role in upregulation of atmospheric temperature that affects quality and quantity of secondary metabolites (Jamloki et al. 2021). As plants are facing the major challenge of climate change, they are developing resistance against the influence of climate. The increasing arrival of ozone, harming substances principally CO_2, is the principle justification for climatic changes (Patni and Bhattacharyya 2021). Various pathways involved in secondary metabolite production due to environmental variations, are regulated by various genes and transcription factors involved (Li et al. 2020).

Li et al. (2016) observed that stress inducible factors upregulation elicit the maximum secondary metabolites along with the high temperature encoding genes. Cawood et al. (2018) reported that *Amaranthus cruentus* grown at high temperature showed the maximum production of secondary metabolite from the encoding genes.

Sobuj et al. (2018) compared *Populus tremula* with control plants and found that due to warming of climate the total phenolic content in stem decreased by 55%.

13 Effect of Pesticides

Pesticides exposed to various plant parts use vascular system and cell to cell movement and hence indirectly affect the plant's secondary metabolite biosynthetic pathway (Jamwal et al. 2018). Certain insecticides, such as alphamethrin deltamethrin and lambda-cyhalothrin, have been found to induce oxidative stress, activate the antioxidant defence mechanism and affect the metabolic pathways in plant tissues (Bashir and Iqbal 2014; Bashir et al. 2007, 2014). For instance, lambda-cyhalothrin-induced oxidative stress altered the ascorbate-glutathione cycle in *Glycine max* (Bashir and Iqbal 2014). Pretali et al. (2016) analyzed the metabolome of tomato plants subjected to a pesticide and identified polyphenols, flavonoids, phytoalexins and hormones produced in response to it. Few herbicides, such as alachlor and glyphosate, directly affect plant secondary metabolite biosynthetic pathway, where alachlor reduces favonoid synthesis while glyphosphate blocks synthesis of cinnamate of shikimate pathway. Even sulsonylureas and p-nitro diphenyether herbicides increase the cinnamate-derived phenolic compounds and terpenoid stress metabolites (Jan et al. 2021). Moreover, pesticides induced impact on the rhizospheric community of plants, where changes occur in the plant secondary metabolites (Musilova et al. 2016). Usage of pesticide manifested the negative effect on various nitrogen -fixing bacteria, phosphate-solubilised bacteria that critically achieve the growth and productivity of plant secondary metabolites (Ansari et al. 2017). Due to stressful conditions mostly pesticide toxicity, plant growth regulators elicit various responses such modulation of gene expression, and activation of stress-signaling pathways to tackle the pesticide toxicity (Ali and Baek 2020). Plants have intrinsic mechanism such as breakdown and transformation of pesticide compounds, even detoxification of pesticide augmenting from the pesticidal damage (Wang et al. 2017). Organophosphorus pesticides, such as glyphosate, limit the plant growth, reduce photosynthesis, disturb the root nodules of leguminous plants and plant metabolic pathways (Gomes et al. 2017; Lushchak et al. 2018). Further, pesticides form resilient complexes with metal ions and reduce the organic content in the soil (Kaur et al. 2017). The pesticide application of Abamectin (ABM) and Thiamethoxam (TXM) cause transcriptomic changes in rice and found limited number of genes that dramatically affect rice growth and development. Notably, significant upregulation of Os12g27220 gene in rice encodes Spermidine hydroxyl cinnamoyl transferase 1 enzyme, which is responsible for the biosynthesis of alkaloids, terpenoids and phenolics (Muhammad et al. 2019).

14 Conclusion

Tolerance to abiotic stresses in plants depends upon the plant anatomy, physiology and genetic factors. Plants synthesize secondary metabolites by modifying primary metabolism pathway so as to adapt to the stressful environmental conditions. Apart many other metabolic pathways are involved in the production of secondary metabolites under different growth conditions that in turn facilitates various signal transduction pathways. Even the spatial and temporal changes have a significant role in the production of plant secondary metabolites which differs across species, genotypes and cultivars. Further, several genes are responsible for the biosynthesis and accumulation of secondary metabolites that enhance the tolerance level and helps the plant to cope up the environmental stressful conditions.

Acknowledgements The authors thankfully acknowledge various funding agencies (Govt. of India) such as DST, JK-DST, UGC, NMPB for research-grants in the form of major research projects and the School of Biotechnology, University of Jammu, for facilities.

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Effect of Temperature (Cold and Hot) Stress on Medicinal Plants

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Abstract Environmental factors such as light intensity, humidity, microbial attack, and temperature etc. are prominent in causing stress to medicinal plants which results in altered physiological processes. We know that medicinal properties in plants are attributed to the phytochemicals (secondary metabolites) present in them, which are governed by the various internal and external factors a plant is acted upon. One of the major factors that influence secondary metabolite production in plants is temperature under which a plant has to grow. Temperature extremes induce various physiological, morphological, and molecular changes in medicinal plants and these changes need to be addressed to find out approaches in order to empower medicinal plants' growth and healthy survival. High temperature induces direct and indirect damage to plants *via* protein denaturation and inactivation of chloroplast enzymes respectively. Cold temperature stress induces reduction in water uptake by plants, thus leading to cellular dehydration. Thus, there is a need to develop suitable engineered medicinal species of plants by creating desired genetic modifications for optimum growth, survival, and productivity.

Keywords Herbal plants · Metabolic phenomena · Extreme temperature · Abiotic stress · Plant response

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https://doi.org/10.1007/978-981-19-5611-9_5

1 Introduction

The biotic and abiotic stresses lead to activation of defence mechanisms that trigger various biochemical processes in plants (Husen 2021a, b). Environmental stresses such as temperature, light intensity, pollution, microbial attack etc. trigger in plants the production of phytochemicals called secondary metabolites (SMs) (Deepti et al. 2022a, b; Rahman and Husen 2022). Medicinal properties of plants are mainly attributed to the quality and quantity of these secondary metabolites (Parveen et al. 2020; Husen 2021c, 2022a, b). They not only find use as medicine, flavours, and in dyes and pesticides, but also enable plants to defend themselves from different types of stress (Ncube et al. 2012; Pagare et al. 2015). It is therefore interesting to understand how the quality and quantity of these metabolites are regulated.

Stages of plant growth and development have a bearing on SM production (Ahmad et al. 2004), but the understanding of this relationship, which involves regulation of genes and enzymes, is still incomplete. However, Li et al. (2020) have compiled the changes in secondary metabolites in relation to different developmental stages of plants, such as fruiting stage, whole growth stage, older tree etc. Environmental factors such as temperature, drought, salinity, and pollutants (gases and heavy metals) also influence the synthesis and accumulation of secondary metabolites in plants (Ahmad et al. 2007; Ali et al. 2008; Arshi et al. 2006; Ashraf et al. 2018; Iqbal et al. 2004; Singh et al. 2000).

Plants respond to environmental stresses, including temperature stress, by expression of specific plant growth regulators in the form of hormones and metabolites (Borges et al. 2017; Diwan et al. 2010). Studies have shown that temperature has a considerable influence on the physiological regulation, signalling pathways and defence responses in plants by alteration in their secondary metabolites (Al-Jaouni et al. 2018; Pandey et al. 2018). Alteration in photosynthetic activities is one of the important physiological changes that occur in plants to combat stresses (Bashir et al. 2015). High temperatures increase the vegetative development (appearance of node and leaf) while cold temperatures cause decrease in growth, development of leaves as well as photosynthetic activities in plants (Pant et al. 2021). Therefore, it is important to review and explore how the temperature stress causes alterations in secondary metabolites eventually affecting the properties, production and yield of potential medicinal plants, and what could be the potential innovative strategies to combat temperature stresses.

2 Temperature Stress on Medicinal Plants

When temperature rises above a threshold point for an extended period of time, heat stress occurs, causing irreparable impairment to plant development and function (Jemaa et al. 2010). Temperature stress can produce a variety of physiological, biochemical, and molecular alterations in plant metabolism, including protein denaturation and membrane integrity disruption. As a result, these changes can affect the SM concentration in plant tissues, which indicates the stress injury in plants (Levitt 1980; Zobayed et al. 2005). Temperature variations affect the expression of metabolic processes through regulation, permeability, and intracellular response rates. This bears a substantial impact on growth, cytodifferentiation, and metabolite synthesis (Isah 2019). Heat stress initially enhances secondary metabolite accumulation hence it could be an approach for increasing secondary metabolite concentrations in medicinal plants.

For optimum growth and development, each plant requires a specific range of temperatures. A certain level of temperature favorable to one plant may be harmful to another. Plants adapted to warm environments, for example, have been observed to produce damage signs when exposed to low (10–15 °C) non-freezing temperatures (Yadav 2010). The various phases of plant life such as germination, development, growth, reproduction, and yield, are affected by heat stress (Hasanuzzaman et al. 2013) and are discussed below.

2.1 *Seed Germination*

Heat stress has a harmful influence on seed germination, but the effective temperature ranges are different for different plant species (Kumar et al. 2011). In a variety of cultivated plant species, heat stress has been associated with lower germination percentages, poor radicle and plumule growth of geminated seedlings, low seedling vitality and deformed seedlings (Hasanuzzaman et al. 2013).

2.2 *Photosynthesis*

In plants, photosynthesis is one of the most temperature-sensitive biological processes. In comparison to C4 plants, high temperatures have a greater impact on C3 plants' photosynthetic potential. It modifies the rate of Ribulose 1,5-bisphosphate (RuBP) restoration by disturbing electron transport and inactivating the oxygen-evolving enzymes of PSII, thereby altering energy generation, and changing the actions of carbon metabolism enzymes, mainly Ribulose-1,5-bisphosphatecarboxylase/oxygenase (Rubisco) (Wahid et al. 2007).Heat stress adversely affects the plant's total photosynthetic performance due to negative impacts on the leaf, such as reduced leaf water potential, shrunken leaf area, and early leaf senescence. Even prolonged heat stress results in a decrease in carbohydrate reserves and plant nutrition (Hasanuzzaman et al. 2013).

Photosynthesis is also affected by low temperatures. Together with alteration in electronic transport in the thylakoids, carbon fixation and chilling conditions produce a drop in photosynthetic rates. Furthermore, cold limits photosynthesis by lowering the accessibility of free phosphate in the chloroplast due to poor utilization rate, resulting in the formation of trioses-phosphate, thus lowering the phosphate availability for photosynthesis. Another effect of low temperatures is observed as a change in the fluidity of cell membranes, which is also caused by heat. Exposure to low temperatures makes the membrane rigid, whereas high temperature makes the membrane more fluid (Nievola et al. 2017).

2.3 Plant Growth and Development

Heat stress has an impact on all plant tissues, on the phenomena of growth and development, and on the final yield of the plant, because photosynthesis is directly correlated to carbon partitioning and the resultant increase of plant biomass (Noor et al. 2019). This impact manifests both in the vegetative (primary as well as secondary) and the reproductive phases of plant growth (Iqbal and Ghouse 1982, 1985). Even the periodic activity of vascular cambium, the lateral meristem responsible for the formation of secondary vascular tissues in woody plants, is regulated largely by the variation in atmospheric temperature, especially in the tropical and sub-tropical climatic conditions. The cambium remains dormant during acute winters when even the differentiation of derivative cells into mature vascular tissues comes to standstill. At the advent of spring season, when the temperature starts increasing, the apical growth of twigs starts and emergence of leaf primordia and branch primordia begins (Iqbal 1994, 1995). The developing young leaves are the main seat for auxin production, which is required for reactivation of the vascular cambium as well as for the formation of flowers/inflorescences (Iqbal and Ghouse 1985). As soon as this auxin reaches the cambium, seasonal reactivation of the cambium begins. In species that have early flowering, cambial reactivation is normally delayed due to non-availability of the required amount of auxins, because in such cases the auxins produced are directed first towards inflorescences to serve the purpose of reproductive growth, and it is only around the end of the flowering phase is over that the auxins are available to feed the cambium to enable it to initiate the seasonal meristematic activity (Iqbal and Ghouse 1985; Iqbal 1995; Iqbal et al. 2000, 2005). This cambial activity as well as the differentiation of newly produced cells is on its zenith when the atmospheric temperature is quite high. In India, it is normally the period during April to August. Later, when the temperature starts decreasing, the cambial activity also declines and normally comes to rest when winter starts (Ajmal and Iqbal 1987a, b; Fahn and Werker 1990; Rao and Rajput 1999). In some hot regions, particularly in the tropical/sub-tropical belt, the cambium of some native species remains active almost throughout the year (Iqbal and Ghouse 1980; Iqbal 1994, Fahn and Werker 1990). Not only the behavior but even the structure of the cambium and of its derivative tissues, *viz*. the secondary phloem and secondary xylem, exhibits variations due to change in climatic conditions (Ajmal and Iqbal 1988; Ghouse and Iqbal 1982; Iqbal and Ghouse 1987).

The reproductive tissues are the utmost sensitive and even a small degree of temperature rise during flowering time can disrupt the entire crop cycles. Even a brief spell of excessive heat may cause damage to floral buds and flowers, depending upon the degree of susceptibility among and within the plant species and varieties (Sato et al. 2006).

Crop productivity is also very sensitive towards the temperature stress. It has such a severe consequence on crop production that just a minor increase in temperature (1.5 °C) may have a big detrimental effect (Warland et al. 2006).

2.4 Effect of Temperature on Medicinal Plants

High temperatures normally cause leaf rolling, leaf senescence, root and shoot growth suppression, reduced seed germination, fruit discoloration, reduced pollen viability, and yield reduction. Such detrimental influences on plant growth are consequences of drastic changes in physiological processes like increased respiration, declined photosynthesis, higher membrane permeability and ROS generation (Paupière et al. 2014). Due to a major collapse of cellular structure, extremely high temperatures can induce substantial damage to cells, often leading to cell death. Protein denaturation and aggregation as well as a rise in membrane lipid fluidity, are examples of direct damage. The inhibition of enzymes in mitochondria and chloroplasts, protein breakdown, and protein synthesis suppression are all examples of indirect heat damage (Wahid et al. 2007).

Cold stress below 20 °C has a harmful impact on plant growth and development, limiting the productivity. It prevents plants from expressing their full genetic potential, which inhibits metabolic responses; water intake is affected, in turn causing the cellular dehydration (Verma and Shukla 2015; Verma et al. 2021). Membrane disintegration is one of the principal consequences of cold stress-induced dehydration, which has a negative impact on all physiological phenomena related to growth and development. Limited germination, stunted seedlings, yellowing of leaves, wilting, and diminished shoot growth are the common cold-stress symptoms. Pollen sterility is another factor caused by cold stress, which results in low productivity (Yadav 2010).

Effect of heat and cold on secondary metabolite production in medicinal plants has been shown in Table 1.

3 Adaptive Responses of Medicinal Plants to Combat Temperature Stress

There are various responses, such as generation of antioxidants and heat shock proteins (HSPs), with the help of which medicinal plants combat temperature stresses.

Table 1 Temperature stress effect on secondary metabolites of medicinal plants

Temperature	Plant species	Medicinal use of plant	Secondary metabolite/ phytochemical	Regulation	References
High	*Panax quinquefolium* L.	Lower blood sugar and cholesterol levels, reduce stress	Ginsenosides	Decrease	Jochum et al. (2007)
Low	*Medicago sativa* L.	Arthritis, kidney problems, diuretic, anti-cancer,	Putrescine (polyamine)	Increase	Nadeau et al. (1987)
Pre chilling treatment	*Artemisia annua* L.	Fevers, inflammation, headaches, bleeding, and malaria	Sesquiterpene Lactone artemisinin	Increase	Yin et al. (2008)
Low	*Ocimumbasilicum* L.	Arthritis, diuretic, anti-cancer,	Phenolic Monoterpenes	Increases	Barickman et al. (2021) Arulmozhi et al. (2019)
High temperature	*Astragalus compactus* L.	Immune-boosting, anti-aging and anti-inflammatory	Phenols Phenolics	Increase	Naghiloo et al. (2012)
High temperature	*Chrysanthemum* L.	Chest pain (angina), high blood pressure, type 2 diabetes	Phenols Anthocyanins	Decrease	Shibata et al. (1988)
Low temperature	*Camellia japonica* L.	Astringent, antihemorrhagic, hemostatic, salve and tonic	Fatty acid α-linolenic acid, Jasmonic acid	Increase	Li et al. (2016)
Low	*Nyctanthesarbortristis* L.	Immune-boosting, anti-aging and anti-inflammatory	Phenolic	Decreases	Gautam et al. (2019) Snijesh and Singh (2014)
Low	*Catharanthus roseus* (L)G. Don	Relieving muscle pain, depression of the central nervous system	Ajmalicine	Increase	Courtois and Guren (1980)

3.1 Antioxidant System

Temperature stress is known to augment the formation of reactive oxygen species (ROS), such as superoxide radical (•O_2), singlet oxygen ($1O_2$), hydroxyl radical (HO•), and hydrogen peroxide (H_2O_2), that all promote oxidative damage in plants (Chaitanya et al. 2002). Heat may cause a rise in ROS production, particularly in the mitochondria and chloroplasts. ROS induce oxidative damage to lipids, nucleic acids and proteins, disrupting the metabolic functions and organelle vitality (Nievola et al. 2017). The membrane lipids and pigments autocatalytic peroxidation, which alters membrane integrity and functions, is also an outcome of RO Sattack (Scharf et al. 2012).

Plants prevent excessive oxidative damage by counterbalancing ROS generation through antioxidant mechanisms that neutralize these naughty molecules (Mittler 2002; Aref et al. 2016). As the heat stress exceeds a certain threshold, the equilibrium between the production and removal of ROS is disrupted, inevitably a rise in ROS production occurs, which causes oxidative damage in plants (Nievola et al. 2017). The plant also comes into action immediately and starts producing antioxidants consisting of a complex enzyme system as well as non-enzymatic components. Normally, ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase and glutathione reductase (GR) constitute the enzyme system, while the non-enzymatic components are ascorbate, glutathione, tocopherols (vitamin E) and carotenoids (Mittler 2002).

3.2 Heat Shock Proteins (HSPs)

High temperature sensitivity signals cause variation at the molecular level, altering the transcript accumulation and gene expression, and as a stress-tolerance technique, this results in the production of stress-related proteins. Heat stress causes reduction in normal protein synthesis, which is followed by an increase in the transcription and translation of novel protein components known as the heat shock proteins (HSPs) (Jemaa et al. 2010). HSPs function as molecular chaperones, preventing protein denaturation and maintaining plant cell homeostasis under adverse conditions (Yin et al. 2008; Mittler 2002). Low temperatures are also associated to protein disfunction and denaturation, which leads to HSP accumulation (Naghiloo et al. 2012; Ul-Haq et al. 2019).

4 Plant Defense Mechanism against Extreme Temperatures

Heat, at least 5 °C above their normal development temperatures, induces plants to produce a unique system of cellular and metabolic defensive mechanisms to cope with the extreme heat (Bita and Gerats 2013). HSPs, osmo-protectants, phytohormones including abscisic acid (ABA), and antioxidant enzymes are all key defense mechanisms in plants when they are exposed to heat stress (Devi et al. 2017). Furthermore, many plant species increase the synthesis of secondary metabolites in response to heat stress to protect them from oxidative damage. Secondary metabolites contain a wide variety of useful natural compounds that help plants survive under unfavorable environmental conditions (Isah 2019). The stimulation of phenolics in tomato and watermelon reveals that thermal stress promotes its production while suppressing the oxidation, indicating that this could be a plant defense mechanism against thermal stress (Rivero et al. 2001). Under high temperature stress, anthocyanins help in the reduction of leaf osmotic potential which is related to increased uptake and decreased water loss through transpiration (Bita and Gerats 2013).

Cold acclimatization occurs when plants cultivated at low temperatures show significant changes at physiochemical and molecular levels (cellular dehydration, water absorption, and metabolic reactions) that allow them to endure low temperatures (Li et al. 2020). Plants that grow in cold climates tolerate low temperature stress by establishing their own microenvironment through change in their morphological structure so as to avoid the effects of free air circulation by shrinking plant size, developing compact growth strategies and selecting sheltered environment. The cold-tolerant plants have higher antioxidant enzyme activity than the sensitive plants (Hasanuzzaman et al. 2012). (Zhang et al. 2008) reported a considerable rise in reduced ascorbate (AsA) and reduced glutathione (GSH) levels in strawberry plants as an outcome of low temperature stress (Zhang et al. 2008).

5 Temperature Induced Changes in Medicinal Plants

Physiological changes induced by temperature stress in medicinal plants include destabilization of various RNA species, proteins, cytoskeleton structures and membranes, and alterations in the efficiency of enzymatic reactions in cells. These changes create metabolic imbalance, thus causing deviation of processes. Changes in biochemical processes lead to molecular changes, such as alteration in gene and protein expression levels, which induces stress-response proteins, like different heat-shock proteins, in order to combat environmental harshness (Iqbal et al. 2015).

Morphological changes are also induced by temperature stress, which normally include sunburns and scorching of leaves, buds, twigs and stems, stunted shoot and root growth, leaf abscission and senescence, and fruit discoloration and quality deterioration. Various physiological and morphological changes observed in different medicinal plants are shown in Table 2.

Table 2 Physiological and morphological changes due to temperature stress in some medicinal plants

Plant species	Physiological changes	Morphological changes	Temperature status	Reference
Lotus criticus L.	NS	Reduction in leaf area and cell size; increase in density of stomata and trichomes; increase in number of xylem vessels in root and shoot	High	Banon et al. (2004)
Vitis vinifera L.	NS	Damage to mesophyll cells: Chloroplast irregular, stroma lamellae disordered, cristae of the mitochondria disrupted	Chilling	Zhang et al. (2005)
Vitis vinifera L.	NS	Damage to mesophyll cells, chloroplast became round in shape, the stroma lamellae swollen, mitochondria outer membrane digested	High	Zhang et al. (2005)
Saccharum officinarum L.	Photosynthesis impairment; rapid leaf respiration reduces the amount of available sugar for translocation.	Smaller internodes, increased tillering, early senescence, reduced total biomass	High	Ebrahim et al. (1998)
Saccharum officinarum L.	Sucrose translocation by phloem is inhibited	Very slow growth, with few and short internodes and few leaves	Low	Ebrahim et al. (1998)
Lycopersicon esculentum Mill.	Reproductive process affected adversely	Poor fruit set, excreted stigma	High	Dane et al. (1991)
Chenopodium album L.	Quantitative variation in chloroplast; Low Molecular Weight -Heat Shock Protein (LMW-HSPs)	NS	High	Barua et al. (2003)
Citrus aurantium L.	Increase in nitric oxide content; increased rate of electrolyte leakage	Injury symptoms	High	Ziogas et al. (2013)
Vigna unguiculata L. Walp.	Early flowering and shorter pod set	Reduced grain yield	High	Ismail and Hall (1999)
	Increased accumulation of osmolytes and	Decrease in plant height and weight	High	Alhaithloul et al. (2020)

(continued)

Table 2 (continued)

Plant species	Physiological changes	Morphological changes	Temperature status	Reference
Catharanthus roseus (L.) G. Don	secondary metabolites			
Sorghum bicolor (L.) Moench	Starch deficiency in microspores, male sterility, reduced germination; impairment of cell wall invertase-mediated sucrose hydrolysis and subsequent lack of sucrose biosynthesis	NS	High	Jain et al. (2007)
Mentha piperita L.	Increased accumulation of osmolytes and secondary metabolites	Decrease in plant height and weight	High	Alhaithloul et al. (2020)

NS not studied

6 Conclusion

Medicinal plants under extreme temperature stress undergo significant changes in their physiology, anatomy, morphology, and biochemistry, which pose a serious challenge to their growth, survival as well as their medicinal properties. Plants utilize adaptive responses as a way of dealing with these challenges and try to protect their medicinal benefits. Mechanisms such as activation of antioxidant enzymes, expression of heat shock proteins and generation of secondary metabolites are attributed to plants ability to cope with heat and cold stress. The changes at gene or protein levels lead to changes observed even at morphological level. Morphological changes act as indicators of specific environmental stresses apart from being the adaptive responses and helping in acclimatization. Figure 1 presents an overview of the temperature-stress-induced tolerance mechanisms in medicinal plants.

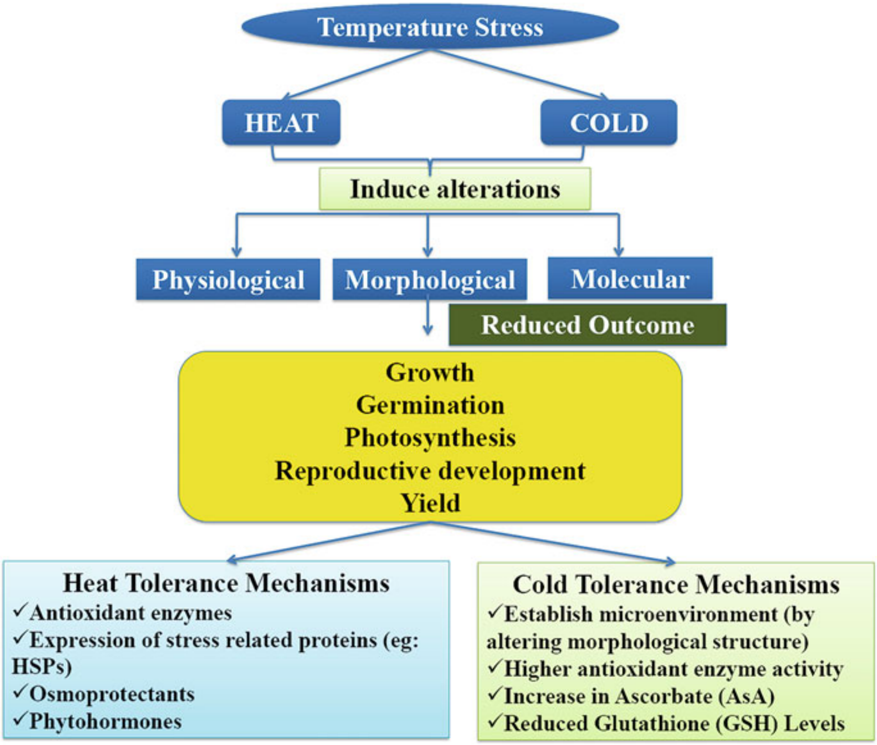

Fig. 1 Temperature stress induced tolerance mechanisms in medicinal plants

Acknowledgement Encouragement received from IMS Engineering College, Ghaziabad (UP), and Sankalchand Patel University, Visnagar, Gujarat, during preparation of this review, is gratefully acknowledged.

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Effect of Water Stress (Drought and Waterlogging) on Medicinal Plants

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Abstract Climate change causes different biotic and abiotic stresses to the plants, water stress being one of them. Water stress includes water-logging (flooding) and water deficit (drought). These stresses restrict the plant's growth, development, and ultimately yield. Approximately 60% of crops, including plants of medicinal importance, are affected by water stress. Water stresses cause physiological disturbance (closure of stomata, decrease in photosynthesis and transpiration) and biochemical alteration (protein denaturing, ROS production, increase in antioxidant enzymes and osmoprotectants) in plants. Water stress can alter the yield of secondary metabolites in medicinal plants. To cope with these stresses, tolerant genotypes have been produced, but more efforts are required for modification of medicinal plant genomes that can help us maintain plant growth and increase yield to meet the requirements of an increasing world population. This chapter deals with the physiological and molecular mechanisms of medicinal plants under waterlogging and water-deficit conditions.

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A. Husen, M. Iqbal (eds.), *Medicinal Plants*,
https://doi.org/10.1007/978-981-19-5611-9_6

Keywords Flooding · ROS · Secondary metabolites · Water deficit · Molecular response

1 Introduction

Stress is a condition which disturbs the physiology of plants as against the balance existing in normal situations. Stress induces alterations at the metabolic levels leading to injury in plant parts, irregular changes in plant physiology and biochemistry, and the incidence of diseases. Plants have a variety of mechanisms for dealing with short-term changes. Plant response to environmental stressors depends on the type and duration of stress, and the nature of plant species, and involves physiological and biochemical changes (Khalid et al. 2019). Plants genotype often decides the extent of damage caused by environmental stresses to plant growth and yield. Plants exposed to such environmental factors as high and low temperatures, water shortages, heavy metals, air pollutants, salinity, nutrient deficiency, and light stress can generate reactive oxygen species (Aref et al. 2016a; Arshi et al. 2012; Husen 2021a, b, c).

Through physiological adaptation, plants cope with these abiotic stresses and, in the process, undergo changes in their internal features at the biochemical, molecular, morphological, and anatomical levels (Aref et al. 2013; Bagheri et al. 2015; Gizińska et al. 2015; Husen et al. 2016, 2017, 2018, 2019; Hussein et al. 2017; Nighat et al. 2020; Noor et al. 2019; Husen 2010; Husen 2021d). Both the primary and secondary metabolisms exhibit biochemical adaptation in plants under stress (Ali et al. 2008; Beigh et al. 2008; Singh et al. 2000). Reduction in leaf growth, leaf pigments, and stomatal conductance, and a resultant low photosynthetic productivity lead to reductions in yield (Aref et al. 2016b; Husen and Iqbal 2005; Husen et al. 2014).

Medicinal plants are the most common kind of crop widely utilized for illness treatment, either as ethnomedicine medicine or commercial herbal treatments (Parveen et al. 2020a, b). Based on a variety of cultural, religious and regional traditions, medicinal plants have been used by tribal, rural, and nomadic people to manage various physical disorders and diseases for thousands of years (Atique et al. 1985a, b, Atique and Iqbal 1992; Anis et al. 2000; Beigh et al. 2002, 2003), but their popularity has never waned, with 80% of the world still relying on herbal remedies. The quality of these medicinal herbs is, in fact, determined by the secondary metabolites and aromatic chemicals they contain (Husen 2021e, 2022). It has been shown that the habitat conditions have a significant influence on the synthesis of these chemical compounds in plants (Wilhelm and Selmar 2011). This review is likely to contribute to a better understanding of the effects of drought stress on medicinal plants.

Drought tolerance is found in all plants, though its level differs among the species and sometimes within the species. Root length, stem height, leaf area, chlorophyll content, photosynthesis, and transpiration are hampered by water deficit conditions in the soil and, consequently, in plants. Water stress also delays seed germination.

Water stress restricts the plant's growth and performance. The average of this restriction due to low water level hovers around 60% worldwide. Plant growth processes depend on the rate, intensity, and duration of exposure to stress, and the stage of the crop also affects water stress. Oxidative stress induced within plants affects the physiological and biochemical processes in plants, and secondary compound concentration rises (Hussain et al. 2018; Khalid et al. 2021a, b). In order to combat the production of free radicals and their toxicity and improve their defence systems, plants are forced to produce enzymatic and non-enzymatic mechanisms under stressful conditions (Aref et al. 2016a). During these mechanisms to maintain the ion balance in the vacuole and cytoplasm, osmolytes, (low molecular mass compounds) are developed in the cytoplasm.

In medicinal plants, water stress can alter the yield of secondary metabolites; the rate of photosynthesis in higher plants is decreased. When relative humidity and leaf water potential are restricted, drought stress induces opening and closing of stomata, indicating low leaf transpiration and photosynthetic rate. Also, the absorption and digestion of food and carbon consumption with the help of Rubisco are concealed and the amount of carbohydrates is reduced. Water stress also affects the potential of active ingredients in medicinal plants.

2 Drought Stress Effects on Medicinal Plants

Climate change threatens plant development and production. Rapid changes in the environment pose a direct danger to plant productivity while short-term changes show a cumulative effect. Plants, on the other hand, can adapt to modest alterations. The plant reacts to various stressors by undergoing physiological, biochemical and molecular changes. Alavi-Samani et al. (2015) performed a research trial on thymes species and concluded that the minor water deficit conditions affect medicinal plants, including oil quality enhancement, the compounds of which are affected by irrigation levels. In medicinal plants, accessibility and absorbance of nutrients are decreased. This is a secondary effect of stress. The rapidly growing demand for herbal medicines has inspired plant collectors and traders to destroy the environment and the populations of medicinal plants. The number of flowering plant species that are used for medicinal purposes is about one out of eight (Schippmann et al. 2002). On a global scale, specifically in the west, the industry of pharmacy intensifies the damage to the natural plant population (Van de Kope et al. 2006).

Excessive harvesting of medicinal plants is the main reason for the reduction of genetic diversity and the destruction of homes of all plants (Guo et al. 2009). The long-lasting solution to all these problems is the cultivation of medicinal plants. However, it is feared that growing the healing potential of cultivated medicinal plants may be affected due to change in biotic and abiotic factors like herbivores, pathogens, seasonal changes, geographic origin, growth conditions, altitudes, plant phenology, and changing environmental conditions. The quality of antimicrobial activity of plants greatly varies in nature (Pirbalouti et al. 2011). Secondary plant

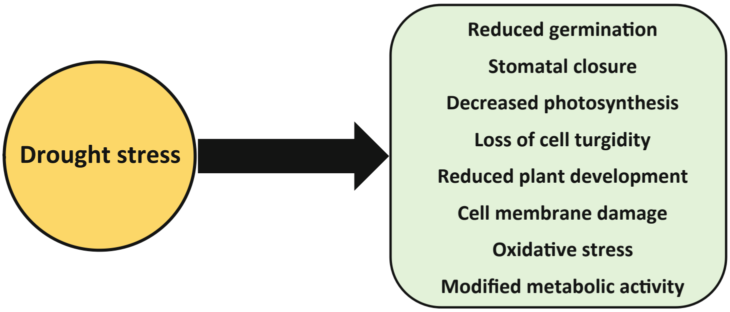

Fig. 1 Impact of drought stress on plant functioning

products and their concentration in medicinal plants also vary widely depending on the condition of plant growth. Stressful situations, have a great influence on the metabolism pathways responsible for the gathering of all natural products.

Peppermint an aromatic plant is used all over the world for many products, including food, flavor, and fragrance. It is also used in industrial products to recover from fungal and other diseases (Ram et al. 2006). Water deficit stress affects interconversion and formation in peppermint essential oil constitutes (Khorasaninejad et al. 2011a, b). Seeds and essential oil of another medicinal plant, *Foeniculum vulgare* L. are used in foods like cheese and bread. Moreover, in aromatic plants, the production quality of essential oil ingredients and plant growth are affected by drought stress. Plants respond to water stress by undergoing phenotypic, morphological, and biochemical changes in order to minimize the moisture loss from the plant surface (Chaves et al. 2011). Plants also use additional methods to improve water absorption to maintain turgor pressure (Geilfus 2019). Water stress has a major impact on various physiological processes in plants, especially on photosynthesis, through its effect on stomata and ATP synthase enzymes (Sharma et al. 2020a, b). Water scarcity often leads to the production of reactive oxygen species (ROS), which affect the redox regulatory mechanism of the cell (Laxa et al. 2019; Sharma et al. 2020a, b) (Fig. 1).

3 Reduced Yield

Following a water shortage, there is a typical drop in plant production. Initially, drought stress decreases medicinal and aromatic plant production via three primary processes: the absorption of photosynthetically active radiation (PAR) of the whole plant canopy is reduced due to lowered leaf area, transient leaf withering or curling at times of extreme stress. Secondly, drought stress reduced the crop's ability to utilize the absorbed photosynthetically active sunlight to create new dry matter (the radiation use efficiency). This may be observed as a drop in crop dry matter accumulation per unit of photosynthetically active radiation absorbed during a particular time period, or as a decrease in the instantaneous whole-canopy net CO_2 exchange rate per unit of consumed solar radiation. Finally, drought stress may restrict grain production by lowering the harvest index (HI).

If a short period of stress coincides with the crucial developmental stage around blooming, this may happen even if there is no significant decrease in overall dry matter accumulation (Kabiri et al. 2014). Plants produce secondary metabolites in response to biotic and abiotic stressors (Anjum et al. 2011). Drought stress also impacts essential oil percentage since drought stress raises volatile oil proportion but reduces shoot biomass, leading to a reduced essential oil content (Chaves et al. 2011).

4 Natural Product Accumulation

Environmental conditions of the habitat, such as temperature, light, and nutrient supply, have a significant impact on plant secondary metabolite synthesis and their efficacy (Falk et al. 2007). Often, unsuitable environments result in imposing stress on plants that has a significant effect on the overall growth and development and, in particular, on the metabolism of the plant (Bohnert et al. 1995). In contrast to biotic interactions, which typically require only a few factors, such as a specific pathogen or a specific herbivore, the situation regarding abiotic stress appears to be more intricate, as several correlations exist between both these factors: High light intensity is associated with higher temperatures, often leading to water scarcity, thus inducing drought conditions.

Research trials have exhibited the effect of a specific abiotic stress on the secondary metabolism of medicinal plants. It has been found that medicinal plants subjected to water stress develop larger quantities of secondary products than those grown in well-watered environments.

In drought-stressed *Hypericum brasiliense* plants, phenolic content levels are much higher than in control plants (de Abreu and Mazzafera 2005). The drought-stressed plants were smaller in length but having 10% more phenolic content. Similarly, in drought-stressed peas (*Pisum sativum*), a 30% greater amount of anthocyanins was produced in comparison to the control (Nogues et al. 1998). In contrast, there was a significant increase in total phenolic proportion in drought stressed peas plants when compared to control plants. Jaafar et al. (2012) found that drought stress enhances not only the accumulation but also the quality of total polyphenols and flavonoids per plant (Table 1). In contrast, reduced flavonoids content was observed in red sage plant (*Salvia miltiorrhiza*) growing under drought stress (Liu et al. 2011).

Regarding terpenoids, just a few studies clearly demonstrate a drought-related increment in terpene levels in plants. Drought stress causes a significant rise in the concentration of monoterpenes in sage (*Salvia officinalis*), which readily compensates for the loss in biomass (Nowak et al. 2010). The total quantity of monoterpenes produced in drought-stressed sage plants is considerably greater than in well-watered

Table 1 Impact of drought stress conditions on some plant secondary metabolites

Crop	Activity	Reference
Sunflower	Tenfold increment of chlorogenic acid levels	del Moral (1972)
Peach, thyme, Hypericum, Ajwain	Induced higher levels of total phenols	Kubota et al. (1988) de Abreu and Mazzafera 2005 Azhar et al. (2011)
Peas	Increased activity of flavonoids	Nogues et al. (1998)
Peas	Higher levels of anthocyanins	Alexieva et al. (2001)
Tea plant	Higher levels of epicathecins	Hernández et al. (2006)
Pines	Significant increase in α-pinene and careen contents	Llusià and Peñuelas (1998)
Peppermint, rosemary	Significant increase in essential oils	Bettaieb et al. (2009)

controls. Likewise, drought stress in parsley (*Petroselinum crispum*) increased the production of essential oils but it was compensated for with a loss in plant biomass (Petropoulos et al. 2008). Also, the catmint and lemon balm plants under drought stress exhibited a modest increase in monoterpene proportion with a slight reduction in growth and yield (Manukyan 2011).

5 Mechanisms of Drought Tolerance

Molecular reactions to stress condition include highly regulated genes and signaling pathways that assist plants in dealing with the stress. C-repeat/dehydration-responsive element binding factors (CBF/DREB), mitogen-activated protein (MYB), cup-shaped cotyledon CUC, no apical meristem NAC TFs, and zinc-finger proteins (ZFPs) have all been identified as important components of resistance mechanism in plants against drought (Cong et al. 2008). The GsZFP1 gene enhanced drought resistance in *Medicago sativa*, implying that it is effective in promoting drought-tolerant plant species. Overexpression of SNAC1 in *Gossypium hirsutum* improves its capacity to deal with water deficits while simultaneously increasing root development, demonstrating that larger roots are beneficial in drought-tolerant breeding (Liu et al. 2014). To improve drought resistance, the BdWRKY36 gene promoted transcription of stress-related genes, reduced electrolyte leakage, lowered ROS levels, and increased chlorophyll quantity, crop water status and the antioxidant enzyme activities (Sun et al. 2015). Late embryogenesis associate (LEA) expression of genes decreased photosynthetic activity while increasing the plant antioxidant defence system to enhance drought stress tolerance in three Linderniaceae species with varying degrees of desiccation resistance (Juszczak and Bartels 2017). The primary stimulatory strategy for high water productivity in drought-tolerant *Malus domestica* involves maintaining C3 cycle activity by enhancing the photosynthetic enzymatic activity, alleviating e-transfer, decreasing ROS portion by attempting to control the photosynthetic e-transport chain, and C2 cycle and ROS mitigation capacity to reduce photoinhibition and enhance photosynthetic activity (Li and Liu 2016).

In response to water deficiency stress, the resultant signal transduction stimulated the production of several components, including phytohormones, in order to react to and adapt to drought stress (Khalid et al. 2022a). ABA improves plant drought tolerance by activating a variety of signaling pathways (Bücker-Neto et al. 2017). ABA-induced genes producing drought-related proteins, such as dehydrins, ROS-detoxifying enzymes, regulatory proteins, and phospholipid signaling enzymes, may enhance drought resistance in addition to promoting stomatal movement, root architecture, and regulating photosynthesis (Khalid et al. 2022a; Fahad et al. 2019). Increased ABA triggered a signaling cascade in guard cells, resulting in guard cell K^+ outflow and decreased turgor pressure, eventually inducing stomatal closure (Salazar et al. 2015). ABA alleviated drought stress and improved wheat endurance by increasing stem lengths and plant biomass while decreasing the H_2O_2 and malondialdehyde levels (Wei et al. 2015). Elevated cytokinin levels in xylem sap promoted stomatal opening by decreasing sensitivity to ABA (Fang et al. 2016). In expressing cell lines of VaNAC26, JA synthesis-related genes were activated, which enhanced ROS scavenging and promoted stomatal closure and root development, ensuring a better drought tolerance (Bielach et al. 2017).

6 Water Logging

Medicinal plants have a higher concentration of natural products. Water is necessary for the growth of plants. Any water distress leads to stomatal closure, and the uptake of CO_2 and water is markedly decreased, thereby reducing photosynthetic rate and nutrient uptake. Base changes can lead to changes in the structure of proteins. Waterlogging is an abiotic stress that can intensely harm crops around the world. The condition can be characterized as the immersion of soil in water. Waterlogging creates low oxygen conditions in the root regions, causing a lack of ATP from the restraint of oxidative phosphorylation (Fig. 2). Waterlogging has adverse effects on the molecular characteristics of plants. Three-dimensional protein structures play an important role in understanding the molecular mechanisms for drug design.

China is known for the production of herbaceous peonies, but waterlogging affects the growth and development of plants. Waterlogging disturbs the soil aeration as well as root respiration. This leads to disturbed water absorption and transportation. Photosynthetic products are also affected. Normally, reactive oxygen species are produced in a dynamic balance, waterlogging disturbs this balance and the ROS are produced in large numbers, promoting protein degradation and lipid peroxidation. Waterlogging disturbs DNA and the cell-membrane functioning. Reactive oxygen species act as signaling molecules and control the plant growth and development.

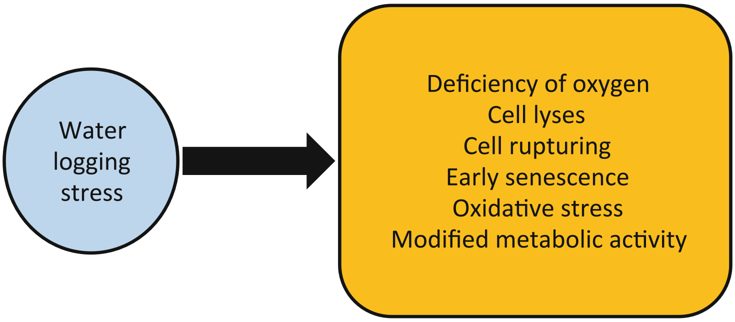

Fig. 2 Impact of water logging on plant functions

Medicinal plants have certain secondary metabolites that tolerate stress. Perennial herbs, such as *Paeonia lactiflora*, are suitable for high as well as dry planting grounds. Being a deep-rooted plant *Paeonia lactiflora* is good for sandy loam. Its roots are used in medicine for curing several diseases. Important components such as paeoniflorin, benzoyl paeoniflorin, albiflorin and oxypaeoniflorin are used to treat swelling, inflammation, as well as tumors. The herbaceous peony has phenolics as well as flavonoids that help fight tumours and aging.

7 Waterlogging Tolerance Mechanism

Plants that can withstand waterlogging conditions have mechanisms such as increased availability of soluble sugar, fermentation enzymes, greater activity of glycolytic pathway and involvement of antioxidant defence mechanisms to cope with the oxidative stress induced by waterlogging. Ethylene production plays an important role in the change mechanism of plants (Khalid et al. 2019). Under waterlogging conditions, plants act differently depending on their growth stages. When a plant is in the development stage, waterlogging conditions severely decrease its yield and productivity. However, when a plant is dormant, the effect is very small and seen only for a short time. Plants exposed to waterlogging stress, are affected in many ways including the reticence of seed germination, decrease in vegetative and reproductive growth, alteration in plant structure, and accelerated senescence (Fig. 3).

8 Molecular Marker

Molecular techniques and molecular markers provide new strategies to scientists, geneticists and plant breeders for the improvement of medicinal plants. DNA-based molecular markers identify the genetic diversity among individuals and populations.

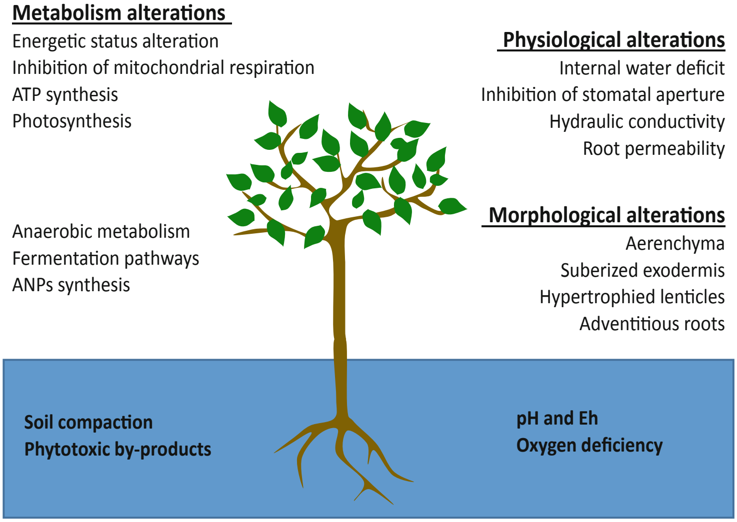

Fig. 3 The response and changes in soil and plants under flooding

Different factors that are considered in the selection of molecular markers include availability of marker system and time, DNA quality and quantity, and technique simplicity.

9 Use of Nanotechnology

During the last few decades, nanotechnology has been widely used in different fields, including agriculture and biomedical (Khalid et al. 2022b). There is a need of seeds that resist bacterial attacks and can be easily germinated, giving rise to healthy plants that can tolerate environmental challenges. Treatment of seeds with nanomaterials has the solution (Husen and Iqbal 2019). Sequencing techniques have been used for genomic analysis. This provides descriptions of gene expression at the genome-scale level with high accuracy and large dynamic ranges. Transcriptome analysis helps in understanding the molecular responses to waterlogging.

10 Conclusion

Medicinal plant growth and development are strictly affected by water stress (drought as well as water logging), which alters physiological phenomena (closure of stomata, decrease in photosynthesis and transpiration) and biochemical characteristics (protein denaturing, ROS production, increase in antioxidant enzymes and osmoprotectants) in plants. To cope with these stresses, tolerant genotypes have been produced, but more efforts are required for genetic modification of medicinal plant genomes that can help us maintain plant growth and increased yield to meet the requirements of the increasing human population in the world.

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Effects of Gaseous Pollutants on Medicinal Plants

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Abstract Plants are regarded as the crucial creatures in the formation of life on the planet Earth. Unfortunately, the climate of the Earth is rapidly deteriorating, primarily because of the increasing concentration of pollutant gases in the atmosphere, and the consequent rise of temperature and its after effects. Emissions from power plants and various factories (mostly a combination of oxides of carbon, nitrogen, and sulphur) and the release of greenhouse gases (carbon dioxide, methane, nitrous oxide, ozone, chlorofluorocarbons, etc.) are mainly responsible for this grave situation. The presence of these unwanted molecules in the atmosphere has a big impact on plants' growth and productivity. Since plants cannot move away from harmful conditions due to their sessile nature, they have to face the harsh environment and undergo various alterations in their form and function. Metabolic alterations, or more precisely, fluctuation in the concentration of secondary metabolites, are thought to be one of the plants' defense mechanisms against unfavorable environments. Secondary metabolites, although not required for a plant's usual functions, do form the immune system of plants. Climate change has the potential to alter the quality of natural products, as well as the flavor and medicinal value of various plant species. Rising temperatures, drought, salinity, and erratic rainfall, which are an outcome of all these gaseous emissions, have an obvious impact on plant growth and physiology. This chapter presents a brief discussion of these atmospheric impacts on the form and function of medicinal plants with a special focus on their secondary metabolism.

Keywords Climate change · Ecosystem · Gaseous pollutants · Medicinal plants · Secondary metabolites

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A. Husen, M. Iqbal (eds.), *Medicinal Plants*,
https://doi.org/10.1007/978-981-19-5611-9_7

Abbreviations

CFC	Chlorofluorocarbons
TCM	Traditional Chinese Medicine
UV light	Ultraviolet light
WHO	World health organization

1 Introduction

Climate change is associated with changes in climate over a comparable length of time that is related to human activities and modifies the composition of the global atmosphere, either directly or indirectly. Over the last century, the global mean temperature of the earth's surface has risen by 0.74 °C. According to the fluctuation in surface temperature, the 1990s decade was the warmest in the past millennium, with 1998 being the warmest year. The temperature rise is ascribed to an alarming increase in atmospheric concentrations of a variety of toxic gases (largely oxides of carbon, nitrogen, and sulphur) emitted from households, various industries, and thermal power plants, and the so-called greenhouse gases such as carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and chlorofluorocarbons (CFCs), primarily as a result of increased industrialization (Fig.1). Carbon dioxide concentrations are expected to be 100% greater in 2100 than they were in the pre-industrial era. With global temperatures anticipated to climb by up to 6 °C by the end of the century in comparison to the pre-industrial levels, this agroclimatic indicator is unlikely to remain steady (Singh 2010). The presence of these toxic elements in

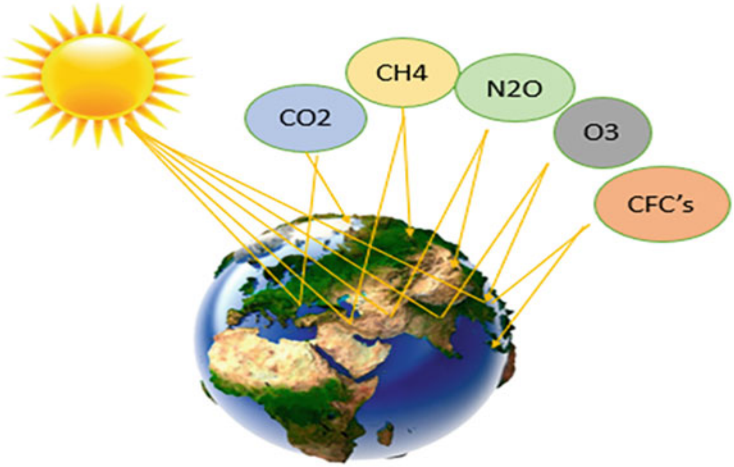

Fig. 1 Diagram showing gaseous pollutants

the air, soil, and water gives rise to some secondary stress factors like increased temperature, rising sea level, drought, salinity, and excess of heavy metals, among others. All these pollutants and the consequent environmental conditions are active in the growing plants individually or collectively at the same time (Aref et al. 2013a, b; Hussein et al. 2017; Iqbal and Ghouse 1982; Iqbal et al. 2000b; Qureshi et al. 2006; Husen 2022a; Husen et al. 2014, 2016, 2017, 2018, 2019; Getnet et al. 2015; Embiale et al. 2016). The physiological status of plants is determined solely by the local climatic factors; for example, photosynthesis is influenced by temperature, carbon dioxide, water, and nutritional ingredients. Planting a crop in an ecologically unsuitable location increases production costs and, as a result, diminishes the likelihood of economic success. Environmental conditions of the habitat determine the size of plants, the duration of phenological stages, and the time and volume of harvest at a specific location (Iqbal and Khudsar 2000; Kumar et al. 2020). Plant growth and development are influenced by a variety of environmental conditions and soil characteristics (Iqbal and Ghouse 1985; Hamdo et al. 2010; Husen 2022a). Climate change is responsible for the variations in environmental conditions across the globe, which have a big impact on chemical constituents, especially the secondary metabolites in plants (Iqbal et al. 2011). Significant biological and pharmacological functions are attributed to these plant components.

Climate change has a wide range of negative consequences for various sectors, including human health, water, air, soil, microbial populations, plants, and their medicinal components (Ahmad et al. 2011). Climate change is caused by several variables, including a growing global population, fast industrialization, and the widespread use of chemical fertilizers and pesticides in agricultural fields. Rising temperatures, drought, and changes in rainfall patterns are all examples of changing climatic conditions. All these characteristics have an impact on how humans, plants, and microbial population function. Anthropogenic activities have played a significant role in causing global climate change. Excessive emissions of gases like CO_2, SO_2, NO_2, and O_3 have resulted in global warming and consequently climatic shift (Mishra 2016). Plants, unlike humans, cannot move away from harmful conditions due to their sessile nature; they have to resort to additional mechanisms to ensure their protection and survival (Iqbal et al. 1996; Anjum et al. 2012b; Husen 2021a, b, c, d). Metabolic alterations, or more precisely, fluctuation in the nature and content of secondary metabolites, are thought to be one of the plant's defense strategies against the unfavorable environments (Pichersky and Gang 2000; Ober 2005). Secondary metabolites are molecules that aren't required for a plant's usual functions, but together with alkaloids, terpenes, and cyanogenic glycosides, they form a plant's immune system (Wink 2003; Hartmann 2007). Climate change has the potential to alter the quality of natural products, as well as the flavor and medicinal value of particular plants (Gore 2006). Secondary metabolite production is increased in stressed situations; nevertheless, secondary metabolite production is influenced by several elements such as plant competition, intensity and duration of light, soil characteristics, and degree of humidity, among others (Das 2012). In comparison to other living organisms, medicinal and aromatic plants are less resistant to climate change. Because climate change has a profound impact on plant life cycles and distributions, many medicinal plants have become indigenous to specific geographic locations. Global warming is supposed to cause a widespread plant extinction around the world. It is estimated that due to further increases in greenhouse gas emissions, more than half of the plants would be damaged by 2080 (Das and Mukherjee 2018). The reaction of plants to climate change varies depending on the plant species and developmental stage. Various plants have different species-specific thresholds, and their reactions, such as root elongation, root growth angle disruption, and yield loss, differ with species (Malhi et al. 2021).

This chapter discusses the impact of gaseous pollutants and the climate change on medicinal plants and their products, particularly on secondary metabolites (SMs). Special attention has been paid to the natural behavior, physiology and metabolism under harsh environmental conditions.

2 Medicinal Plants and their Importance

Plants play a vital role in the medicinal and healthcare regimes of people living in remote locations as in mountainous or desert regions, who often have a strong faith in the efficacy of herbal medicines, and generally lack access to contemporary healthcare facilities (Anis et al. 2000; Beigh et al. 2002, 2003a, b). Ayurvedic, Unani, and other traditional medicinal systems, as well as plant-based pharmaceutical enterprises, amply utilize the medicinal plants (Kumar et al. 2017; Parveen et al. 2020a, b, 2022; Husen 2021f, 2022b).

Medicinal plants are particularly important because of their secondary metabolites of pharmacological qualities, which are widely used in the pharmaceutical, cosmetic, and nutritional industries (Beigh et al. 2002; Hassan et al. 2012). According to the World Health Organization, about 80% of the world's population and 65% of Indians utilize natural and traditional methods of healing and curing with medicinal herbs (Bannerman 1980; Prashantkumar and Vidyasagar 2008). In Indian society, there are people, known as 'Vaidyas' and 'Hakeems', who have a deep understanding of medicinal plants and their applications for healing. They utilize the native herbal plants as a source of raw materials to create medications for disease therapy (Chopra and Khoshoo 1986). Herbal medication is now gaining ground in India as an alternative to modern Allopathy for treating chronic disorders. Plants produce a variety of secondary metabolites with unique properties that help improving the human immune system and treating various ailments (Husen and Iqbal 2022). In terms of toxicity or side effects, plant extracts have more positive points than negative ones (Van Huyssteen 2007). Even those living in developed countries are now opting for herbal medicine because of their low cost and negligible side effects. Terpenoids, phenols, steroids, flavonoids, tannins, and aromatic compounds are only a few of the chemicals derived from plants. Secondary metabolites are employed by plants for immunity against pathogens and herbivores; over 12,000 secondary metabolites have been isolated, with many more in the process of identification. Different components of plants are consumed more frequently than their derived oil during eating. By now, just a small number of medicinal plants, about half a million plants, have been identified; therefore, medical plant research has a bright future.

3 Secondary Metabolites under Changed Climate

Isolated plant metabolites, such as phenols, terpenes, and alkaloids, have been used in a variety of ways, including alterations to the core skeleton of products and medications. The isoprenoid, polyketide, and shikimate pathways are the primary pathways for the synthesis of secondary metabolites in plants (Verpoorte and Memelink 2002). Secondary metabolites have been proven in numerous studies to lessen the risk of a variety of major diseases and syndromes, including diabetes, TB, ulcers, asthma, cancer, Alzheimer's disease, and cardiovascular disease (Basu and Imrhan 2007; Holst and Williamson 2008; Crozier et al. 2009; Fang et al. 2011; Akula and Ravishankar 2011; Miller and Snyder 2012). According to studies, within 10 years (2005–2015), around 60 plant extracts and 110 purified compounds were obtained from 112 medicinal plants, and they showed efficacy in the treatment of multidrug-resistant pathogenic disorders (Gupta et al. 2019). Anthropogenic activities of the modern world have played a major role in polluting the atmosphere with a variety of toxic gases and particulate matters, although some natural events taking place occasionally also cause environmental degradation (Yunus and Iqbal 1996; Iqbal et al. 2000b). The pollutants so produced are responsible for increase in the atmospheric temperature, thus affecting the climatic condition. The gaseous as well as particulate pollutants not only remain suspended in the air but also settle down on the earth surface, thus rendering the air, soil and water toxic and unhealthy for life activities (Ansari et al. 2012; Iqbal et al. 2000a). These atmospheric changes result in conditions like drought, salinity, flooding, and extreme low or high temperature swings (Gupta et al. 2019). Plant growth and development are bound to be influenced by abiotic variables, as each plant species requires specific environmental conditions to thrive (Table 1).

Under harsh environmental conditions, plants tend to alter their set patterns of metabolic and functional activities in order to adapt to the changed environment (Anjum et al. 2012a; Iqbal and Khudsar 2000; Iqbal et al. 2005). This results in alteration of their physiological, structural and developmental traits (Aquil et al. 2003; Dhir et al. 1999; Singh et al. 2000). Finally, both the primary and secondary growth patterns get affected and exhibit a drastically modified picture (Hussein et al. 2017; Iqbal and Ghouse 1982; Iqbal et al. 2000b, 2010c; Verma et al. 2006), to the extent that even the schedule and duration of the formation of secondary vascular tissues (wood and bark) and also their composition (i.e. relative proportion of the component cell types, like axial parenchyma, fibres, tracheids/vessel elements, sieve-tube elements, ray cells, etc.) may undergo alteration (Gupta and Iqbal 2005; Iqbal et al. 2000a, 2010a, b; Mahmooduzzafar et al. 2010).

Currently, increase in normal temperature is a common and predictable feature all over the globe (Bhatla and Tripathi 2014). Temperature increases up to 5 °C have

Table 1 Impact of elevated carbon dioxide and ozone on secondary metabolites of some well-known medicinal plant species

Sr. No	Plant name	Disease treated	Pollutant	Alterations caused	References
1	*Ginkgo biloba* L.	Alzheimer disease	Elevated CO_2	Altered terpenoid content, 15% increase in quercetin aglycon and a 10% decrease in kaempferol aglycon, 15% in isorhamnetin and bilobalide to some extent	Gupta et al. (2019)
2	*Quercus ilicifolia* Wangenh.	Gynecological problems	Elevated CO_2	Increase in tannins and phenolic content	Stiling and Cornelissen (2007); Ibrahim and Jaafar (2012)
3	*Melissa officinalis* L.	Dementia, Anxiety and Central nervous system (CNS) related disorder	Elevated ozone	Increased in total anthocyanins to a substantial extent along with phenolics and tannins	Pellegrini et al. (2011); Shakeri et al. (2016)
4	*Capsicum Baccatum* L.	Asthma and digestive Problems	Elevated ozone	50% decrease in capsaicin and dihydrocapsaicin, seeds showed significantly reduction in capsaicin but no change in dihydrocapsaicin	Bortolin et al. (2016)
5	*Papaver setigerum* DC.	Eye and lung inflammation	Elevated CO_2	Enhancement of four alkaloids viz. morphine, codeine, papaverine and noscapine	Ziska et al. (2008)
6	*Hymenocallis Littoralis* (Jacq.) Salisb.	Neoplastic diseases and viral infections	Elevated CO_2	Increase in three types of alkaloids (pancratistatin, 7-deoxynarciclasine and 7-deoxy-trans dihydronarciclasin)	Idso et al. (2000)
7	*Salvia officinalis* L.	Gastritis, diarrhea, bloating, and heartburn	Ozone stress	An increase in phenolic content, notably in Gallic acid, Catechinic acid, Caffeic acid and Rosmarinic acid	Pellegrini et al. (2011)

been observed recently, and this can have a drastic impact on many plant species with reference to their survival, growth and yield (Cleland et al. 2012; Noor et al. 2019). Temperature spikes affect plant metabolic and growth performances due to changes in metabolic pathways that control signaling, functioning and defense programs within the plant. Consequent upon these conditions, production of primary metabolites, such as amino acids, carbohydrates, and Krebs cycle intermediate products, and also of various nitrogenous as well as non-nitrogenous secondary metabolites, gets affected. In general, an increased production of secondary metabolite protects plants from biotic stress, thus providing a connecting link between the biotic and abiotic stresses (Arbona et al. 2013). Some genotypic adjustments or changes could aid in the damage mitigation or plant adaptation to changing environmental conditions (Springate and Kover 2014). By way of an early activation of metabolic reactions, plants can overcome chemical imbalances, which is a must for their survival. Plants capable to modify their morphology and physiology in response to environmental changes can survive well under harsh environments (Millar et al. 2007; Noor et al. 2019).

3.1 Impact of CO_2 on Secondary Metabolites

Since the industrial revolution, CO_2 levels have risen substantially, posing a serious threat to human life and plant physiology. Since 1750, CO_2 emissions have increased considerably as a result of anthropogenic activity (Gupta et al. 2019). Although CO_2 basically favours photosynthesis and hence the phenomenon of plant growth (Ruhil et al. 2015), yet its excessive concentrations become toxic for plants. Medicinal plants have the ability to adapt to changing environmental circumstances. Secondary metabolites provide elasticity to their metabolic pathways, but this may have an impact on metabolite production, which is the foundation of their therapeutic efficacy (Mishra 2016). Secondary metabolite concentrations in plants are regulated not only by CO_2 concentration, but also by the exposure period. *Digitalis lanata* is used to treat heart failure and contains medicinal qualities (Rahimtoola 2004). When exposed to high levels of CO_2, however, digoxin (a cardenolide glycoside) concentrations increased by 3.5-fold, whereas other glycoside concentrations, such as digoxin-monodigitoxoside, digitoxin, and digitoxigenin, declined dramatically (Table 1). According to Weinmann et al. (2010), *Ginkgo biloba* is used to treat Alzheimer's disease, vascular dementia, and mixed dementia. When *G. biloba* is exposed to high levels of CO_2 and O_3 together, the terpenoid content changes, with a 15% increase in quercetin aglycon but a 10%, 15%, and to some extent, a drop in kaempferol aglycon, isorhamnetin, and bilobalide concentrations, respectively. Ghasemzadeh et al. (2010a, b) reported an increase in the concentrations of phenolic and flavonoid in *Zingiber officinale* due to increases in CO_2 levels. (Stiling and Cornelissen 2007) observed elevation in the concentrations of phenols and tannins in *Quercus ilicifolia* related to increases in CO_2 levels. Similar studies with *Elaeis guineensis* (oil palm) revealed an increase in phenols and flavonoids, and also in the primary metabolite phenylalanine, which is a precursor of various secondary metabolites (Ibrahim and Jaafar 2012; Rehman et al. 2021).

3.2 Impact of Ozone on Secondary Metabolites

Ozone layer in the stratosphere absorbs damaging ultraviolet light with wavelengths in the UV-B band between 280 and 320 nm, which can injure plants and animals (Montzka et al. 2018). Although ozone is prevalent in the stratosphere, it is considered a pollutant when present in the lower atmosphere (troposphere). It should, therefore, have harmful effects on plants also. Because the effects of O_3 on medicinal plants are little studied, it is important to extend future research in this direction (Table 1). *Melissa officinalis* is utilized to treat central nervous system issues, dementia, and anxiety. However, when exposed to high levels of O_3, its levels of phenols, tannins, and anthocyanins were slightly enhanced (Pellegrini et al. 2011; Shakeri et al. 2016). When a suspension culture of *Pueraria thomsonii* was exposed to O_3, it showed no elevation in the production of puerarin after 20 hours of exposure (Sun et al. 2012). However, a maximum of 2.6-fold increase in puerarin could be obtained after 35 hours (Gupta et al. 2019).

3.3 Plant Response to SO_2 and NO_x

Plants have long been used to pattern the degree of ambient air pollution because they are the first recipients of contaminants and act as their scavengers (Kaler et al. 2017). Pollutants emitted from various sources are normally the oxides of carbon, nitrogen and sulphur in gaseous form. They accumulate or impose themselves on the plant's leaf surface in particular and enter the leaf through stomata. Thus, they penetrate into the intercellular spaces of mesophyll cells and progressively diffuse into the cell sap. Air pollution has a negative impact on the health of plants; plant cells become inactive when pollutants are present in large concentrations (Iqbal et al. 1996; Munsif et al. 2021). Pollutants such as SO_2, NO_2, and H_2S cause a greater depletion of soluble sugars in the leaves of plants grown in polluted locations. Changes in the biochemical parameters of plant tissues are normally proportional to the load of contaminants inside the plant. Plant symptoms produced by air pollution may be chronic or acute, depending on the nature and extent of injury or damage (Dhanam et al. 2014). A chronic injury can kill a whole tissue or ruin the entire area of a leaf or needle. Acute damage occurs when a plant is overly sensitive to a particular pollutant or is exposed to high levels of pollution for a brief period of time. SO_2 is oxidized inside the leaf to sulphur trioxide (SO_3), which then reacts with water to generate sulfuric acid (H_2SO_4). As a result, acid production in the plant's body disrupts metabolic activities and reduces the plant's output (Sharma et al. 2017). Similarly, NO_2 interacts with the cell walls to create nitrous acid (HNO_2) and nitric acid (HNO_3), which lower the cellular pH, inhibit metabolism, and cause toxicity and growth suppression. Discolored spots or light brown hue, as well as bleached or necrotic spots in interveinal sections of leaves, are the morphological signs induced by NO_2 (Das and Mukherjee 2018; Adak and Kour 2021).

Sulphur gases are substantial air pollutants that can be created naturally by volcanic activity, but large concentrations owe to anthropogenic emissions from fossil fuel combustion (Sun et al. 2018). Plant metabolism can be significantly altered by air pollutants such as SO_2, H_2S, NO_2, or O_3, which affect a variety of molecules such as sugars, polyamines, phenylpropanoids, and several specialized phytochemicals (Khaling et al. 2015; Papazian et al. 2016). The majority of air pollutants, such as CO, SO_2, NO_2, and O_3, interact with plants near the leaf surface, where they can diffuse through stomatal pores and enter intracellular regions (Castagna and Ranieri 2009; Räsänen et al. 2017). Once absorbed, these hazardous chemicals disrupt stomatal functioning, leaf transpiration, gas exchange, and CO_2 fixation (Nighat et al. 2000; Rai et al. 2011). Chlorophyll content and photosynthetic rate are the main target and the major sufferers, and their disruption affects the entire form and function of the plant (Dhir et al. 2001; Wali et al. 2004, 2007). The reduced CO_2 availability and photosynthetic efficiency have a significant impact on plant central carbon metabolism. Oxidative stress caused by the production of reactive oxygen species (ROS) in tissues then compels the plant to develop appropriate responses to the pollutants load through activation of enzymatic and/or non-enzymatic antioxidant system and the production of specific secondary metabolites (Ainsworth et al. 2012; Yendrek et al. 2015; Aref et al. 2016).

3.4 *Role of Methane and CFC's*

Methane (CH_4), one of the most significant greenhouse gases, was previously thought to be a physiologically inert gas. However, the discovery that CH_4 has a variety of biological activities in animals, including anti-inflammatory, antioxidant, and anti-apoptosis activities, has cast doubt on this viewpoint. Meanwhile, it has been identified as a potential gaseous signaling molecule in plants, however the biosynthetic and metabolic pathways, as well as the mechanisms of CH_4 signaling, are yet unknown. Plants have traditionally been thought of as conduits for CH_4 transport and emission from the soil to the atmosphere (Li et al. 2020). Agricultural soils are the major source of methane and nitrous oxide gases and a sink of carbon dioxide. About 30% and 11% of the global agricultural output of methane and nitrous oxide, respectively, come from rice fields. Alterations in the conventional crop management regimes may likely cause reductions in the emission of these gases from the rice field. Organic soil amendments reportedly increase CH_4 emission from rice fields and improve the flag leaf photosynthesis of the rice crop over the control (NPK application alone). The combined application of NPK and Azolla compost caused a 15.66% higher CH_4 emission with 27.43% more yield over the control and increased the capacity of soil carbon storage, with a high carbon efficiency ratio (Bharali et al. 2018; Gupta et al. 2021). FCs (chlorofluorocarbons) are normally harmless and non-flammable compounds made up of carbon, chlorine and fluorine atoms. However, they are known to destroy the ozone layer, and this is likely to allow greater amounts of the sun's radiation reach the earth and affect the plants. It is, therefore, apprehended that the plants may consequently experience abnormal and reduced growth due to possible protein denaturation and DNA damage (Gupta 2018).

4 Conclusion

Plants have a wide range of species and produce a large number of secondary metabolites, many of which are physiologically active and extremely beneficial to humans, being utilized mainly for therapeutic purposes. Medicinal plants have been used to develop new allopathic medicines for the past few decades. Changing climatic circumstances and abiotic stress factors have an impact on plants' natural behavior and physiology, which has an impact on essential secondary metabolites. Gaseous pollutants (such as SO_2 and NO_2) and greenhouse gases (like CO_2, ozone, methane, and CFCs) have direct toxic effects on plants and also change climatic conditions, affecting water, pH level, and salinity, which again have a bearing on metabolite production in plants. Some environmental factors, such as temperature and elevated CO_2, basically enhance the secondary metabolism in plants, whereas extreme temperatures (too hot or too cold), drought, and high salinity negatively affect the metabolites, growth, and productivity of plants.

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Impact of Salinity Stress on Medicinal Plants

Muhammad Zulqurnain Haider, Muhammad Arslan Ashraf, Rizwan Rasheed, Iqbal Hussain, Muhammad Riaz, Freeha Fatima Qureshi, Muhammad Iqbal, and Arslan Hafeez

Abstract The growing of medicinal plants has proven to be financially beneficial to the pharmaceutical and medical industries. These plants are rich in active ingredients that can be turned into a wide range of medicines, many of which are life-saving. However, since most cultivable lands are primarily used to produce essential food crops, it is hard to start massive cultivation of these plants. However, non-cultivable lands are often subjected to various abiotic stresses, the most common of which is salinity. Salinity affects a large portion of land worldwide; over 45 million hectares (M ha) of irrigated area, or 20% of total land area, have been degraded by salt, and 1.5 million ha are pulled out of cultivation yearly due to increased salt accumulation in the soil. The highly productive use of saline agriculture in developing countries is an extensive scientific issue for local governments and agriculturists. Salinity is significant stress that reduces the growth and yield production potential of medicinal plants worldwide. The osmotic effects of Na^+ and Cl^-, alongside oxidative stress, are responsible for the harmful effects of salinity on plant growth. Plant metabolic activities are negatively impacted by salinity stress, which is caused by DNA damage and membrane lipid peroxidation as a consequence of reactive oxygen formation. Elevated salt levels trigger various adverse effects on agricultural output, including plant growth and development delays, impairment of metabolic processes, and a fall in photosynthetic efficiency.

Keywords Phytochemicals · Antioxidant defense · Polyphenols · Flavones · Flavonoids · Nutrient acquisition · Growth · Artichoke

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A. Husen, M. Iqbal (eds.), *Medicinal Plants*,
https://doi.org/10.1007/978-981-19-5611-9_8

1 Introduction

Global water scarcity, severe environmental contamination and salinization of land and fresh water marked the beginning of the twenty-first century. Increased human population and a loss in arable land are the two key risks to agriculture sustainability (Gupta et al. 2021). The global population will reach 9.6 billion by 2050 and further aggravate the food security issues throughout the world (Sita and Kumar 2020). Food security is considered as a difficult challenge in developing countries. According to Food and Agriculture Organization (FAO), Asian countries are mostly facing food insecurity issues, showing that it may be hard to provide enough food to the increasing global population in the near future (Mukwada et al. 2020). Around 70% more food production is required to meet the demand. Abiotic stresses are causing more than 50% loss of average crop yield throughout the world (Fahad et al. 2015; Malhi et al. 2021).

Water deficit, toxic metals, cold, heat, and salt stress are the main abiotic stresses prevailing in the environment, affecting plant growth and development. Salinity is the excessive accumulation of salts in soil that disturbs the normal growth of plants (Sabagh et al. 2020). Soil and irrigation water with an electrical conductivity of 4 dSm^{-1} or higher is considered as being saline (Farooq et al. 2019). The primary salinity originates from natural means of rock weathering that discharge soluble salts like NaCl, Ca, Mg, SO_4^{2-}, and CO_3^{2-} into water that becomes part of the soil medium via rain and wind. Besides, anthropogenic activities such as the substitution of perennial crops with annual ones, usage of salt-concentrated water for irrigation purposes, and application of chemical fertilizers enhance the salinity problem of an area (Giordano et al. 2021). More than 20% of agricultural land is affected by salinity, spoiling around 954 million hectares of total land area all over the globe (Saddiq et al. 2021; Tufail et al. 2021). It is expected to rise up to 50% by 2050 (Das and Tzudir 2021). Pakistan's entire geographical area is 79.61 million hectares, of which 80% land area is irrigated with canal water. Salinity stress has affected about 14% of irrigated land, whereas salt stress causes a 64% yield drop in Pakistan. As a result, only around 23 million hectares of land remain suitable for the agricultural system in Pakistan (Syed et al. 2021).

Factors including the quantity of evaporation (that could increase salt level), and precipitation (resulting in reduced salt level) define the extent of salt stress in an area. Excess deposition of salts, particularly sodium (Na) ions, can change the soil's basic structure, causing a reduced soil porosity, thereby resulting in low soil aeration and water conductance. Plants may vary in terms of their tolerance to salt stress as mirrored in their different growth stages. For instance, glycophytes, also known as salt-sensitive plants, such as rice, maize, soybean, or beans and halophytes (salt tolerant plants) such as salt bush and alfalfa (Porcel et al. 2012; Sodani and Mundiyara 2021). Salt stress affects health of soil, plant growth and yield globally. Salt-mediated reduced plant growth results through impaired photosynthetic rate, imbalanced ion uptake, osmotic stress, higher ROS synthesis, oxidative injury and hormonal imbalance (Akbar et al. 2021). However, different factors such as type of salts, time period, the way of salt application to plants and plant category (species and genotype) determine the extent of salt-induced injury in plants (Sehrawat et al. 2015). Salts over-accumulation results in specific ion toxicity, in turn affecting water relations of plants. Therefore, plants in response to hyper-osmolarity environment faces various metabolic and physiological problems. The disturbed plant metabolism thereby gives rise to oxidative injury through excess reactive oxygen species production in plants (Shafiq et al. 2021). Salt stress reduces plant vegetative growth and yield through causing hyper-ionic and hyper-osmotic impact on soil rhizosphere (Bilal et al. 2020).

The response of plants to salinity stress may vary with the developmental stages of plant. For example, plants are more sensitive to salinity stress at their vegetative stage than at the reproductive stage. Salt tolerance is measured as the plant-survival percentage during seedling stage. Elevated levels of salts reduce the growth of seedlings, flowering, formation of spikelet, germination of pollen grain and fertilization of plants (Sodani and Mundiyara 2021). Therefore, management measures could be achieved by better knowing plant tolerance to salt at various phases of development (Ashraf et al. 2015). Salt stress-mediated delayed seed germination may be taken as indicator of plant sensitivity (Abdel-Farid et al. 2020; Shafiq et al. 2021). A marked reduction is also caused in leaf area, internodal length, and the overall root and shoot (Kumar et al. 2021).

Exposure to higher doses of salts affected the reproductive cycle in wheat, which impeded spike development resulting in a considerable yield drop (Läuchli and Grattan 2007). Salt stress diminished essential ions uptake by affecting root growth and interfering with the transporters present in root cell membrane (Ha-Tran et al. 2021). Garcia et al. (2019) reported that elevated levels of salts in Florida diminished the K^+ and Mg^{2+} ions availability in soil medium, thereby causing wilting of plants and other nutritional issues. Plants adopt a number of tolerance mechanisms to mitigate the toxic impacts of salt stress. They cope with high salt levels by hindering Na ions access into root, its transportation and distribution in the leaves and sequestration into vacuoles. Two types of Na^+/H^+ antiporter, namely, vacuolar Na^+/H^+ and plasma membrane Na^+/H^+ antiporters facilitate Na ions transfer from cytoplasm to vacuole or apoplast (Porcel et al. 2012). Generally, plants uptake Na passively, causing their maximum concentration in cytosol (Ashraf et al. 2015).

Vacuolar antiporters sequestrate Na ions inside the vacuoles, where they aid in maintaining a reduced cellular Na concentration. However, the lateral antiporter facilitates exclusion of Na ions from the cytosol and their redistribution in root and shoot of plants (Evelin et al. 2019). Schematic representation of salinity effects and tolerance mechanisms in plants is shown in Fig. 1

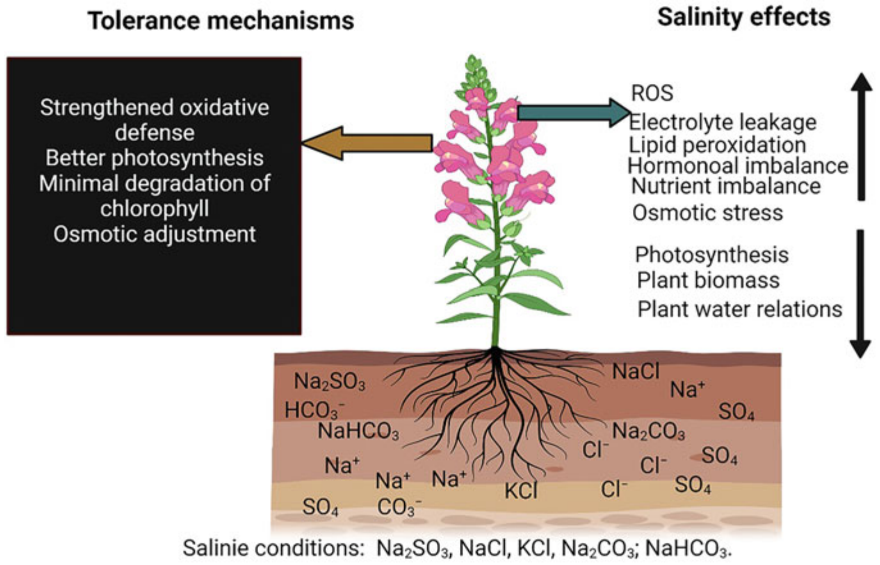

Fig. 1 Schematic representation of salinity effects and tolerance mechanisms in plants

2 Effects of Salinity Stress on Plant Growth

Salt stress affects plant growth through (1) decreased osmotic potential of soil, causing water-deficit condition in plants, (2) mineral nutrients imbalance, (3), specific ion impacts and (4) oxidative injury (Laxmi et al. 2021).

3 Osmotic Stress

Osmotic stress decrease plant growth and productivity against salt stress (Sairam and Tyagi 2004; Ludwiczak et al. 2021). It is the initial stage of salinity stress that begins when salts concentration rises above a certain threshold limit around the roots of plants (Ilangumaran and Smith 2017). Excessive salt-mediated decrease of water potential (James et al. 2008; Upadhyaya et al. 2013) and turgor pressure (Arif et al. 2020) is responsible for osmotic stress in plants. Generally, plants may uptake reduced amount of water and nutrients in response to greater osmotic potential of saline soil/water containing higher amount of Na and Cl ions (Yuan et al. 2020). Salt stress-mediated osmotic stress results in suppressed development of shoot and reproductive organs; limited leaf growth, delay in new leaves emergence, withering of older leaves, production of less branches or lateral shoots and start of earlier blooming of plants (Munns and Tester 2008). Increased leaf temperature and delayed shoot elongation are also caused due to low water potential and thick inner wall of guard cells (Mukhopadhyay et al. 2020). Salt-induced osmotic stress also leads to degradation of protein and other organic substances (Santander et al. 2017), and reduces the vacuolar and cytosolic volume in plants (Ates et al. 2021). It causes hormonal disorders and deteriorates enzymatic activities required for nucleic acid and protein metabolism, in turn, affecting seed reserves during germination process. Water use efficiency and relative water contents are also affected; water potential and relative water contents in wheat plants declined by 3.5% in salt-tolerant cultivar and 6.7% in salt-sensitive cultivar under salinity stress (Hussein and Abou-Baker 2018). Osmotic stress favors closure of stomata, which impedes photosynthetic ability by inhibiting CO_2 supply to plants (Hameed et al. 2021). The arrest in rice growth from germination of seed to maturity phase in response to salt stress might be owing to the increment in osmotic pressure of root and ions impacts in plants (Sheteiwy et al. 2019). On the whole, all these changes caused by salinity stress factors result in reduced plant growth and yield, as observed in a variety of nutritional and medicinal plants, including rice (*Oryza sativa*), wheat (*Triticum aestivum*), faba bean (*Vicia faba*), Brahmi (*Bacopa monniera*), Senna (*Cassia angustifolia*), chamomile (*Matricaria chamomilla*), etc. (Ali et al. 1999; Arshi et al. 2002, 2004; Heidari and Sarani 2012; Hussein et al. 2017; Hussein and Abou-Baker 2018). The degree of impact varies depending on the mode of stress (slow or fast), exposure time and the nature of plant species/genotype. External administration of osmoprotectants and stimulation of internal osmoprotectants synthesis could also help the plant to confront toxic effects of osmotic stress, when the plant is in early stage of salt salinity stress (Ashraf et al. 2015).

4 Specific Ion Toxicity

By definition, ion toxicity is the ion buildup in the shoot of plants and the inability to control the ions accumulation. It is the 2nd phase of the effect of salt stress indicating the accumulation of ions above the exclusion rate in plants. These ions are translocated from the below-ground mass to the xylem, whereby they translocate towards the leaves to accumulate in cytoplasm and vacuoles to toxic limits. This situation leads to death of the leaf and if the rate of new leaf emergence is lower than mortality, the photosynthetic capability of the plant is hampered (Ayuso-Calles et al. 2021). The specific ion toxicity impairs the ions balance with the increase in concentration of tissue Na and Cl ions and a decline in tissue K ions (Soliman et al. 2020). The accumulation of Na ions becomes toxic for older leaves, rendering them unable to expand and detoxify the salt's toxic impact, as compared to younger leaves. If this situation continues, the photosynthetic capacity of the plant does not suffice to supply proper amounts of carbohydrates that are needed by young growing leaves, hence plant growth is inhibited (Carillo et al. 2011). Specific ions (Na and Cl) toxicity demolishes the plant growth and productivity. For instance, elevated level of Cl ions hamper the plant chlorophyll contents though Na ions may or may not decrease the chlorophyll contents in plants, suggesting that Na accumulation is not the primary reason behind chlorophyll deterioration in plants. Chloride ions are responsible for chlorosis in salt-affected plants (Tavakkoli et al. 2011). Salt-mediated specific ion toxicity also results in altered K/Na ratio in plants, like the exogenous Na can drastically affect the intracellular influx of K in plants. Salt stress cause over-accumulation of Na and Cl ions in cytosol that becomes lethal for plants cells. The Na can affect the membrane potential and accelerate the Cl ion uptake down the gradient in plants under salt toxicity (Tuteja 2007). The Cl ions are an essential micronutrient involved in regulation of several enzymes' activities in cytoplasm, function as cofactor in photosynthetic system, regulate pH and turgor in plants. However, their excess quantity becomes lethal for plant growth and development under salt stress. Na ions may drastically damage the stability of membrane and cause leakage of ions from the membrane in plants under NaCl stress. However, Luo et al. (2005) found that two cultivars of soybean showed more toxic effects of Cl ions on plant membrane rather than Na ions accumulation in response to NaCl application (Kumar and Khare 2015). Cucumber genotypes (Aramon and line-759), which differ in Na and Cl exclusion were used in a mixed-salt experiment with similar osmolarity and equimolar levels of ions between treatment. They displayed reduced photosynthetic rate and stomatal conductance in response to Na ions than Cl ions under NaCl stress. In another study, the limitation of Na uptake by pumpkin roots has been characterized as the most obvious physiological event of evolved salinity tolerance in plants. Na ions accumulation impaired the activity of enzymes involved in photosynthesis. Na ions also interacted with plant physiological events maintained by K, such as stomatal regulation. The plant roots did not discharge Na ions, in turn improving K balance and tolerance of salinity (Chen et al. 2020).

5 ROS Generation in Plants

Reactive oxygen species (ROS) are a class of oxygen-derived free radicals constantly produced as by-products of basic metabolic activities like photosynthesis and respiration in plants. Abiotic stresses including salt stress favor the production of different kinds of ROS including singlet oxygen ($^1O_2$), hydroxyl radical ($OH^\bullet$), superoxide radical ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) in plants (Kanojia and Dijkwel 2018). The incomplete or partial reduction of oxygen (O_2) molecule is responsible for ROS synthesis in plants (Hasanuzzaman et al. 2021). The O_2 in its ground state is considered as harmless molecule since it possesses two unpaired electrons with parallel spin, making it paramagnetic, and thus unlikely to participate in organic molecules processes unless activated. The O_2 activation takes place through absorption of ample amount of energy to reverse the spin on one of the unpaired electrons in turn, leading to the production of $^1O_2$ while in second step, O_2 is reduced to $O_2^{\bullet -}$, H_2O_2 and $OH^\bullet$ radicals. $O_2^{\bullet -}$ radical being the first ROS produced by monovalent reduction in the electron transport chain (Anjum et al. 2015). This $O_2^{\bullet -}$ radical is then converted into H_2O_2 by the action of either superoxide dismutase (MnSOD) or ascorbate peroxidase (APX) in mitochondria. H_2O_2 is further converted to $OH^{\bullet}$ radical in the Fenton reactions (Sharma et al. 2012). Both $O_2^{\bullet -}$ and H_2O_2 are synthesized from the fatty acid oxidation and photorespiration reactions. ROS has dual role in plant life; signaling or stressor, depending on the balance between the ROS synthesis and their quenching (Hasanuzzaman et al. 2021). Salt stress-mediated ROS generation affects the discharge of photorespiratory H_2O_2, photosynthetic and respiratory electron transport systems in plants. Also, various enzymes' activities such as peroxidases, glucose and xanthin oxidase, particularly NADPH oxidase, are affected under salt stress in plants (Tomar et al. 2021). The over-production of these ROS species leads to oxidation of macromolecules such as carbohydrates, DNA, proteins and lipids, causing membrane injury and cell death (Anjum et al. 2012b, 2015; Nguyen et al. 2017; Luo et al. 2021). For instance, $O_2^{\bullet -}$ radical generation results in oxidative injury, modulation of enzymes having iron-sulfer centers and amino acid oxidation in plants under salt stress (Mushtaq et al. 2020). H_2O_2 (10μM) inhibited 50% of CO_2 fixation owing to the oxidation of thiol groups of Calvin cycle enzymes (Kreslavski et al. 2012; Sachdev et al. 2021). $^1O_2$ produced as a result of photo-oxidative injury can harm photosystem (PS) II through interacting with D1 protein. $OH^{\bullet}$ radical, being the most potent ROS species, causes damage to membranes of various plant organelles through lipid peroxidation in plants. Its elevated level can also damage protein and DNA, and generate cytotoxicity causing cell death in plants under salinity stress (Banerjee and Roychoudhury 2018). Higher H_2O_2 and $O_2^{\bullet -}$ radical production was found in mustard (Sami et al. 2021), sweet pepper (Abdelaal et al. 2020), tomato (Ahanger et al. 2020), rice (Long et al. 2020), cowpea (Desoky et al. 2020) and cucumber (Jiang et al. 2019) plants under salt stress. Similarly, wheat plants also manifested increased H_2O_2 production in response to salt stress (Mohsin et al. 2020).

6 Lipid Peroxidation

Salt stress-induced ionic as well as osmotic toxicity and nutrients imbalance resulted in impaired metabolic system and increased oxidative injury in plants (Bhargava and Srivastava 2020). ROS acts as the second messenger to regulate plant normal growth at their minute level. However, their maximum generation creates oxidative burst due to their incomplete quenching by antioxidant defense system in plant under different environmental constraints (biotic and abiotic). Lipids and proteins act as the main target sites for oxidative injury in response to ROS generation in plants (Anjum et al. 2015). The oxidative breakdown of PUFA (polyunsaturated fatty acids), also called as lipid peroxidation, occurs in all living beings and evaluates the severity of lipid injury under abiotic stresses. Generally, lipid peroxidation initiates a chain reaction, causing production of other reactive products including hydroxyl acids, ketones and aldehydes, and in turn, can modify proteins through oxidation of some amino acid residues. The protein activity changes owing to carbonylation, nitrosylation, disulfide bond creation and glutathionlyation in plants (Xie et al.

2019). Salinity stress-mediated ROS synthesis also affects plant cell through lipid peroxidation in the form of MDA (malondialdehyde) production, amino acid oxidation, enzymes denaturation and damage of nucleic acid (Hossain and Dietz 2016; Mishra et al. 2021). MDA is the breakdown product of PUFA of biological membrane. Further, MDA is used as a biomarker of lipid peroxidation, plasma membrane and plant structural injuries under salt stress (Vasilakoglou et al. 2021). Rasheed et al. (2014) found maximal increment in MDA contents in canola plants under salt stress. Cucumber plants displayed higher contents of MDA alongside the maximum electrolyte leakage in response to salt toxicity (Khan et al. 2013). Elevated levels of salt significantly enhanced the electrolyte leakage and MDA contents, and showed higher lipid peroxidation in *Plantago ovata* plants (Reza et al. 2020). The increased electrolyte leakage might be owing to salt-induced membrane injury and selective permeability in plants under salt toxicity (Abdelaal et al. 2020). A marked amplification in MDA and electrolyte leakage was observed in radical and coleoptile region of barley plant seeds under salt stress, which was indicative of both membrane and oxidative injuries at their cellular level, causing a retarded seed germination in plants (Ben Youssef et al. 2021). Milk thistle plants exhibited higher oxidative injury in the form of MDA and H_2O_2 in response to salt toxicity (Zahra et al. 2021). Salinity stress augmented the $H_2O_{2,}$ MDA and electrolyte leakage in cucumber leaves. The elevated level of H_2O_2 caused lipid peroxidation, which affected plant membrane, thereby resulting in electrolyte leakage and MDA contents in plants. Similar to MDA, electrolyte leakage also acts as indicator of membrane integrity under abiotic stresses. However, H_2O_2 level is a measure of ROS quenching ability of plants under salt stress (Wang et al. 2016).

7 Hormonal and Nutritional Balance

7.1 Role of Hormones

Plant hormones are organic compounds mainly synthesized in one part of plant and transported towards others, where they trigger physiological responses at their minute level. Being signal chemicals, they have a role in induction of stress responses in plants (Kaya et al. 2009). There are nine well-recognized plant hormones including salicylic acid, ethylene, jasmonic acid, abscisic acid, strigolectone, cytokinins, auxins, brassinisteroids and gibberellins. Some of these, like abscisic acid (ABA), indole acetic acid (IAA), salicylic acid (SA) and ethylene, are relatively better studied in relation to salt stress. Abscisic acid plays effective role in providing defense to plants under salt stress. ABA quantity increases in response to salt and osmotic stress in plants. Salt-stress-mediated impaired nutrient uptake is also regulated by sufficient ABA level in plants. For instance, it accelerates the cytosolic free Ca ions level in plants under salt stress. ABA generates tolerance through incorporating with Ca by exciting channels that are attached with plasma membrane or discharging Ca from intracellular Ca-store area in plants under salt stress (Yu et al.

2020). Ethylene, cytokinin and gibberellins also regulate plants' normal growth and development under salt stress condition (Dey et al. 2021; Rhaman et al. 2021). However, salinity stress diminishes the gibberellins and cytokinin contents in plants (Kaya et al. 2009). Soybean plants showed a higher level of ABA under salinity stress (Khan et al. 2021). Although ABA is a growth-inhibiting hormone, its small quantity can enhance growth of plants under stressed environment (Martínez-Andújar et al. 2021). Elevated level of salt enhanced the ABA contents in strawberry leaves could affect gene expression in plants. Higher ABA contents also maintains water level in plants under salt stress (Zahedi et al. 2020). Salt stress-mediated accumulation of ABA causes closure of stomata in plants (López-Serrano et al. 2020; Acet and Kadıoğlu 2020). ABA-induced alterations of ion fluxes in guard cells are responsible for stomatal closure in plants under salt stress (Raghavendra et al. 2010). ABA decreases the turgor of guard cells through the efflux of K and other anions, converting malate to starch that is osmotically inactive substance in plants under salt stress (Amjad et al. 2014).

In plants, ethylene acts as a stress-responsive and growth-stimulating agent under stress environment. Its mild accumulation favors plant growth, but elevated levels suppress the growth, causing death of plants. Endogenous synthesis of ethylene is required for plant response to salt stress. Since ethylene is known as salinity response hormone, its level in plants increases under salt stress. It is also actively involved in stress signal transduction in plants in response to salt stress. For instance, various kinds of genes (ethylene receptors, signaling and MAPK cascade genes) were all activated in response to salt stress in cotton plants. It confers salt stress tolerance via ameliorating the Na/K balance in plants (Tao et al. 2015). Tolerant genotypes of tomato plants displayed elevated amount of ABA and ethylene in response to salt toxicity, compared to sensitive genotypes. Higher ABA content was due to osmotic impact of NaCl and its *de novo* production in plant roots that enters into apoplast via transpiration stream (Amjad et al. 2014).

Husen et al. (2016) reported negative impact of salt stress on dry mass production, relative water content and leaf characteristics such as pigment concentration, maximum quantum yield of PSII, stomatal conductance, net photosynthetic rate, transpiration rate and water use efficiency in two-month-old pea plants (*Pisum sativum* L. cv Adi). All these parameters were suppressed under salinity, but the effect of salinity was much less on plants that received a foliar spray of IAA. Salinity stress also hampered the overall growth performance of cultivars Adet and Merawi of Ethiopian mustard (*Brassica carinata*) in a dose-dependent manner. It reduced the stomatal conductance, chlorophyll fluorescence, net photosynthetic and transpiration rates, nitrate reductase activity, and plant water status. However, SA application alleviated the adverse effects of salinity and improved the overall performance of both the cultivars. Higher dose of salinity increased proline production but SA application mitigated this impact. However, activity of antioxidant enzymes increased by salt stress as well as SA treatment (Husen et al. 2018). Similarly, salt treatments significantly inhibited growth parameters of niger (*Guizotia abyssinica*), an oilseed plant, as was evident from a dose-dependent reduction in size and biomass of roots and shoots. Salinity stress also reduced photosynthetic efficiency by inhibiting chlorophyll synthesis, and hampering other related aspects. High salinity increased proline production and the activity of antioxidant enzymes, viz. superoxide dismutase, catalase, and peroxidase. Application of salicylic acid (SA) in combination with salt stress mitigated the salinity-induced adverse effects on growth attributes, chlorophyll contents and photosynthetic parameters, but the upregulation of antioxidant enzymes evoked by salinity was further enhanced. Thus, SA application fortified the salt-tolerance capacity of *Guizotia abyssinica* by alleviating the membrane injury, accelerating assimilatory activities, improving plant water status, and activating the defense arsenal (Husen et al. 2019). Irrigation with salt (NaCl) caused drastic damage to growth parameters of lemongrass (*Cymbopogon flexuosus*) through formation of ROS, but different SA concentrations applied exogenously in the form of foliar spray significantly minimized the damage seemingly by increasing the POD, CAT and SOD activities (Rehman et al. 2022).

7.2 Role of Nutrients

Salt stress resulted in maximal accumulation of Na and Cl ions that hinder acquisition as well as balance of essential nutrients ions and trigger oxidative injury in plants (Iqbal et al. 2015). Higher Na ions accumulation in plants results in impaired nutrient uptake, including phosphorus (P), nitrogen (N), calcium (Ca), magnesium (Mg), and potassium (K). Generally, higher uptake of both Na and Cl ions causes hyperosmotic stress that, in turn, declines water potential thereby reducing the uptake of water by roots. Salinity treatments caused significant reductions in the root, shoot, leaf, pod biomass, leaf area, stomatal conductance, photosynthetic rate, concentration of K^+ and Ca^{2+} and the sennoside concentration and yield in senna (*Cassia angustifolia*), whereas individual $CaCl_2$ treatments had a favourable effect. Under combination ($NaCl+CaCl_2$) treatments, the extent of reduction was much less, compared to NaCl treatments alone. The combined treatments thus mitigated the adverse effects caused by NaCl. On the contrary, proline accumulation in the leaves increased with NaCl treatment and further increased with combined combination of NaCl and $CaCl_2$. Thus, calcium could alleviate the salt-induced inhibition of plant growth via the maintenance of net K^+ to Na^+ selectivity and the enhancement of proline accumulation in leaves (Arshi et al. 2005, 2006a). Similar effects of Na and Ca were also recorded on different growth parameters of some other medicinal plants including chicory (*Cichorium intybus*) and soybean (*Glycine max*) (Arshi et al. 2006b, 2010a, b).

Calcium, being an essential element, plays several important roles in plants in establishing the structure of cell walls, and regulating the ion selectivity and membrane integrity in plants. Potassium (K) is the most common cation in plant cell that sustains the integrity of cell and plasma membrane. Under salt stress, Na ions replace K ions owing to their mutual resemblance, thereby inhibiting several metabolic events and enzymatic activities that are regulated by K in plants. For instance, replacement of K by Na ion in ribosomes results in a decline of protein synthesis in response to salt stress (Bello et al. 2021). Further, K is also an essential element for plant growth due to its involvement in maintenance of turgor pressure and support to photosynthetic system. Therefore, K deficiency becomes lethal for plant survival under salt stress (Al-Murad et al. 2020). Plant tissues need an acceptable level of K under salt stress, which is maintained through selective absorption of K and compartmentation of Na and K, followed by their allocation in shoot (Nawaz et al. 2010; Ashraf et al. 2015). Salt stress inhibited growth parameters, including biomass, chlorophyll content, protein content and NR activity, in Indian mustard (*Brassica juncea*) plants (Yousuf et al. 2015). Membrane damage was induced with a concurrent increase in antioxidant defence system and proline content. Individual application of K and Ca mitigated the negative influence of the stress, with the maximum alleviating potential exhibited by the combined application of these nutrients. Results obtained on real time expression of genes encoding enzymatic antioxidants (SOD, APX, CAT and GR), NR and proline confirmed the findings with biochemical assays (Yousuf et al. 2015).

Magnesium ions are important for stable chlorophyll molecule formation in plants. Fe plays effective role in plant nitrogen fixation and respiration, and forms part of cytochromes and non-heme iron proteins that are involved in photosynthesis (Okon 2019). Excessive amount of Na ions replaces the Ca ions found in cell wall and cell membrane, thereby decreasing their transportation in plants under saline environment. Plants uptake N in the form of nitrate and ammonium ions, and salt stress inhibits N uptake by immobilizing them in plants. The solubility and mobility of Fe ions are affected under salt stress, producing a depletion zone around the root, which hinders Fe uptake in plants. P contents are demolished in saline-affected plants owing to their precipitation with other cations including Ca, Mg and Zn rely on the pH of the soil medium. This condition favors P deficiency in plants mirrored as growth retardation (Evelin et al. 2019). Alnusairi et al. (2021) reported that over-accumulation of NaCl decreased the N, Ca and K ions uptake in wheat plants under saline condition. Salt stress-mediated inhibition in essential ions uptake might be due to their competition with other ions transporters present in root plasma membrane. A decline in osmotic potential of soil solution reduces the bulk flow of nutrients in roots thereby causing their deficiency in plants (Rasheed et al. 2020). Elevated level of Na ions in cell membrane disrupted the uptake of K and Ca ions in thyme species. Conversely, the antagonism between Na, K and Ca levels can decrease Ca ions availability and their competition for binding sites in plants under salt stress (Bistgani et al. 2019). Spearmint plants displayed higher P and Na ions but lower contents of N, K, Mg and Zn in response to salt stress (Chrysargyris et al. 2019). Soybean plants manifested lowered K, Ca, and P contents as well as Ca/Na and K/Na ratio under salt stress. The reduced Ca contents might be due to replacement of Ca with Na present in plant cell membrane. The impact of ionic strength and reduced solubility of calcium-phosphorus minerals is involved in diminished P availability in plants. Further, phosphate level is highly controlled by sorption process in the soil solution that lowers P contents in salt-affected plants. Elevated level of Na salt is responsible for reduce Ca/Na and K/Na ratio in plants under salt stress (Weisany et al. 2014). Similarly, salt stress caused a maximal drop in K, Ca and K/Na ratio in tomato plants (Taffouo et al. 2010). Thus, plants undergo acute nutritional deficit when growth medium contains substantial amount of NaCl. Further, the Na-mediated decrease in mineral uptake, K uptake in particular, could be responsible for nutrient imbalance in plants.

7.3 Proteomics and Metabolomics

Of late, various steps involving genes, transcripts, proteins and metabolites, are being taken to find ways to overcome the challenge put to crop productivity by environmental stresses. Proteomics and metabolomics have been used in several studies to unravel the impact and mode of action of salinity stress in plants (Hakeem et al. 2013). Sensitivity and modulation of plant proteome regulate the proper signal execution and adaptation to abiotic stress via molecular responses, thus strengthening the plant defence system. In a study by Bagheri et al. (2015), peptide mass fingerprinting (PMF) enabled identification of maturase K and PPD4 with increased abundance in Spinach (*Spinacia oleracea* L.) exposed to individual and combined stresses of salinity and cadmium. Salinity stress and combined ($NaCl+CdCl_2$) stress silenced the presence of one protein (polycomb protein EZ2) and two proteins (cellulose synthase-like protein and ubiquitin conjugation factor E4), respectively (Bagheri et al. 2015). The identified proteins were functionally associated with signal transduction (15%), protein synthesis (16%), stress response and defence (33%), photosynthesis (13%), plant growth/cell division (9%), energy generation (4%), transport (4%), secondary metabolism (3%), and cell death (3%), thus clearly maintaining a high ratio of defence and disease-responsive proteins and suggesting that plants have differential mechanisms, with unique sets of proteins, to respond to stresses caused by Cd, salinity, and their combination (Bagheri et al. 2015).

A comparative proteome analysis of shoots of two genotypes (CS-52 and Pusa Varuna) of Indian mustard {*Brassica juncea* (L.) Czern. and Coss.} brought out a differential expression of 21 proteins during two-dimensional electrophoresis (2DE). These proteins were linked to osmoregulation, photosynthesis, carbohydrate metabolism, ion homeostasis, protein synthesis and stabilization, energy metabolism, and antioxidant defense system. Salt-tolerant genotype (CS-52) showed a relatively higher expression of proteins involved in turgor regulation, stabilization of photosystems and proteins, and salt compartmentalization, as compared to salt-sensitive genotype (Pusa Varuna). These findings suggest that modulating the expression of salt-responsive proteins can pave the way for developing salt tolerance in the Indian mustard plants (Yousuf et al. 2016b). Likewise, analysis of differential expression of chloroplast proteins in Pusa Agrani (salt-sensitive) and CS-54 (salt-tolerant) genotypes of *Brassica juncea*, grown under salinity stress, enabled identification of proteins related to a variety of chloroplast-associated molecular processes, including oxygen-evolving process, PS I and PS II functioning, Calvin cycle and redox homeostasis. Expression analysis of genes encoding differentially expressed proteins through real time PCR supported the findings obtained with proteomic study.

Thus, modulating the expression of chloroplast proteins associated with stabilization of photosystems and oxidative defence could play major roles in adaptation to salt stress (Yousuf et al. 2016a).

Yousuf et al. (2017) observed that productivity of 14 genotypes of Indian mustard (*Brassica juncea*) was markedly reduced by salt stress, as it inhibited biomass accumulation, reduced the protein and chlorophyll contents and triggered a concentration-dependent overproduction of reactive-oxygen species and a concurrent upregulation of the expression of different antioxidants. Genotypes CS-54 and Pusa Agrani, showing the minimum and maximum damage respectively, were investigated through gel-based proteomic approach, which resulted in the identification and quantification of 42 salinity-responsive proteins related to different metabolic modifications. Molecular processes, including photosynthesis, redox homeostasis, nitrogen metabolism, ATP synthesis, protein synthesis and degradation, signal transduction and respiratory pathways, showed significant changes. The identified stress-responsive proteins could pave the way to develop salt tolerance in Indian mustard genotypes (Yousuf et al. 2017).

Metabolomes, the derivative of the metabolic system, comprise of many primary and secondary metabolites (molecular weight less than 1500 Da), which originate following a definite biosynthetic pathway of the physiological system of plants, and are indicative of the responses of biological organisms to their environment. Metabolites like saponins, proline, sugars, flavonoids and other phenolic compounds, GABA, threonine, leucine, glutamic acid, glycine, mannose, and fructose with the intermediates of TCA cycles help develop tolerance against salinity in different plant species. In rice leaves, for instance, salinity stress increases serotonin and gentisic acid levels in tolerant varieties (Borrelli et al. 2018; Gupta and De 2017). Metabolites synthesized in response to abiotic stress mostly exhibit antioxidative activity. They reduce the overactivity of reactive oxygen species and protect the biomolecules like lipid, protein, and DNA, from falling prey to oxidative stress (Details in Chaps. 2 and 3 of this book).

8 Salinity Tolerance Mechanism

8.1 Oxidative Defense System

The synthesis of reactive oxygen species (ROS) is an inevitable event after exposure to O_2 in plants. Plants pose a well-developed enzymatic and non-enzymatic defense system to resist salinity-mediated osmotic stress. Enzymatic defense system is mainly composed of superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), and peroxidases (GPOX) to inhibit toxic impacts of salinity stress in plants (Kaya et al. 2018; Sarker and Oba 2020). The non-enzymatic system includes flavonoids, α-tocopherol, ascorbic acid and carotenoids. Vitamins, phenols, glutathione and flavonoids are among the natural antioxidant found in plants. These antioxidants are mainly involved in free-radical quenching, and inhibition of enzymes and peroxide decomposers in plants (Tuna et al. 2013).

8.2 Enzymatic Defense System

Among enzymatic antioxidant, SOD is the first line of defense against oxidative injury that dismutate $O_2^{\bullet -}$ radical into H_2O_2 in plants. SOD is further classified into other isoforms based on the cofactor availability in plants. Fe-SOD is found mainly in chloroplast, Mn-SOD in mitochondria as well as in peroxisome, and Cu/Zn SOD in cytosol and chloroplast. All these isoforms actively quench $O_2^{\bullet -}$ radical in plants under salinity stress. The end-product of SOD, which is H_2O_2 is further detoxified by CAT into H_2O and O_2 in plants under salt stress. Plant peroxisomes and mitochondria are the main sites of CAT production. Similar to CAT, APX also quenches H_2O_2 under stress (Anjum et al. 2012a, b). APX utilizes ascorbate as reducing agent and is found in stroma of chloroplast, cytosol, mitochondria and peroxisomes. Peroxidases further quench H_2O_2 in plant apoplastic region under stress conditions (Bose et al. 2014). APX actively quench the H_2O_2 contents by following Mehler and glutathione-ascorbate pathway (Anjum et al. 2014; Moradbeygi et al. 2020). Tolerant cultivars of canola plants displayed higher SOD, CAT and POX activities in response to salt stress (Farhoudi et al. 2012). Similarly, salt stress enhanced the SOD, POD and CAT activities in rice and maize plants (Kim et al. 2014; Kaya et al. 2018). Two different cultivars of rapeseed (Yangyou 9 and Zhongshuang 11) showed different response in terms of SOD, POD, CAT and APX activity under salinity stress. In this context, SOD, POD and APX activity was higher in Yangyou 9 cultivar than in Zhongshuang 11. However, CAT activity decreased in both cultivars with the maximum reduction in Zhongshuang 11 (El-Badri et al. 2021). Salinity stress deteriorated the CAT and enhanced the SOD activity in tomato plants. The decline in CAT activity showed a lesser capability of this plant towards effective H_2O_2 removal, in turn, generating more oxidative injury in plants under salt stress (Alzahib et al. 2021).

8.3 Non-Enzymatic System

Non-enzymatic antioxidants directly remove the ROS as well as other radicals and decrease the substrate availability for these enzymes' activities in plants (Ramadoss et al. 2021). Among non-enzymatic antioxidants, ascorbic acid is a potent antioxidant found abundantly in all parts of plants, especially in leaf. It acts as an enzyme cofactor in plants. Being a cofactor, it is also involved in production of some plant hormones and their transduction mechanism. It also regulates cell division and elongation, and ultimately the growth of plants under stress (Semida et al. 2021). Ascorbic acid reacts not only with H_2O_2 but also with $O_2^{\bullet -}$, $OH^{\bullet}$ and hydroperoxides of lipids in plants under salinity stress. An elevated level of ascorbic acid is crucial for the maintenance of antioxidant system capable to provide protection to plants against oxidative injury (Jaleel et al. 2007). Wheat plants manifested a decline in ascorbic acid contents under salt stress (Alnusairi et al. 2021). However, ascorbic acid-induced defense system was seen in canola (Bybordi 2012), okra (Raza et al. 2013) and barley (Agami 2014) under salt stress. α-tocopherol effectively quench the $^1O_2$ and $OH^•$ radicals found in thylakoid membrane. It can also decrease the oxidation of fats by hindering lipid peroxyl through converting them into alternate hydroperoxides and improves photosynthetic system by maintaining PSI system of plants under salt stress (Al-Hayani and Al-Behadili 2021). Carotenoids are quenchers of $^1O_2$ and an important part of PSI system, supporting thylakoid membrane and light-harvesting complex of plants. They are lipid soluble antioxidants (Ramadoss et al. 2021). They also stabilize the membrane, decline the oxidative injury and boost up plant growth under salt toxicity (Behzadi Rad et al. 2021). Plant phenolics are also important in effective ROS removal under salt stress. As the potential quenchers of $^1O_2$, they actively decompose the peroxide and hinder lipids auto-oxidation in plants. They provide protection to plants against the escalation and advancement of stress-mediated oxidative injury. They also protect plant membrane under stressful environment. Anthocyanins are not only involved in ROS removal but also hinders their synthesis in plants. Plants that can synthesize and accumulate a large quantity of anthocyanins in their epidermal layers can reduce oxidative injury more effectively. It also functions as an osmotic solute under salt toxicity (Moradbeygi et al. 2020) and acts as an osmo-regulator in plants (Behzadi Rad et al. 2021). Flavonoids being non-enzymatic antioxidants are effective scavengers of ROS in plants under salt stress (Ahmad et al. 2020). Salt enhances the phenolics and flavonoids contents, as observed in *Leucojum aestivum* (Ates et al. 2021), yam plants (Cécile et al. 2020) and grapevine (Gohari et al. 2021). Elevated level of salt exacerbated the ascorbic acid and α-tocopherol contents in wheat plants, which in turn, demolished the stress-induced toxic impacts in plants (Desoky et al. 2021). In another study, carotenoids and anthocyanin contents went down in *Pistacia* species against salt stress (Behzadi Rad et al. 2021).

8.4 Production of Compatible Solute

Plants adopt several defense mechanisms to maintain turgor pressure in tissues under salinity stress. Regulation via accumulation of compatible solutes is considered as the best strategy to counteract salt toxic effects (Rady et al. 2018). Compatible solutes, also termed as osmoregulators, are low-molecular weight organic solutes (Estaji et al. 2019), which include soluble sugars, amino acids, glycine betaine, polyols and proline. They accumulate in large quantities in plants under salt stress and mitigate the adverse effects of ROS-mediated oxidative injury in response to salt stress (Qureshi et al. 2013; Alasvandyari et al. 2017). They involved in preservation of cell water potential, improving plant tolerance against salt stress (Al-Mushhin et al. 2021; Ahmad et al. 2020). Salt stress activate the amylase, sucrose synthase, invertase and sucrose phosphate synthase activities, that maximize the starch accumulation and some other soluble sugars content, thus increase the tolerance in plants (Qureshi et al. 2013; Ahmad et al. 2020). Soluble sugars, also known as reserve sugars, can function as key substances in response to salt toxicity in plants. Abiotic stress after inhibiting the photosynthetic process stimulate starch remobilization for providing carbon and energy to plants (de Oliveira et al. 2022). Higher quantity of sugars involves in activation of growth promoting genes by inhibiting stress genes in plants under salt toxicity. Therefore, soluble sugars play dual role in plant metabolic regulation under salinity stress (Al-Mushhin et al. 2021). Proline is a compatible osmolyte in plants under stress environment. It is an antioxidant, ROS quencher and provide protection to several enzymes in plants. It also stabilizes structure of cell membrane and proteins in plants. Proline also acts as a carbon and nitrogen storage molecules following stress reduction, regulates cytosolic pH and is a signaling molecule that can activate defensive responses in plants (Goharrizi et al. 2020). Proline accumulation protects the photosynthetic system by stabilizing chlorophyll and turgor of plant cells under salt stress (Arora et al. 2020). Glycine betaine is mainly produced in plant chloroplast under salt stress. It has diverse roles; protecting thylakoid membrane and improving photosynthetic performance in plants. It also maintains osmotic equilibrium, stabilizes chlorophyll contents and thus help in generating tolerance in plants against salt-mediated toxic impacts in plants. The mitochondrial and cytoplasmic enzymes are also stabilized through accumulation of osmoregulators in plants (Estaji et al. 2019). Higher soluble sugars, proline and glycine betaine contents were found in alfalfa plants in response to salt stress (El Moukhtari et al. 2021). Several reports have shown salt-stress-mediated increment in proline, soluble sugars and glycine betaine accumulation in plants. For instance, mango (Laxmi et al. 2021), soybean (Agha et al. 2021; Elkelish et al. 2019; Farhangi-Abriz and Ghassemi-Golezani 2018), maize (Iqbal et al. 2018) and chickpea (Ahmad et al. 2016) plants displayed maximum accumulation of osmolytes in response to salt stress. By contrast, elevated level of salts prevented the soluble sugars accumulation in quinoa plants. However, quinoa plants displayed maximum aggregation of proline and glycine betaine contents under salt stress (Al-Mushhin et al. 2021).

9 Effect of Salinity Stress on Some Specific Medicinal Plants

*9.1 Chamomile (*Matricaria chamomilla *L.)*

Chamomile is an important medicinal plant with significant modulation in physiology and biochemistry under saline conditions. As a drug, it finds use in flatulence, colic, hysteria, and intermittent fever. It has anti-inflammatory, antiseptic, antispasmodic and mildly sudorific properties. It is used internally for sluggish digestion, diarrhoea and nausea; rarely but effectively for inflammation of urinary tract and painful menstruation also (Singh et al. 2011). Heidari and Sarani (2012) reported the effect of salinity and iron deficiency on nutrient acquisition, carbohydrate, proline and growth of chamomile plants. Salinity decreased shoot fresh weight and increased root fresh weight. The levels of carbohydrates and proline were higher in plants under salinity. Toxic Na levels were several folds greater in plants alongside a significant decline in K (37.6% in shoot and 46.1% in roots). Salinity induced a significant drop in shoot and root Fe contents in chamomile plants. Fe administration to the nutrient solution mitigated the effects of salinity stress causing remarkable overall improvement. In another study, salinity resulted in a significant reduction in peduncle head diameter and length, flowers per plant, number of branches per plant, and height. Higher salinity doses resulted in a significant reduction in essential oil and flower yield (Razmjoo et al. 2008). Askari-Khorasgani et al. (2017) reported diverse responses genotypes Isfahan, Ahvaz and Shiraz of chamomile and recognized Isfahan as the most tolerant genotype to salinity. Na exclusion was the most efficient mechanism used by plants to avoid salinity. The higher Mg/Na and Ca/Na ratio in roots and leaves along with the efficient sequestration of Na ions in the vacuole appeared as an effective adaptation in chamomile plants to tolerate salinity.

Salimi et al. (2016) studied the interactive effects of methyl jasmonate and salinity on physiological and morphological attributes in chamomile plants. They reported better growth, upregulated antioxidant enzymes activities, photosynthesis, membrane stability index, proline and potassium contents in plants treated with methyl jasmonate under salinity. Plants treated with 75 μM methyl jasmonate showed the maximum tolerance to salinity. A study of oil quality, productivity and growth changes in chamomile grown in two seasons at four different locations with variable irrigation water and soil salinity revealed that the yield and oil quality remained unaffected in saline soils. Higher salinity conditions resulted in a significant essential oil content and yield of chamomile plants. Plants grown under low salinity conditions produced essential oil rich in sesquiterpene, while higher salinity levels made the oil rich in oxygenated sesquiterpene. The major oil components were variable across different locations. The major components were bisabolol oxide, bisabolol oxide B, cis-β-farnesene, and bisabolol oxide A. The productivity and quality of chamomile plants is affected significantly by soil quality. Higher salinity results in more flower yields and essential oil contents. Some reports on chamomile plants under salinity stress are given in Table 1.

9.2 Echinacea *Species*

Echinacea, commonly referred to as coneflower, belongs to family Asteraceae and has nine species. These species have long been recognized as important medicinal plants, customarily used for treating common cold, coughs, bronchitis, upper respiratory infections, some inflammatory conditions and wound infections (Percival 2000). Salinity effects on growth, ion acquisition, gas exchange, chlorophyll contents, electrolyte leakage and antioxidant enzymes of *Echinacea angustifolia*,

Table 1 Effect of salinity on growth and physiochemical responses of chamomile plants

Salinity	Plant response	References
0, 25, 50, 75, 100, 125, and 150 mM NaCl	The growth and essential oil contents dropped in plants.	Ali et al. (2007)
0,4, 8, 12 and 16 dS m^{-1}	Salinity induced a substantial drop in fresh flower weight, whereas salinity did not show any impact on oil quality, oil quantity or apigenin contents. Chamomile plants preserved all the medicinal values when grown under saline conditions and the authors recommended the use of this plant in saline soils.	Baghalian et al. (2008)
0, 25, 50, 75, 100, 125, and 150 mM	Application of different polyamines protected plants from salinity effects.	Ali et al. (2009)
0, 40, 80, 120, and 190 mM	The flavonol-O- glycosides and flower dry weight were reduced under salinity.	Afzali et al. (2007)
0, 4, 8, 12, and 16 dS m^{-1}	Salinity significantly decreased different germination traits, including time to 90% germination and 50% germination percentage.	Fatholahi et al. (2016)
0, 4, 8, 12, and 16 dS m^{-1}	Salinity resulted in a significant decline in chlorophyll, anthocyanins and carotenoids contents. The endogenous levels of proline were many folds greater in plants under salinity.	Lotfollahi et al. (2015)
0, 0.5, 1 and 1.5%	Salinity induced a substantial drop in length of hypocotyl and germination percentage. Salicylic acid priming significantly improved germination attributes and seedling growth under salinity.	Tavassoli et al. (2010)
0, 3 and 6 dS m^{-1}	Salinity induced a significant reduction in chlorophyll content, membrane stability alongside a notable improvement in proline, soluble sugars and antioxidant enzyme activities. The accumulation of Na^+ was significantly higher in plants under salinity. Gibberellic acid and mycorrhizal applications significantly protected plants from salinity effects.	Ali and Hassan (2014)

Echinacea pallida and *Echinacea purpurea* were examined by Sabra et al. (2012b). The shoot and root biomass remained unaffected in all the three species. *Echinacea angustiflolia* showed a significantly higher electrolyte leakage, manifesting the oversensitivity of this species. It also showed a significant decline in photosynthesis-related parameters, and higher Na and Cl contents than in other *Echinacea* species. Khorasaninejad and Hemmati (2020) studied the salinity effects on the contents of chlorogenic acids, caffeic acid, phenol, proline and flavonoids in roots and shoot of *Echinacea purpurea* L. plants. The chlorogenic acids significantly affected as a result of salinity. The caffeic acid was also modulated in roots, but not in shoots under salinity. Exogenous silicon (Si) application protected plants from salinity effects. Similarly, hydroponic experiment was undertaken to examine the alterations in ketones, alkamides and caffeic acid derivatives in *Echinacea angustiflolia*, *E. purpurea*, and *E. pallida*. The phytochemical profile of alkamides, ketones and caffeic acid derivatives were changed remarkably due to salinity (Sabra et al. 2012a).

In another study, salinity and osmotic stress effects on germination and seedling growth were studied and a significant abridge in germination attributes and seedling growth was recorded (Amiri et al. 2011). Exposure of *Echinacea Purpurea* plants to salinity resulted in a significant reduction in germination parameters and seedling growth (Miri and Mirjalili 2013). Likewise, salinity induced a conspicuous reduction in plant biomass and chlorophyll contents, while lipid peroxidation, phenolics and activities of antioxidant enzymes also increased several folds. Exogenous supplementation of nitric oxide in the form of sodium nitroprusside (SNP) significantly mitigated salinity effects on plants (Asadi-Sanam et al. 2018). Another study (Choirunnisa et al. 2021) recorded variation in flavonoids and morphological attributes in *Echinacea Purpurea* plants under salinity.

*9.3 Jerusalem Artichoke (***Helianthus tuberosus** *L.)*

Jerusalem artichoke (*Helianthus tuberosus* L.) contains anti-fungistatic, anti-carcinogenic and antioxidant components, and is used in cardiovascular diseases, chronic infectious diseases, chronic fatigue syndrome, gut-flora disorders, and immune system disorders. It lowers high cholesterol, triglycerides and glucose levels; facilitates weight loss; lowers uric acid levels; has immune-stimulating properties; protects the gastric mucosa, prevents constipation and acne, and has cytotoxic properties in breast cancer (Sawicka et al. 2020). Yang et al. (2016) reported that salinity can alter root distribution and increase the diversity of bacterial communities in the rhizosphere soil of Jerusalem artichoke plants. Shao et al. (2016) found that mild salinity stress (2.2 g NaCl kg^{-1} soil) did not affect the growth of artichoke plants, while the rise in soil salinity to 2.7 g NaCl kg^{-1} soil significantly decreased the distribution of dry matter to tubers and stems. Further, mild salinity induced sugar accumulation in tubers, whereas higher salinity reduced the tuber dry matter accumulation and fructan accumulation efficiency. Induction of salinity resulted in a conspicuous rise in the levels of GA3 and IAA alongside a decline in ABA levels. The authors reported a significant drop in tuber yield due to increase in salinity. Lucini et al. (2016) reported that the induction of salinity stress resulted in the activation of valuable phytochemicals with significant therapeutic and nutraceutical values in artichoke plants. Plants subjected to 30 mM KCl showed a prominent decrease in leaf dry biomass alongside a significant decrease in macro- and micronutrients acquisition. The levels of Cl and K increased several folds in plants under salinity. Further, indole-3-acetic acid conjugates, cytokinin precursor N6-(Delta-2-isopentenyl)-adenosine-5′-triphosphate and 6-deoxocastasteron (brassinosteriod hormone) increased significantly in plants under salinity. The phenolic profile of salinity stressed-artichoke plants also displayed decline in isoflavones and flavones and increase in glycosides. Also, peroxidation of membrane lipids, accumulation of mannitol phosphate and degradation of ascorbate took place. Similarly, in another study, Saleh et al. (2005) used three different strategies to induce salinity tolerance in artichoke. Plants were subjected to saline nutrient medium (6.5 dS m^{-1}) in the sand culture and plants were inoculated with *Bacillus subtilis* strain, Ca was supplemented in the nutrient solution and with foliar application of Zn, Mn and Fe. Salinity decreased bud growth and yield quality. The endogenous levels of Cl and Na increased sharply in different plant parts. However, inoculation of salinity stressed-plants with *Bacillus subtilis* significantly improved yield and yield quality. Alleviation of salinity stress by bacterial inoculation ranked first, followed by the Ca supplementation in the nutrient solution, whereas foliar spray of Zn, Mn and Fe did not show any prominent effect on plants under salinity (Saleh et al. 2005). Xue and Liu (2008) also studied the impact of NaCl salinity on chlorophyll contents, gas-exchange, net photosynthetic rate, cell membrane stability, lipid peroxidation and antioxidant enzyme activity in two artichoke cultivars with differential salinity tolerance, *viz.* Wuxi (salt-sensitive) and Dafeng (salt-tolerant). These cultivars were subjected to different NaCl levels in the nutrient solution (75, 150 and 225 mM) for one week. The activities of antioxidant enzymes increased several folds in Dafeng cultivar, while Wuxi cultivar did not show any significant changes. Peroxidation of membrane lipids and injury to cell membranes were higher in both the cultivars. The net photosynthesis and stomatal conductance decreased in both cultivars, while Dafeng cultivar had a comparatively less reduction in photosynthesis, compared with Wuxi cultivar. In another study, salinity induced a notable reduction in Artichoke seedling growth and germination (Jorenush and Rajabi 2015). Likewise, salinity stress increased proline accumulation by regulating the biosynthesis and degradation of proline in artichoke plants (Huang et al. 2013). In another study, KCl treatment significantly enhanced polyphenols, antioxidant activity, flavonoids and total phenolics in artichoke leaves (Borgognone et al. 2014). Similarly, a significant decrease was observed in growth of two artichoke cultivars having differential salinity tolerance. The negative impact was evident from relative growth rate, chlorophyll content, plant dry weight, leaf area, stomatal conductance and photosynthesis rate. The levels of Ca and Na were higher in salt-affected plants than in the control (Long et al. 2010).

*9.4 Peppermint (*Mentha *species)*

Peppermint (*Mentha piperita* L.), a perennial and herbal plant of Lamiaceae family, possesses antiseptic, antimicrobial, antitumor, antiallergenic and antioxidant attributes. It has long been a classic choice for the treatment of nausea. It is used internally as an antispasmodic (upper gastrointestinal tract and bile ducts) and to treat irritable bowel syndrome, catarrh of the respiratory tract, and inflammation of the oral mucosa. Externally, peppermint oil is used for myalgia and neuralgia. The oil may also act as a carminative, cholagogue, antibacterial and secretolytic, and has a cooling action (Balakrishnan 2015). When exposed to salt stress, it markedly inhibits total chlorophyll and menthol contents and enhances the plant MDA, proline and essential oil contents. Activity of the enzymatic antioxidants, including SOD and CAT, increases in response to salt stress (Fathi et al. 2020). In a study by Danaee and Abdossi (2021), salt stress markedly inhibited the fresh as well as dry weight of both root and shoot, and the chlorophyll, protein and phenolics contents of *Mentha piperita*. Besides, the SOD and POD activities were altered and the proline content increased. However, the salt stressed-plants, when treated with silicon and nano-silicon, exhibited relatively improved growth. Similarly, salt stress inhibited the uptake of essential mineral ions (N, K, P, Ca, Mg, Fe and Zn) in peppermint plant. However, application of organic media (coco, peat, palm) or magnetized water improved the nutrient uptake in plants, the latter doing better than the former (Alavi et al. 2021). Salinity stress reduced the length as well as the biomass of root and stem internodes, and inhibited the essential oil content (Khorasaninejad et al. 2010). In another study, salt stress markedly reduced the biomass and the K, Fe, Zn and Ca ions uptake in the peppermint plants, and enhanced the Na and P ions uptake, lipid peroxidation in the form of more MDA production, and proline contents in plants. Activity of antioxidant enzymes such as DPPH, SOD, CAT and GPOX also increased under salt stress (Askary et al. 2017). Salt stress declined the fresh and dry weight of roots and aerial parts of peppermint plants. Shoot diameter, number of lateral branches, number of nodes and leaves, shade of canopy and length of internodes were also affected. However, addition of salicylic acid improved all these parameters (Nourafcan 2015).

Ghorbani et al. (2018) noted that salt stress markedly suppressed the different foliar characteristics related to size, pigments and water content. The essential oil contents of the plant were also reduced. Similarly, salt stress resulted in cell death along with condensation of chromatin and caspase-3-like activation in peppermint plants. Salt stress accelerated the ROS generation which is responsible for death of plant cells. Mitochondria and chloroplasts are the major sites for ROS production. Similar observations on ROS generation, and altered activity of antioxidant enzymes were reported by Li et al. (2015). In another study, salinity stress increased the minimum fluorescence (F_0), non-photochemical quenching (NPQ), quantum yield of regulated energy dissipation (YNPQ) and quantum yield of non-regulated energy dissipation (YNO) values of peppermint plant. However, plant chlorophyll a and b, maximum fluorescence (Fm), variable fluorescence (Fv), Fm/Fv ratio and electron transport rate (ETR) were reduced under salt stress. Salinity favored more accumulation of Na ions than K and P ions, and reduced the essential oil contents of peppermint plant. However, plants inoculation with endophytic fungi like mychorriza and *Piriformospora indica* improved the plant growth attributes and oil contents via mitigating the toxic impacts of salt stress (Khalvandi et al. 2021). Likewise, salt stress reduced the root and shoot length, their fresh and dry weights, photosynthetic parameters and anthocyanins in peppermint plants. Plant antioxidant system such as CAT and guaiacol peroxidase activities were enhanced. Both leaves and roots accumulated higher amount of total soluble sugars under salt toxicity.

Similarly, *Mentha pulegium* L., when exposed to salinity stress, manifested heavy accumulation of total phenol, glycine betaine, proline, H_2O_2 and malondialdehyde alongside the enhanced activities of antioxidant enzymes. On the contrary, photosynthetic pigments and leaf water content declined significantly. Foliar spray of salicylic acid did not influence the accumulation of total phenol and glycine betaine under salinity, but proline content was intensified. Salicylic acid administration enhanced antioxidant enzyme activities and diminished lipid peroxidation and H_2O_2 production (Farhadi and Ghassemi-Golezani 2020). Azad et al. (2021) also reported decline in plant growth, chlorophyll content, relative water content and peroxidase activity in *Mentha pulegium*. Application of salicylic acid showed a positive effect. Oueslati et al. (2010) reported that salinity caused decline in growth, tissue hydration and K^+ uptake alongside an enhanced Na^+ uptake in *M. pulegium* plants. The response of leaves and roots was different with reference to antioxidant response. The total phenol content dropped significantly in plants under salinity. Merati et al. (2016) also obtained similar results. In another study, salinity increased lipid peroxidation, ROS production and proline in *M. pulegium*. Ghalkhani et al. (2020) observed significantly prompted antioxidant activities due to salinity stress. Likewise, Merati et al. (2014) recorded reduction in growth and relative water content (RWC), but prominent increase in activities of antioxidant enzymes, H_2O_2 levels and lipid peroxidation in *M. pulegium* plants under salinity stress.

In a study of peppermint (*Mentha haplocalyx*) and blessed thistle (*Silybum marianum*), salt stress caused high lipid peroxidation in the form of MDA in both the species, but the photosynthetic pigments remained unaffected. Total protein contents and K/Na as well as Ca/Na ratios were also affected, and proline accumulation markedly increased. However, addition of vermicompost in growth medium demolished the MDA contents and improved the K/Na and Ca/Na ratios and the total protein content of the plants. Proline contents was not improved by vermicompost but increased by inorganic fertilizers' application (Xu et al. 2016). Treatment with Au-Ag nanoparticles reduced the negative effects of stress and improved growth parameters in *Mentha* as well as several other plants (Husen and Iqbal 2019; Singh and Husen 2019, 2020; Husen and Jawaid 2020; Aliakbarpour et al. 2020).

9.5 Hyssop (Hyssopus officinalis *L.)*

Hyssop (Hyssopus officinalis L.), a member of Lamiaceae family, is abundantly found in eastern Mediterranean to central Asia. The plant is rich in polyphenolics such as flavonoid and their derivatives. It is known to be a stimulant, carminative and expectorant and is used in colds, coughs, asthma and lung complaints. It is also effective in digestive problems, uterine and urinary troubles. Traditionally, it has been used as a carminative, tonic and antiseptic. A tea made from its leaves is beneficial in nervous disorders and toothache (Ravindran et al. 2012). HPLC (High-performance liquid chromatography) analysis for diosmin purification from its leaves and shoots, and NMR (Nuclear magnetic resonance) spectroscopy studies have manifested that salt stress markedly enhanced the total flavonoids and diosmin contents (Soheilikhah et al. 2021a, b). Salt stress also affected plant growth by decreasing the chlorophyll contents. The plants accumulated more proline (4.5-fold) and soluble sugars (1.6-fold) under salt stress. Salinity stress also enhanced the SOD, CAT and POD activities, especially in the aerial plant parts. Accumulation of secondary metabolites, including flavonoids, phenolics, iridoids, saponins and anthocyanins, increased and the total antioxidant activity enhanced in response to salt stress. The findings exhibited that hyssop plant is capable to grow under elevated level of salt (Soheilikhah et al. 2021a, b). In another study, it was found that saline water augmented the plant enzymatic antioxidant system including the SOD, POD and CAT activity. Similarly, the non-enzymatic antioxidants such as anthocyanin and phenolics were enhanced under salt stress. Salinity stress also improved the free proline contents of plants. Thus, the plant was well-equipped to effectively quench the salt-induced ROS production (Jahantigh et al. 2016a, b). The elevated level of salinity significantly enhanced the essential oil contents of hyssop plants (Jahantigh et al. 2016a, b).

*9.6 Neem (*Azadirachta indica*)*

Neem (*Azadirachta indica* L.), a well-recognized medicinal plant, belongs to the family Meliaceae. It is utilized as a good shade tree and is capable to decrease soil erosion. All parts of neem tree are used as anthelmintic, anti-fungal, anti-diabetic, antibacterial, antiviral, contraceptive and sedative. Neem is highly beneficial in treating skin diseases, liver function, and blood toxicity, dental disorders, cough, asthma, ulcers, piles, intestinal worms, and urinary problems. Neem leaf paste is applied to the skin to treat acne, and measles etc. Patients suffering from chicken pox are advised to sleep on neem leaves. A decoction prepared from neem roots is ingested to relieve fever in traditional Indian medicine. Studies based on animal model have established that the chief constituents of neem play pivotal role in anticancer management through the modulation of various molecular pathways including p53, pTEN, NF-κB, PI3K/Akt, Bcl-2, and VEGF (Alzohairy 2016). The famous bioactive compounds of neem include Nimbin, Nimbolide, Gedunin, Azadirachtin, Mahmoodin, Gallic acid, Epicatechin, Catechin, Margolone, Margolonone, Isomargolonone, Cyclic trisulphide and Cyclic tetrasulphide (Balaji et al. 2018). The seeds of this plant are used as biological control of pests and fungi in agricultural crops. Neem plants exposed to salt stress showed a significant reduction in leaf pigments, and in the reducing and non-reducing sugars contents. A significant reduction in mineral ions, including the Mg, Ca, K, P, and N contents, was also noticed. However, the proline, total phenolics and Na ion contents were enhanced under salt stress (Hamayl et al. 2020). Similarly, Jahan et al. (2018) noted a marked reduction in growth of the neem plant due to salt stress. The total dry matter and water contents as well as the green pigments and carbohydrate contents were suppressed, but the proline and phenolics contents were enhanced under salt stress (Jahan et al. 2018).

*9.7 Garlic (*Allium sativum *L.)*

Garlic (*Allium sativum* L.) from Alliaceae family is high in sulphur contents and bears significant therapeutic and prophylactic value. It is used to treat several ailments including fevers, diabetes, rheumatism, intestinal worms, colic, flatulence, dysentery, liver disorders, tuberculosis, facial paralysis, high blood pressure, and bronchitis (Kuete 2017). Astaneh et al. (2019) studied the effect of salinity on growth, oxidative defense and lipid peroxidation in hydroponically grown garlic plants. Salinity stress caused a notable reduction in growth attributes and chlorophyll content, while plants treated with exogenous Se manifested a conspicuous rise in chlorophyll contents and the fresh and dry biomass of bulb. Similarly, Shama et al. (2016) found growth attributes, and the extent and quality of yield greatly reduced in garlic plants under salinity. Salicylic acid application significantly reduced the inhibitory effects. Astaneh et al. (2018b) exposed garlic plants to different levels of NaCl salinity, which remarkably reduced photosynthetic pigments, relative water contents, and K uptake. However, Se supplementation significantly improved growth, chlorophyll contents, relative water contents and K levels together with a significant drop in the uptake of Na. In another study, salinity stress reduced growth and yield potential in garlic plants, while foliar applications of potassium, proline and biostimulants increased growth and yield production (Awad-Allah et al. 2020). Likewise, in a study conducted by Astaneh et al. (2018a), phenolics contents increased by several folds in garlic plants under salinity; Se supplementation protected plants from damaging effects of salinity on cell membranes.

*9.8 Flax (*Linum usitatissimum*)*

Flax (*Linum usitatissimum* L.), an ancient plant known for its oilseed and for fiber production, belongs to family Linaceae. It is a rich source of micronutrients, lignin, proteins, vitamin B1 and essential fatty acids. Flaxseed oil, fibers and flax lignans have potential health benefits and are used treating the cardiovascular disease, atherosclerosis, diabetes, cancer, arthritis, osteoporosis, autoimmune and neurological disorders. Flax protein helps in the prevention and treatment of heart disease and in supporting the immune system (Goyal et al. 2014). Salt stress reduces the growth of flax plants (Dubey et al. 2020). It was observed by El Hariri et al. (2010) that salt stress remarkably reduced the length and the fresh dry weights of stem, relative water and indole acetic acid contents of flax seedlings. Fiber yield was also greatly reduced. However, treatment with ascorbic acid and α-tocopherol ameliorated the growth attributes of plants under stress (El Hariri et al. 2010). In another study, three genotypes (Tabare, Golchin, and 375Ha) of flax were exposed to salt stress, which caused a substantial increment in SOD, CAT, guaiacol peroxidase, H_2O_2, MDA and proline contents of the plants. Proline contents and CAT activity were affected earlier the activity of SOD and guaiacol peroxidase and the MDA contents.

Genotype (375Ha) gave more prominent results (Alaei et al. 2020). Similarly, three cultivars of flax plant (Sakha-1, Sakha-3 and Sakha-5) were exposed to salt stress and ascorbic acid. It was noted that salt stress suppressed the growth indices such as fiber ratio, and seed yield. The physiological attributes including leaf pigments, Na/K ratio and water relation were also inhibited under salt stress. The stress also triggered the CAT and POD activity. However, treatment with ascorbic acid improved the plant growth, and the various physiological and biochemical attributes. Cultivar Sakha-5 was more resistant than the other two cultivars. The elevated level of salt stress disturbed the formation of xylem and arrangement of its component cells (EL-Afry et al. 2018).

When flax seeds were supplied with six different salts (*viz*. Na_2CO_3, $CaCO_3$, Na_2SO_4, KCl, NaCl and $CaCl_2$) with varying doses like 0, 50, 100 and 200 mM, it was noted that Na_2CO_3, $CaCO_3$ and Na_2SO_4 caused more toxic impacts compared to the other salts. The impact of salts on seed germination was in the order NaCl $>$ $CaCl_2 > KCl > Na_2CO_3 > Na_2SO_4 > CaCO_3$. Although the seeds were able to grow even at 200 mM NaCl dose, they could germinate at very low (50 mM) level of other salts (Na_2CO_3, $CaCO_3$, Na_2SO_4) or in the distilled water (Moghaddam et al. 2018).

*9.9 Feverfew (*Tanacetum parthenium *L.)*

Feverfew (*Tanacetum parthenium* L.), a perennial herbaceous plant native to the Mediterranean region and Kazakhstan Central Asia, belongs to the family Asteraceae and is widely distributed in different parts of America, Asia and Europe. It contains substantial amount of sesquiterpene lactones as secondary metabolites with parthenolide as the main active constituent element of the plant (Pourianezhad et al. 2016). It has multiple pharmacologic properties, such as anticancer, anti-inflammatory, cardiotonic, antispasmodic, and emmenagogue. It has been traditionally used for the treatment of fevers, migraine headaches, rheumatoid arthritis, stomach aches, toothaches, insect bites, infertility, and problems with menstruation and labor during childbirth. It also finds use in treating psoriasis, allergies, asthma, tinnitus, dizziness, nausea, and vomiting (Pareek et al. 2011). Mallahi et al. (2018) studied the effects of salinity on feverfew (*Tanacetum parthenium*). Salinity was applied in the form of $CaCl_2$ and NaCl and salicylic acid was applied as foliar spray. Salicylic acid supplementation significantly reduced the salinity effects and enhanced growth, sugar, proilne, essential oil and antioxidant enzyme activities; the essential oil content increased by several folds.

*9.10 Thyme (*Thymus *species)*

Thymus, a genus from the family Lamiaceae, is known for its medicinal importance. This genus contains 250 species with extensive use as traditional medicine. *Thymus* *vulgaris* is most famous as a medicine. The volatile essential oils of thyme are packed with antiseptic, anti-viral, anti-rheumatic, anti-parasitic and anti-fungal properties. Hence, it is used as an expectorant, diuretic, fungicide, and antibiotic. Being rich in Vitamin K, iron, calcium and manganese, thyme can improve bone's health. It cures bronchitis, cough and fever, stomach ache, diarrhoea, dermal disorders, tooth decay, hypertension and snoring, and also prevents hair loss (Javed et al. 2013; Rizwan et al. 2020).

In a study, *Thymus daenensi* and *Thymus vulgaris* manifested decline in growth and the K^+ and Ca^{2+} contents and increase in Na^+ uptake under salinity stress. Salinity increased the amount of rosmarinic acid and gallic acid, while concentrations of vanillic acid, syringic acid and caffeic remained unaffected. Accumulation of phenolics was remarkably high. Antioxidant capacity of plants was also enhanced significantly (Bistgani et al. 2019). Likewise, Zrig et al. (2019) observed that salinity stress decreased plant growth, osmotic potential and relative water content that resulted in ion imbalance and nutrient deficiencies. However, accumulation of amino acids and soluble sugars improved significantly in salinity-stressed plants. In another study, salt stress diminished plant growth and photosynthesis in *Thymus vulgaris* plants. Salinity-stressed plants displayed higher lipid peroxidation and ROS generation, which caused oxidative injury. The activities of antioxidant enzymes were accordingly upregulated (Zrig et al. 2021). Likewise, thyme plants manifested high carbon and nitrogen contents under salinity. The production of essential oil was also higher on exposure to salinity (Cordovilla et al. 2014).

9.11 Summer Savory (Satureja hortensis L.)

This annual plant, belonging to the family Lamiaceae, is native to western Asia and Mediterranean region. It is consumed as a spice and traditional herbal medicine. This plant possesses antimicrobial, sedative, antioxidant, antidiarrheal and antispasmodic, anticancer and analgesic properties. It forms a good remedy for nausea, diarrhoea, flatulence, bloating, sore throat, colic, asthma and irregular menstruation and is also used as an aphrodisiac and to . A liniment of the herb is used externally to ease the pains of inflamed joints (Fierascu et al. 2018). Savory herbs are rich in pyridoxine, thiamin, niacin, vitamin-C and vitamin-A (Gursoy et al. 2009). Estaji et al. (2018) have reported a significant decline in growth and in chlorophyll, carotenoids, Ca^{2+} and K^+ contents of *Satureja hortensis* under salinity stress. On the other hand, there occurred a remarkable improvement in Na^+, Cl^-, total phenolics, soluble carbohydrates and proline contents. Salinity stress induced a conspicuous increase in lipid peroxidation and electrolyte leakage. The activities of antioxidant enzymes were also greater (Mehdizadeh et al. 2019). Further, seed germination percentage and seedling growth were found to be markedly reduced in this species due to salinity (Saberali and Moradi 2019; Nejatzadeh 2021). In another study, salinity caused a diminished growth and chlorophyll content, alongside a higher proline level in *Satureja hortensis* plants (Arvin 2015).

*9.12 Garden Sage (*Salvia officinalis *L.)*

Salvia officinalis also belongs to the family Lamiaceae. As a folk medicine, it has been used for treating numerous disorders including seizure, ulcers, gout, rheumatism, inflammation, dizziness, tremor, paralysis, diarrhoea, and hyperglycaemia. Recent pharmacological studies have established that it has anticancer, anti-inflammatory, antinociceptive, antioxidant, antimicrobial, antimutagenic, antidementia, hypoglycaemic, spasmolytic and hypolipidemic effects (Ghorbani and Esmaeilizadeh 2017). The extracts are also chemoprotective and antigenotoxic (Tounekti et al. 2011). Es-sbihi et al. (2021) have reported salinity-induced drop in growth, photosynthetic pigments, nutrient acquisition and essential oil yield in Sage plants. In another study, salinity diminished plant growth, water potential, photosynthesis, chlorophyll content and stomatal conductance, but there was a substantial increase in endogenous Na^+. However, lipid peroxidation was not significant (Tounekti et al. 2012). Kulak et al. (2020) reported the presence of camphor, β-thujone, α-thujone, 1, 8-cineole, camphene and α-pinene in *Salvia officinalis* plants subjected to salinity. The chemotype of essential oil changed remarkably as a result of imposition of salinity stress. Taarit et al. (2010) also reported similar findings on growth parameters. Oleic, palmitic, gadoleic and alpha-linolenic acids were the major fatty acids present in plants under salinity. The primary composition of essential oil under salinity was based on manool, viridiflorol, α-humulene, camphor, 1,8-cineole, α and β-thujone (Taarit et al. 2010).

10 Conclusion

Salinity stress is a significant abiotic constraint that impedes growth, development, photosynthesis, chlorophyll biosynthesis and nutrient acquisition in medicinal plants. Plants subjected to salinity manifested a significant damage to membranes due to the peroxidation of membrane lipids and ROS generation. The impaired nutrient uptake reduced the chlorophyll and carotenoids level in plants under salinity. However, the salinity-affected medicinal plants showed a markedly significant accumulation of active phytochemicals with therapeutic values. The medicinal plants can be grown in marginal lands, because saline conditions activate the accumulation of phytochemicals in their tissues. However, their yield is significantly reduced under salinity stress. The treatments of medicinal plants with organic and inorganic chemical compounds significantly improved their tolerance to salinity.

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Impact of Aridity on Specialized Metabolism: Concentration of Natural Products in Plants

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Abstract Spice, medicinal and herbal plants grown in semi-arid regions usually have higher amount of natural products than the identical plants cultivated in moderate climatic conditions. Three causes have been identified to be responsible for the enhanced concentrations of natural products: (1) Due to reduced growth, the biomass under drought-stressed is lower than in the well-watered control plants. Therefore, even if the rate of biosynthesis of natural products is identical in stressed and control plants, their concentration is different. (2) When plants suffer drought stress, their stomata are closed, which generates a shortage of CO_2 within the leaves. In consequence, the reduction status of the leaves is strongly elevated, and all processes consuming $NADPH+H^+$, including the biosynthesis of highly reduced specialized metabolites, are boosted. (3) In various cases, drought stress enhances the activity of enzymes responsible for the biosynthesis of specialized metabolites. The increased consumption of $NADPH+H^+$ might significantly contribute towards preventing the generation of toxic oxygen radicals. Furthermore, drought stress might alter the spectrum of natural products by inducing the conversion of typical phytoanticipins to generate phytomodificines. Drought stress can be utilized to improve the quality of spice and medicinal plants.

Keywords Drought · Plant metabolites · Natural products · Qualitative changes · Medicinal plants

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A. Husen, M. Iqbal (eds.), *Medicinal Plants*,
https://doi.org/10.1007/978-981-19-5611-9_9

1 Introduction

Drought is responsible for massive impairments of plant growth and development. Accordingly, water deficiency causes severe decreases in biomass production and thus represents the gravest problem in agriculture. Nonetheless, in some cases, water shortage might even be advantageous for agricultural manufacturing. This most notably accounts for the cultivation of spice and medicinal plants. It is well established that plants grown in semi-arid areas viz. the Mediterranean regions commonly had much more aroma in comparison to equivalent plants cultivated in moderate climates (Kleinwächter and Selmar 2014; Selmar et al. 2017). This well-known phenomenon is frequently explained by simple and non-scientific statements, such as "*In Southern Europe, plants are exposed to far more sunlight, resulting in increased rates of biosynthesis of natural products*" (Abouzeid et al. 2022). Indeed, at first sight, such a deduction seems to be reliable; but plant scientists are aware that, in general, light intensity is already so high that any enhancement of irradiance is not increasing the rate of biosynthesis (Kleinwächter and Selmar 2014; Selmar et al. 2017). This insight also accounts for plants growing in Central Europe in open areas without any shade. The opposite is true, i.e., most plants absorb far more light energy by their leaves than they require for their photosynthetic CO_2-fixation (Wilhelm and Selmar 2011). Accordingly, there must be alternative clarification for the phenomenon of the higher accumulation of natural products in plants growing in Southern Europe. Meanwhile, we know that drought stress is of particular relevance to the increase in the concentration of natural products (Selmar and Kleinwächter 2013a, b). This chapter expounds on the various principles of operation, and outlines the basic scientific background of the entire episode. Although the related contemplations are well established (e. g. Selmar and Kleinwächter 2013b; Kleinwächter and Selmar 2014; Kleinwächter and Selmar 2015; Abouzeid et al. 2022), these have not been considered adequately in the past.

2 Concentrations of Natural Products in Drought-Stressed Plants

Various environmental conditions, namely temperature, light intensity, water availability, etc., massively affect plant growth and development by impacting their metabolism and metabolic abilities. Similarly, biosynthesis and accumulation of natural products are affected. Many reports on the effect of environmental factors on specialized metabolism have been published (Gershenzon 1984; Falk et al. 2007; Das and Bhattacharya 2016). Severe environmental perturbations, such as various stress conditions, which massively impact the general metabolism and growth aspects (Bohnert et al. 1995; Iqbal et al. 2000a, b; Sampaio et al. 2016; Husen 2021a, b, 2022), also influence the specialized metabolism. In this context, a great many papers have been published describing the induction of natural products synthesis against to pathogen or herbivore attack (Harborne 1988; Hahlbrock et al. 2003; Hartmann 2007; Namdeo 2007; Wink 2010), and to many abiotic stresses situations (Singh et al. 2000; Arshi et al. 2004, 2006; Ahmad et al. 2007; Ali et al. 2008; Husen 2021c, d; Rahman and Husen 2022; Deepti et al. 2022a, b; etc.). Recent researches have elucidated the molecular responses of plants to pathogen and herbivore attacks (e.g. Davies and Schwinn 2003; Zhao et al. 2005; Nascimento and Fett-Neto 2010; De Geyter et al. 2012) and the stress-related alterations of the general metabolism, causing a reduced biomass gain (Zhu 2002; Shinozaki and Yamaguchi-Shinozaki 2007; Baldoni et al. 2015). However, the insights on the metabolic basis responsible for the stress-related effect on specialized metabolism are still limited (Ramakrishna and Ravishankar 2011; Selmar and Kleinwächter 2013a, b; Kleinwächter and Selmar 2014). Nonetheless, a vast number of investigations have outlined that the plants under drought stress generally accumulate higher concentrations of specialized metabolites than the related control plants cultivated under well-watered conditions (Table 1). The concentration of simple and complex phenols has been reported to be significantly more in plants under water shortage. The bandwidth of the stress-related increase ranges from slight to massive enhancements (Table 1). Similarly, the concentration of terpenes is increased in stressed plants than in the controls (Table 1). Furthermore, the concentrations of various nitrogen-containing substances, such as alkaloids, cyanogenic glucosides, or glucosinolates, are also enhanced under drought stress conditions (Table 1). Thus, in general, the concentration of specialized metabolites increases under drought stress.

3 Factors Responsible for the Enhanced Concentrations of Natural Products

Indeed, drought situation often augments the concentration of natural products. Though, it also severely reduces plant growth (Husen 2010; Aref et al. 2013; Husen et al. 2014; Embiale et al. 2016; Getnet et al. 2015). Accordingly, plants under stress conditions reveal far lesser biomass than the well-watered controls. In consequence, in all cases where the rate of biosynthesis of natural products is not affected by the stress situation, concentrations of natural products on dry or fresh weight basis are inevitably elevated. In other words, even without any increase in the rate of metabolite biosynthesis, because of the differences in the reference values, i.e., dry or fresh weight, the numeric value of the concentration of the natural products in drought-stressed plants is logically higher, compared to that of the well-watered controls. Since this effect is not related to any change in the rate of biosynthesis, and is just due to the lower reference values in stressed plants, it is taken to be an indirect effect (Fig. 1). In order to decide whether or not the biosynthesis of specialized metabolites is also affected by the stress situation, the entire amount of natural products per plant has to be estimated. In various cases, a

Table 1 (**a–d**) Increased concentrations of specialized metabolites in drought-stressed plants

A: Phenolic compounds		
Massively enhanced concentrations in stressed plants		
Crataegus spp.	Chlorogenic acid	Kirakosyan et al. 2004
Crataegus spp.	Catechins / epicatechins	Kirakosyan et al. 2004
Echinacea purpurea	Total phenols	Gray et al. 2003a
Helianthus annuus	Chlorogenic acid	del Moral 1972
Hypericum brasiliense	Total phenols	de Abreu and Mazzafera 2005
H. brasiliense	Rutine / quercetin	de Abreu and Mazzafera 2005
H. brasiliense	Xanthones	de Abreu and Mazzafera 2005
H. perforatum	Total phenols	Gray et al. 2003b
H. perforatum	Rutin	Gray et al. 2003b
Labisia pumila	Total phenols	Jaafar et al. 2012
Olea europaea	Various flavanoids	Mechri et al. 2020
Pisum sativum	Anthocyanins	Nogués et al. 1998
P. sativum	Flavonoids	Nogués et al. 1998
Scutellaria baicalensis	Baicalin	Cheng et al. 2018
Trachyspermum ammi	Total phenols	Azhar et al. 2011
Significantly enhanced concentrations in stressed plants		
Achillea pachycephala	Various phenols	Gharibi et al. 2019
Camellia sinensis	Epicathecins	Hernández et al. 2006
Carica papaya	Various phenols	Espadas et al. 2019
Hypericum perforatum	Hyperoside	Gray et al. 2003b
Labisia pumila	Anthocyane / flavonoids	Jaafar et al. 2012
Melissa officinalis	Total phenols	Ahmadi et al. 2020
Ocimum basilicum	Methyleugenol	Abdollahi Mandoulakani et al. 2017
O. basilicum	Methylchavicol	Abdollahi Mandoulakani et al. 2017
O. basilicum	Rosmarinic and caffeic acid	Zare et al. 2021
Prunella vulgaris	Rosmarinic acid	Chen et al. 2011
Prunus persica	Total phenols	Kubota et al. 1988
Salvia miltiorrhiza	Furoquinones	Liu et al. 2011
Thymus capitatus	Phenolics	Delitala et al. 1986
Triticum aestivum	Total phenols	Ma et al. 2014
B: Isoprenoids / essential oils		
Massively enhanced concentrations in stressed plants		
Cistus monospeliensis	Caryophyllene	Llusià and Peñuelas 1998
Cymbopogon pendulus	Geraniol and citral	Singh-Sangwan et al. 1994
Glycyrrhiza glabra	Glycyrrhizin	Nasrollahi et al. 2014
Hypericum brasiliense	Betulinic acid	De Abreu and Mazzafera 2005
Petroselinum crispum	Essential oils	Petropoulos et al. 2008
Petrosilinum crispum	Essential oils	Kleinwächter et al. 2015
Picea abies	Monoterpenes	Turtola et al. 2003
Pinus halepensis	α-Pinen, carene	Llusià and Peñuelas 1998
P. silvestris	Monoterpenes	Turtola et al. 2003

(continued)

Table 1 (continued)

Salvia officinalis	Essential oils	Bettaieb et al. 2009
S. officinalis	Monoterpenes	Nowak et al. 2010
Solanum tuberosum	Steroid alkaloids	Bejarano et al. 2000
Thymus citriodorus	Geraniol	Tátrai et al. 2016
T. Citriodorus	Thymol	Tátrai et al. 2016
T. Citriodorus	Carvacrol	Tátrai et al. 2016
Significantly enhanced concentrations in stressed plants		
Bupleurum chinense	Saikosaponin	Zhu et al. 2009
B. chinense	Saikosaponin	Yang et al. 2020
Mentha x piperita ssp.	Essential oils	Charles et al. 1990
Nepeta cataria	Essential oils	Manukyan 2011
Ocimum basilicum	Essential oils	Forouzandeh et al. 2012
Origanum vulgare	Essential oils	Ninou et al. 2017
O. vulgare	Essential oils	Morshedloo et al. 2017
O. basilicum	β-Myrcene	Abdollahi Mandoulakani et al. 2017
O. basilicum	α-Bergamotene	Abdollahi Mandoulakani et al. 2017
Salvia nemorosa L.	Essential oils	Bidabadi et al. 2020
Satureja hortensis	Essential oils	Baher et al. 2002
Scrophularia ningpoensis	Iridoid glycosides	Wang et al. 2010
Prunella vulgaris	Triterpenes	Chen et al. 2011
Thymus vulgaris	Monoterpenes	Kleinwächter et al. 2015
C: Alkaloids		
Massively enhanced concentrations in stressed plants		
Capsicum spp.	Capsaicinoids	Phimchan et al. 2012
Catharanthus roseus	Indole alkaloids	Jaleel et al. 2007
C. roseus	Total alkaloids	Amirjani 2013
C. roseus	Vincristine	Amirjani 2013
C. roseus	Vinblastine	Amirjani 2013
Lupinus angustifolius	Quinolizidin alkaloids	Christiansen et al. 1997
L. angustifolius	Quinolizidine alkaloids	Frick et al. 2018
Nicotiana tabacum	Nicotiana-alkaloids	Çakir and Çebi 2010
Papaver agremone	Benzylisoquinolines	Behnam et al. 2017
P. somniferum	Morphine alkaloids	Szabó et al. 2003
P. somniferum	Benzylisoquinolines	Behnam et al. 2017
Phellodendron amurense	Benzylisoquinolines	Xia et al. 2007
Senecio jacobaea	Pyrrolizidine alkaloids	Kirk et al. 2010
S. longilobus	Pyrrolizidine alkaloids	Briske and Camp 1982
S. tuberosum	Steroid alkaloids	Bejarano et al. 2000
Significantly enhanced concentrations in stressed plants		
Catharanthus roseus	Total alkaloids	Yahyazadeh et al. 2021
Chelidonium majus	Benzylisoquinolines	Kleinwächter et al. 2015
C. majus	Coptisine	Yahyazadeh et al. 2018
Glycine max	Trigonelline	Cho et al. 2003

(continued)

Table 1 (continued)

Lycoris aurea	Galanthamine, lycorine	Xiang and Cao 2020
P. armeniacum	Benzylisoquinolines	Behnam et al. 2017
P. bracteatum	Benzylisoquinolines	Behnam et al. 2017
D: Various classes		
Massively enhanced concentrations in stressed plants		
Brassica napus	Glucosinolates	Jensen et al. 1996
Coffea arabica	γ-Aminobutyric acid	Bytof et al. 2005
Manihot esculenta	Cyanogenic glucosides	De Bruijn 1973
Triglochin maritima	Cyanogenic glucosides	Majak et al. 1980
Significantly enhanced concentrations in stressed plants		
B. carinata	Glucosinolates	Schreiner et al. 2009
B. rapa	Glucosinolates	Park et al. 2021
B. oleracea	Glucosinolates	Radovich et al. 2005
Phaseolus lunatus	Cyanogenic glucosides	Ballhorn et al. 2011
Tropaeolum majus	Glucotropaeolin	Bloem et al. 2014

stress-related increase of natural product biosynthesis has also been reported, which indicates a "*direct*" impact on the rate of biosynthesis (Fig. 1). In these cases, the products of biomass x metabolite concentration are enhanced in the stressed plants in comparison to those of the well-watered controls (Abouzeid et al. 2022). However, such evaluation of a "*direct*" enhancing effect might sometimes be quite problematic, particularly when the time range is quite long. Due to massive growth reduction and thus the far lesser biomass in stressed plants, any putative stress-related increase in the biosynthesis of specialized metabolite does not inevitably result in higher products of biomass x concentration, and thus in the total amount of natural products. However, we have to consider that the amount of newly synthesized natural products is generated in a far lesser quantum of biomass. This issue has been discussed in detail by Paulsen and Selmar (2016) and Yahyazadeh et al. (2021). These complex situations are little exposed in the literature and a stress-related increase in the concentration of natural products is frequently considered as a proof of stress-induced increase in biosynthesis. However, as mentioned below, many examples clearly state that the total amount of specialized metabolites, i.e., the content per entire individual, is higher in stressed plants than in the well-watered controls.

4 Drought Stress Increases the Total Amount of Specialized Metabolites

Most of the statements on the stress-related impact on the metabolism of specialized products are deduced from comparing the concentration of active compounds in the stressed and control plants, a deduction, which in turn ignores the stress-related

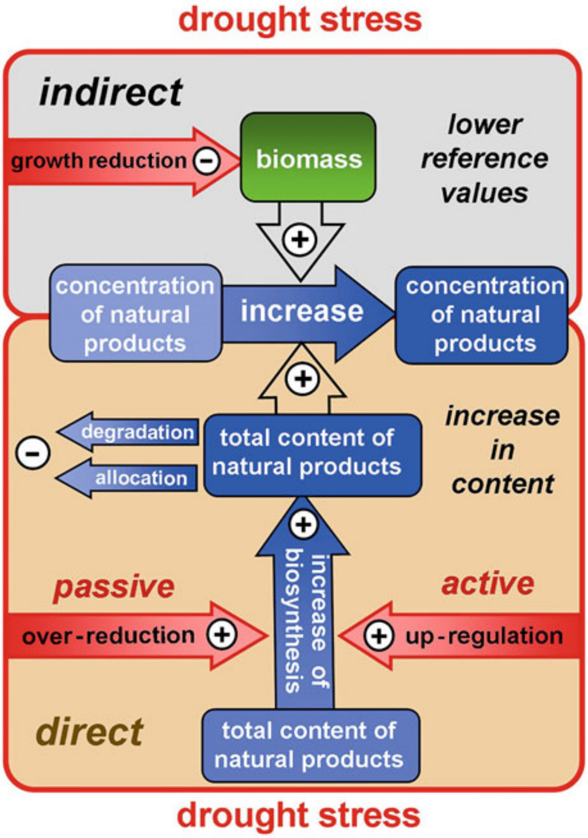

Fig. 1 The increase of natural product concentration in stressed plants results from the interaction of various processes and factors. A sound reflection reveals that it is inevitable to differentiate decidedly between "*concentration*" and "*content*" of specialized metabolites. Three effects contribute to the phenomena of a stress-induced increase in specialized metabolites. First, an "*indirect*" impact by the reduced production of biomass of the stressed plants: due to the corresponding lower reference values - even without any enhancements of the content of natural products – their concentration increase. Alternatively, the concentration increase could be caused by an enhancement in the total content. For this "*direct*" effect, two options have to be considered, a passive shift or an active enhancement of the biosynthetic capacity. The "*passive shift*" is a consequence of the strongly elevated concentration of $NADPH+H^+$, which boosts the biosynthesis of highly reduced specialized metabolites. Thus, although the enzyme activities are not enhanced, the rate of biosynthesis is increased just according to the mass action law. In contrast, in the case of an "*active*" up-regulation, the activity of enzymes responsible for the biosynthesis of natural products is enhanced in response to the stress situation

growth reductions. In the majority of these investigations, data on the overall biomass per plant are lacking. In consequence, a reliable evaluation of the entire amounts of natural products per plant is seem to be impossible. Indeed, this lack of information seems to be due to the fact that generally only the particular plant parts or plant organs, for instance, roots, leaves, flowers, or seeds, are analyzed. In several papers, the total contents of natural products per entire plant could retroactively be calculated based on the data of their concentration and biomass.

A unique report on a drought stress-related increase in the total amount of phenolic compounds has come from De Abreu and Mazzafera (2005). The authors revealed that in drought-stressed *Hypericum brasiliense* plants, both, the concentration and the total content of the phenolic compounds are dramatically enhanced. Indeed, in these plants, the biomass gain is far lower than in the controls. However, due to their tremendously higher concentration of phenolic compounds, their overall content (i.e., the product of biomass and concentration) was significantly higher in the stressed *H. brasiliense* plants than in the well-watered controls (De Abreu and Mazzafera 2005). These results verify that drought situations increase the biosynthesis of phenolic products. Hence, in addition to an indirect cause, i.e., the consequence of the lower reference value (biomass), there is also a direct impact of drought stress via the enhanced extent of biosynthesis (Fig. 1). An analogous deduction also applies for drought-stressed pea plants (*Pisum sativum*), in which the total amount of anthocyanins was about 25% higher than that of well-watered controls (Nogués et al. 1998). Furthermore, the same situation has been reported for *Labisia pumila* (Jaafar et al. 2012). In addition to the concentration, the overall content of total phenolics and flavonoids/plant was significantly higher in plants facing drought stress in comparison with the well-watered controls.

Biosynthesis of various isoprenoids is similarly increased in response to drought stress. In young sage plants (*Salvia officinalis*), drought stress causes a significantly higher overall amount of monoterpenes per plant in comparison to the well-watered controls (Nowak et al. 2010). Although the growth of the stressed sage plants was significantly reduced, the lesser biomass was over-compensated by a massive increase in monoterpene biosynthesis. Hence, both phenomena described above, i.e., indirect and direct stress-related enhancements (Fig. 1), markedly contribute to the stress-related increase in the concentration of monoterpenes.

Unfortunately, while thoroughly contemplating the entire stress syndrome, conjunctures tend to become ambiguous. This difficulty is vividly outlined by drought stress investigations with parsley (*Petroselinum crispum*) and oregano plants (*Origanum vulgare*). At the first sight, the situation seems to be quite clear: the overall contents of essential oils are similar in drought-stressed and control parsley plants, whereas in both trials the concentration of monoterpenes is significantly higher in the stressed plants (Petropoulos et al. 2008). Accordingly, the enhanced monoterpene concentration seems to result just from the lower reference value, i.e., the lesser biomass of the stress-plants, and not from a stress-related increase in the monoterpene biosynthesis. The same situation was reported by Ninou et al. (2017) for oregano plants: although the monoterpene concentrations were significantly higher in stressed plants than in well-watered ones, the overall essential oils content per plant was nearly the same in both groups. At the first sight, more or less identical total amount of monoterpenes seems to expound that drought did not increase the rate of monoterpene at all. However, we must consider that in the drought-stressed plants, an equal amount of monoterpenes is produced by a far lower biomass than in the well-watered controls. In consequence, we have to state that the biosynthetic activity per dry weight is in fact strongly enhanced. These coherences vividly outline the problem of using appropriate reference values, when the extent of natural product biosynthesis is compared between the stressed and control plants. Based on a corresponding case study with thyme plants, Paulsen and Selmar (2016) demonstrated these conjunctures by calculating reliably the putative increase in monoterpenes biosynthesis on a plant and a dry weight basis. It turned out that the rate of biosynthesis on a dry weight basis was indeed quite different in stressed and well-watered plants: when calculated on a dry weight basis, in the first phase of the experiment, the biosynthetic activity was higher in the stressed plants than in the well-watered controls. Though, when the stress continued, the situation reversed (Paulsen and Selmar 2016). This complex situation became even more critical when – in addition to the direct impact via increased biosynthetic activity – an indirect effect due to a decrease in the biomass gain is considered, thereby expounding the requirement to employ appropriate reference values (Abouzeid et al. 2022).

Although only a limited amount of data on the increase of biosynthetic activity is available, we can assume that drought situations provoke an upsurge in biosynthesis of natural products. In this context, it is not relevant whether this statement is based on an enhancement of the total content of specialized metabolites/plant or on a dry weight basis. Thus, the question arises, which metabolic processes and linkages are responsible for the enhancement of the corresponding biosynthetic activity.

Often the issue of a stress-related impact on concentration and content of specialized metabolites becomes even more complex, as many metabolites are not synthesized in the same organ, in which they are finally accumulated (e.g., Hartmann et al. 1989; Kajikawa et al. 2011; Nowak and Selmar 2016). Accordingly, transport processes also need to be considered (Fig. 1). In fact, translocation processes are also strongly impacted by the actual stress situation (e.g. Rötzer et al. 2012). Up to now, information is lacking on how stress impacts the putative *source-* or *sink*-character of the various organs, and in which manner allocation of specialized metabolites is influenced by stress. We should also consider that degradation processes of specialized metabolites may occur and that these catabolic reactions might also be influenced by the stress situation. Indeed, it is well known that various alkaloids are degraded within vital plants (Robinson 1974; Ashihara 2006). However, till now, comprehensive investigations encompassing the putative stress situations are lacking.

5 Complex Metabolic Processes Increase Natural Product Biosynthesis under Stress

As outlined, the so-called direct impact of drought stress is due to an increase in the biosynthetic processes generating specialized metabolites. Meanwhile, we know, that this enhancement could be either "*active*" or "*passive*" (Selmar et al. 2017; Yahyazadeh et al. 2018; Abouzeid et al. 2022).

Passive Shift The issue of energy saving represents one of the most significant paradigms, which is omnipresent in our daily life. At the first sight, it seems reasonable to adopt this assertion also in plant biology. However, taking into account that plants are autotrophic organisms, it becomes undeniable that plants indeed do not suffer from any energy shortage. By contrast, plants generally absorb much more energy than they require for their photosynthetic CO_2-fixation (Wilhelm and Selmar 2011). Although this fundamental claim might sound incredulous, it can easily be documented: on raising the ambient CO_2 concentration, the photosynthetic rate accelerated considerably (Wilhelm and Selmar 2011). This indicates that, under standard conditions, the photosynthetic electron transport chain provides far more reduction equivalents ($NADPH + H^+$) than the plants require for the actual CO_2 fixation and reduction. Thus, plants are faced with a massive oversupply of energy and without any defensive mechanism, a huge overflow of the electron transport chain would be generated and the surplus amounts of electrons would be transferred to oxygen. Nevertheless, in order to avoid such generation of oxygen radicals the oversupply of energy must be dissipated efficiently, i.e., by non-photochemical quenching, photorespiration, or xanthophyll cycle (Reddy et al. 2004; Szabó et al. 2005). Indeed, under regular environmental conditions, these protective mechanisms function suitably and the generation of oxygen radicals is largely inhibited (Fig. 2a). However, any stress situation, most notably drought stress, generates massive imbalances (Selmar and Kleinwächter 2013b): due to water shortage, stomata are closed and the CO_2-influx into the leaves decreases heavily (Fig. 2b). In consequence, the lower internal CO_2 the consumption of $NADPH + H^+$ in the Calvin cycle is strongly reduced. Despite an up-regulation of the energy dissipating mechanisms, i.e., non-photochemical quenching, xanthophyll cycle and photorespiration, the reduction status of the chloroplasts accelerated (Selmar and Kleinwächter 2013a, b; Kleinwächter and Selmar 2015). The excess electrons generated in the photosynthetic electron transport chain are directly transferred to oxygen, and a large number of superoxide radicals are generated (Selmar and Kleinwächter 2013b), which – in turn - are promptly detoxified by SOD and APX. The occurrence of large amounts of oxygen radicals vividly expounds the highly over-reduced status of the chloroplast, which in turn is associated with a strongly enhanced ratio of $NADPH + H^+$ to $NADP^+$ (Fig. 2b). As a result, according to the law of mass action, all processes consuming $NADPH + H^+$ are increased. This also applies to the processes responsible for the biosyntheses of highly reduced specialized metabolites, e.g. isoprenoids, phenols or alkaloids (Selmar and Kleinwächter 2013b;

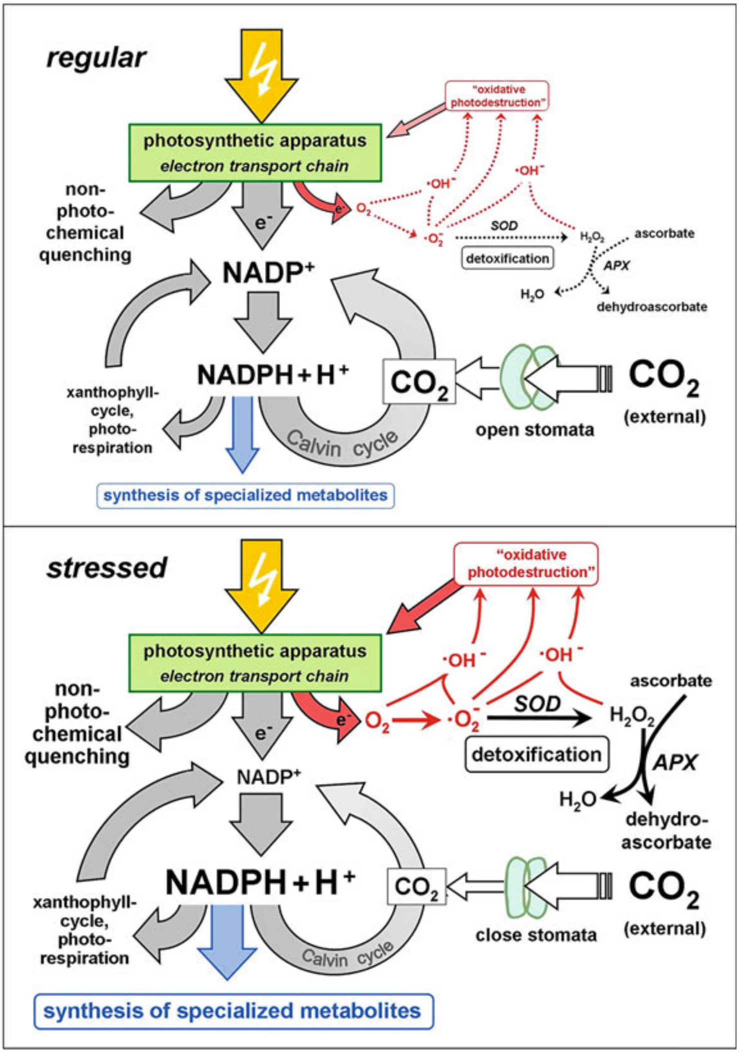

Fig. 2 Energy dissipation in plants. According to Selmar and Kleinwächter (2013b), the light energy absorbed by the photosynthetic apparatus is much higher than the energy required for CO_2-fixation. In consequence, large amounts of energy have to be dissipated via non-photochemical quenching and effective re-oxidation of NADPH $+H^+$, e.g. via photorespiration and the xanthophyll cycle. As a result, the great surplus of reduction equivalents does not generate a significant amount of oxygen radicals, which might be produced in the course of an electron transfer to molecular oxygen. In contrast, the energy fluxes in stressed plants are markedly shifted. The closed stomata effectuate a strongly enhanced diffusion resistance; consequently, the internal concentration of CO_2 is much lower in stressed leaves in comparison to well-watered controls. Hence, much less NADPH + H^+ is consumed by the Calvin cycle and, a far higher share of the energy has to be dissipated. Although non-photochemical quenching and photorespiration are elevated by feedback mechanisms, a waste of electrons is transferred to molecular oxygen. Yet, the generated superoxide radicals are detoxified via superoxide dismutase and ascorbate peroxidase, which are up-regulated in response to the stress. According to the law of mass action, the strongly elevated

Kleinwächter and Selmar 2015; Abouzeid et al. 2022). In other words, the biosynthesis of these compounds is enhanced as a direct consequence of the strongly increased concentration of NADPH + H^+ due to the over-reduced status of the stressed plants (Fig. 2b). Since this enhancement is realized without any increase of enzyme activity, it is denoted as "*passive*" (Fig. 1).

Active Up-Regulation In contrast to the passive enhancement of natural product biosynthesis due to the stress-induced over-reduced status, the extent of biosynthesis could also be increased actively by an up-regulation of genes encoding the enzymes responsible for the biosynthesis of specialized metabolites (Fig. 2). In this context, ample information is available on the expression of related genes in response to pathogen or herbivore attack (e.g. Dixon 1986; Kurusu et al. 2010). In contrast to the impact of biological stress on specialized metabolism, far lesser investigations had been conducted on how drought stress impacts the expression and regulation of genes encoding the enzymes involved in the biosynthesis of natural products.

In *Scutellaria baicalensis*, the increased baicalin accumulation is correlated with enhanced expression level and enzymes activities of flavonoid biosynthesis, i.e., the phenylalanine ammonia-lyase and the chalcone synthase (Cheng et al. 2018). An analogous correlation is observed for the drought-induced increase of flavonoids biosynthesis in *Bupleurum chinense* (Yang et al. 2020). Similarly, in wheat leaves, the drought-induced flavonoids accumulation matches with increased expression level of genes encoding various enzymes of flavonoid biosynthesis (Ma et al. 2014). A similar significant coincidence was verified by Abdollahi Mandoulakani et al. (2017), who found that the drought stress-induced concentration increase of two phenylpropanoids, i.e., methylchavicol and methyleugenol present in the essential oils of basil, is accompanied by an up-regulation of genes encoding key enzymes of the phenylpropanoid-biosynthesis.

Likewise, stress-induced increase of various terpenoids is reported to be – at least in part – due to an increased enzyme activity: in sage, the strong enhancement of the total monoterpene content is correlated with an increase in the expression level of several monoterpene synthases (Radwan et al. 2017). The authors conclude that the rise in monoterpene content is - in addition to a passive shift – also due to elevated enzymatic activities. This deduction is supported by the studies of Palesh and Abdollahi Mandoulakani (2020), who found that in basil, the genes responsible for the monoterpene biosynthesis are up-regulated under drought situations too. In addition, corresponding data are reported for a coincidence of drought-induced increase of glycyrrhizin with an up-regulation of genes encoding the key enzymes in triterpenoid biosynthesis, i.e., squalene synthase and ß-amyrin synthase in *Glycyrrhiza glabra* (Nasrollahi et al. 2014). However, it has always to be considered that different plant genotypes might respond differently in drought conditions. This convincingly was shown by Morshedloo et al. (2017) who demonstrated that the

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Fig. 2 (continued) reduction status, i.e., the ratio of NADPH + H^+ to $NADP^+$, leads to an enhancement of the synthesis of highly reduced natural products drought stress-associated impact on essential oil content and on the related gene expression was quite different in two native subspecies of *Origanum vulgare (*subsp. *virens* and subsp. *gracile).*

Much lesser information is available on alkaloids and drought stress-induced changes in the expression of enzymes involved in their biosynthesis. Notwithstanding, it could be verified that in *Chelidonium majus* the drought stress-related increase of the coptisine concentration (the prevailing isoquinoline alkaloid in greater celandine) is correlated with a corresponding increase in the expression of stylopine synthase, i.e., the key enzyme in the biosynthesis of isoquinoline alkaloids (Yahyazadeh et al. 2018). There is a stringent necessity for further investigations on alkaloidal plants to elucidate if these findings indeed represent a more general feature, i.e., whether or not the stress-related increase of alkaloid concentration is - in addition to the passive shift – also due to a stress-related up-regulation of related genes.

While evaluating the findings on a stress-related up-regulation of genes involved in the biosynthesis of various specialized metabolites, the question arises on the biological relevance of this effect. Ecological biochemistry outlines that the significance of specialized metabolites is based on their functions within the interactions of plants with their environment (Harborne 1988). Specialized metabolites (formerly called "secondary metabolites") repel herbivores, protect plants against pathogens, or attract pollinators (Hartmann 2007; Wink 2010). Though, the bases for the stress-related active up-regulation of natural product biosynthesis cannot be explained by the functions mentioned above. Although drought frequently reduces the extent of pathogen attack, the active up-regulation of phenols, terpenes, and alkaloids was manifested in the course of evolution, indicates an additional evolutionary advantage. In this sense, various specialized metabolites are described to fend off many abiotic stresses, e.g., the protection of plants against UV-light or too high light intensities, the reduction of the transpiration, the scavenging of toxic oxygen radical and the support of osmotic potential by compatible solutes (e.g. Edreva et al. 2008; Wink 2010). Accordingly, the up-regulation of related genes protects the plants against abiotic stresses. Though, most of the examples displayed above are specialized metabolites, which are relevant for the plants' interactions with other organisms and not to protect them against abiotic stresses. A reliable explanation for the observed drought stress-related up-regulation of these specialized metabolites is still lacking. A clue for such significance could be given by the massive emission of isoprene from numerous trees (e.g., Fall 1999; Sharkey and Yeh 2001). Under standard conditions, the emission of isoprene is neglectable. In contrast, when plants are exposed to elevated temperatures, the isoprene synthesis increases dramatically. Now it accounts for more than 25% of the energy used for net photosynthesis (Magel et al. 2006). The authors deduce that the enhanced isoprene emission entails massively the re-oxidation of NADPH + H^+ and thus significantly contributes to the dissipation of the excess of photosynthetic energy. Furthermore, besides the high relevance for dissipation of the surplus of energy, the emission of isoprene also contributes to cooling down the stressed leaves (Behnke et al. 2007). In addition to isoprene, numerous other volatile organic compounds (VOCs, such as terpenes, alkanes, alkenes) are known to be emitted by stressed plants (Kesselmeier and Staudt 1999). In addition to all the well-established ecological functions, the drought stress-related enhancement of the biosynthesis of reduced specialized metabolites evidently contributes to dissipating a surplus of energy (Wilhelm and Selmar 2011). This shows that apart from their function as radical scavengers (Grace and Logan 2000), highly reduced natural products might also protect the plant against stress-related damage just by their enhanced biosynthesis.

6 Induction of Drought Stress

The insights outlined above suggest that in medicinal and spice plants stress could be employed to deliberately enhance the biosynthesis of specialized metabolites in order to increase product quality (Selmar and Kleinwächter 2013a; Kleinwächter and Selmar 2015). The simplest approach to generate drought stress is to reduce irrigation (Radovich et al. 2005). Unfortunately, such an attempt is not applicable in moderate climates, where the water supply by rainfall cannot be modified directly. Accordingly, this approach is restricted to regions where a supplemental watering system is required. Nonetheless, in regions exhibiting frequent rain showers, the soil moisture can also be influenced by choosing appropriate cultivation conditions. Kleinwächter and Selmar (2015) outlined that fields with slopes will retain the water markedly shorter than flat plains, and even in plains, the extent of water drainage can significantly be enhanced by introducing furrows or ridges, which directly improve the drainage properties (Kleinwächter and Selmar 2015). Moreover, drainage could be increased by raising the proportion of sand in the soil. This approach, however, will change the character of the soil irreversibly.

Alternatively, to a reduction of the soil moisture, drought stress can also be induced by applying certain growth regulators and signal molecules (Siddiqi and Husen 2019). In this context, jasmonic acid (JA) is one of the most potent signal transducers, which elicits many genes involved in different stress responses (Turner et al. 2002; Kazan and Manners 2008). Its volatile ester, methyl jasmonate (MeJA), was primarily employed in numerous tissue and cell culture systems to successfully increase the concentration of specialized metabolites (Namdeo 2007). When MeJA is taken up into plant cells, it is hydrolyzed, and the active growth regulator JA is generated (Wu et al. 2008). In this regard, it has to be considered that higher concentrations of JA induce also senescence (Creelman and Mullet 1997). Thus, it is crucial to employ appropriate concentrations of MeJA, which do not yet elicit senescence but which are suitable to mimic drought stress. The application of MeJA successfully increased the concentration of phenols and monoterpenes in *Ocimum basilicum* (Kim et al. 2006), of glucosinolates in *Brassica rapa* (Loivamäki et al. 2004) and in *Tropaeolum majus* (Bloem et al. 2014). In the same manner, the concentration of flavones in parsley and of alkaloids in *Chelidonium majus* increased in response to a MeJA treatment (Kleinwächter et al. 2015). In contrast, in thyme MeJA had no effect on the concentration of monoterpenes, although moderate drought stress resulted in higher monoterpene concentration in comparison to the well-watered controls (Kleinwächter et al. 2015). Obviously, the effectivity of MeJA application depends on its appropriate concentration, which in turn is different for various plant species (Kleinwächter et al. 2015). When *Vinca minor* was treated with MeJA, not only the concentration but also the composition of indole alkaloids is strongly altered (Aerts et al. 1994; Abouzeid et al. 2017). In conclusion, MeJA application represents an alternative for a direct drought exposure in order to improve the quality of medicinal and spice plants.

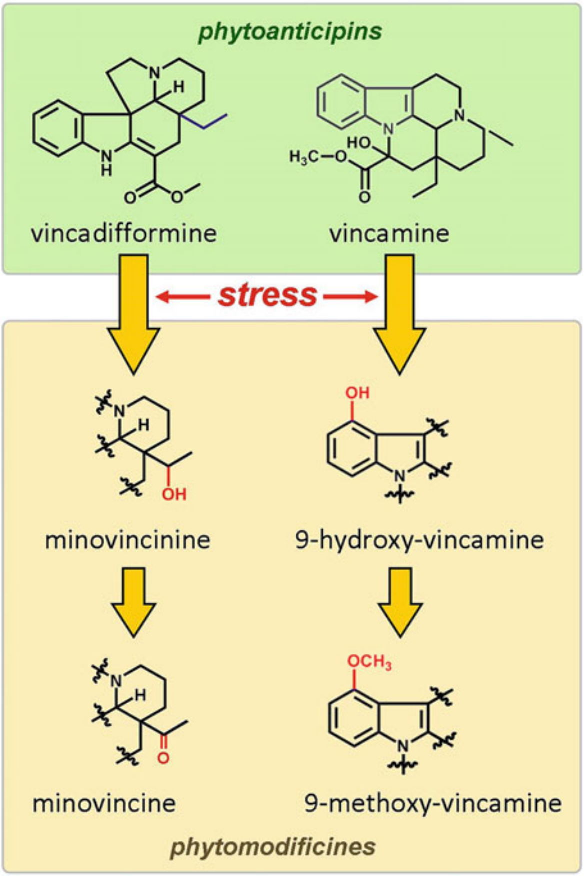
Salicylic acid (SA) represents the key signaling molecule which is responsible for systemic resistance (Durrant and Dong 2004). In analogy to JA also SA and its methyl ester, methyl salicylate (MeSA), has been employed to influence specialized metabolism. When *Echinacea purpurea* leaves were treated with SA, the concentration of phenolic natural products increased significantly (Kuzel et al. 2009). Analogously, application of SA resulted in an increased accumulation of glucosinolates in oilseed rape (Kiddle et al. 1994).

As mentioned, JA and SA also influence primary metabolism and developmental features, in particular by inducing senescence, causing retardation of growth, and alleviating the impact of various stresses (Husen et al. 2018, 2019). Thus, similarly, as discussed for the deliberate induction of drought stress, the application of growth regulators requires a "*thorough balancing of pros and cons*" (Kleinwächter and Selmar 2015). The success of any employment of growth regulators requires the inclusive information of the related scientific background and also a sound determination of their optimal concentration and the best time for their application.

7 Stress Induces Qualitative Changes: Variance in the Spectrum of Specialized Metabolites

Abouzeid et al. (2017) treated lesser periwinkle with MeJA to deliberately increase the indole alkaloids contents. The outcome was noticeable. Whereas 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 Sr et al. 2012), the alkaloid composition of the MeJA-treated plants was greatly different: the genuine alkaloids vincamine and vincadifformine were present only in small amounts. Thus, the contents of these two alkaloids reduced in response to the stress. Contrary to this, minovincinine, minovincine, and 9-methoxyvincamine contents were dramatically enhanced. Due to the very similar structures of vincamine and 9-methoxyvincamine and their inverse changes in concentrations, it was assumed that vincamine was converted to 9-methoxyvincamine in response to the stress (Fig. 3). In analogy, vincadifformine was first converted to minovincinine and finally to minovincine. It is well established that the required hydroxylations are catalyzed by cytochrome P450 enzymes, which are reported to be induced by stress (e.g., Narusaka et al. 2004; Pandian et al. 2020).

Fig. 3 Conversion of indole alkaloids in *Vinca minor*. According to Abouzeid et al. (2019), in stressed *Vinca* plants, their typical phytoanticipins, i.e., vincamine and vincadifformine, are modified by cytochrome P450 enzymes. The resulting derivatives represent a new category of specialized metabolites, denoted as phytomodificines



When naproxen, a well-known inhibitor for cytochrome P450 enzymes was applied together with the MeJA, the conversion of the two major *Vinca*-alkaloids was effectively suppressed (Abouzeid et al. 2019). This verifies that the alkaloids genuinely accumulated in *V. minor* plants are modified in response to the stress induction. It is well known that various specialized metabolites are synthesized in response to pathogen or herbivore attack. However, these natural compounds are generated *de novo* from primary metabolites and accordingly denoted as phytoalexins (Pedras et al. 2011). In the case of the modification of the two major *Vinca*-alkaloids, specialized metabolites, which already are constitutively present in a plant (phytoanticipins) are modified in response to stress. Since the modified indole alkaloids are neither phytoanticipins nor phytoalexins, they represent a novel category of specialized metabolites, the phytomodificines. These specialized metabolites are generated by the stress-induced modification of previously accumulated phytoanticipins (Abouzeid et al. 2019). The production of phytomodificines outlines that stress does not only induce quantitative changes in the biosynthesis of specialized metabolites but may also alter their composition. Further intensive investigation is needed to elucidate the molecular coherence of this fascinating issue.

In conclusion: there is a tremendous potential to deploy the stress-related changes in the spectrum of specialized metabolites. With respect to the exposure to growth regulators to medicinal plants, the elicitation of new, to date still unknown natural products, that might reveal pharmacological activities, opens new doors in research.

8 Implementation into Practice

Drought drastically impairs plant cultivation and causes yield losses in agriculture crops. Though, when considering spice, herbal and medicinal plants, the condition is ambiguous, since not the overall yield, but the product quality mainly determines their benefit. The latter one is determined by the content and concentration of relevant natural products. Accordingly, for increasing the quality of these plant-derived commodities, the content of specialized metabolites has to be enhanced. In the recent past, a couple of related approaches had been successful (Bloem et al. 2014; Kleinwächter et al. 2015). In agricultural practice, the interference of the stress-related reduced biomass gain and the increased concentrations of relevant compounds have to be balanced. In this context, it has to be clarified: what is required, a large overall yield of a particular substance or a high concentration of relevant compounds in the commodity? In the latter case, the focus lies on a high concentration of relevant natural products, and deficits in biomass could be accepted. In contrast, when a high total yield of a particular substance is desired, detriments in biomass production have to be minimized.

In addition to both effects outlined, the stress-induced retardation of growth and the impact on specialized metabolism, it has to be considered that stress also affects the plant's metabolism, e.g. by changing the ratio between generative and vegetative characteristics (e.g., Nederhoff and Houter 2009). In consequence, drought situation alters the *source-sink* properties of the entire plant and thereby also impacts the allocation of specialized metabolites from one organ into another. These considerations especially become relevant when a particular plant-derived commodity is constituted only of one specific organ, e.g., roots, leaves, seeds, or flowers.

A further promising prospect for practical implementation of moderate drought stress in the agricultural practice is related to the generation of phytomodificines. The opportunity to employ stress to induce the generation of new hitherto unknown compounds or to massively alter the spectrum of natural compounds in medicinal plants opens new doors for the search of novel, so far unknown, bioactive natural products. Thus, corresponding approaches will hasten the development of novel, highly desirable drugs.

Apart from medicinal plants, various other well-known examples demonstrate the potential of stress to alter the quality of various plant-derived supplies. This also accounts for all products, whose quality is related to a low content of specialized metabolites. The highest quality of Japanese green teas is linked to very low concentrations of undesired phenolic compounds. For quality improvement, the *Camellia sinensis* plants are normally grown under artificial shading (Wang et al. 2012), subjecting the plants to low light intensities. Accordingly, the surplus of energy (Fig. 2) is far less in the shaded plants than in those irradiated by full sunlight. In consequences, due to the far lesser over-reduced status in the shaded tea plants (Fig. 2), the synthesis of secondary plant products is massively decreased. This was confirmed by Shimoda et al. (1995), who showed that the content of monoterpenes as well as that of coumarins is lower in the shaded, non-stressed tea plants compared to the related controls exposed to full sunlight.

When *Nicotiana tabacum* plants grow in Southern and Middle-America, the full sunlight induces the typical stress-related reactions, and the corresponding leaves exhibit the typical dark colors of the so-called Brazil quality of tobacco leaves. Shading of these tobacco plants results in much brighter hues, which indeed are comparable to the leaves of tobacco grown in Sumatra, where - due to the foggy climate - the solar irradiance is inherently much lower. These phenological observations were confirmed by corresponding phytochemical analyses, which showed that the concentrations of total phenols and alkaloids indeed are quite lower in the tobacco leaves grown in the shade (Andersen et al. 1985).

These examples visualize the potential to improve the quality of spice, herbal and medicinal plants by impacting their specialized metabolism through stress exposure. However, in all attempts, we always have to ponder the advantages and the drawbacks of related approaches. In this context, some simple queries should be raised to facilitate the corresponding evaluations (Selmar and Kleinwächter 2013a; Kleinwächter and Selmar 2015):

- What is required for the desired quality, a large bulk of a specific natural product or a high concentration in the commodity? (total amount *versus* high concentrations).
- What stress and which level is appropriate to enhance the biosynthesis of the desired compounds without causing too high losses in biomass?
- Could the accumulation of the desired specialized metabolite be enhanced by the employment of signal transducers or growth regulators (e.g. MeJA, salicylic acid)?
- In which tissues - s*ource* or in *sink* – is the relevant compound accumulated?
- In which organs - generative or vegetative - is the desired natural product synthesized and accumulated, respectively?
- Should the stress be applied within a special developmental phase or whilst a certain phase of cultivation in order to obtain maximal success?

9 Conclusion

Based on the inherent biochemical consideration, three causes for the stress-related increase in the concentration of natural products could be identified. Due to the lesser biomass of the stressed plants in comparison to well-watered controls, the concentration (on a fresh or dry weight basis) could be higher, even when no difference in the extent of biosynthesis takes place. In addition to this "*indirect*" effect, the total amount of specialized metabolites could actually be higher in stressed plants. This "*direct*" effect is due either to an "*active*" up-regulation of the biosynthetic enzymes or to a "*passive*" shift. The latter one is due to the enhanced level of NADPH + H^+, caused by the shortage of CO_2 as a result of the stress-related closing of stomata.

In addition to these quantitative changes, the spectrum of specialized metabolites could also be altered in response to stress. These physiological considerations are the basis for practical approaches employing moderate drought stress or applying growth regulators to deliberately improve the quality of spice and medicinal plants.

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The Role of PGPRs in Medicinal Plants under Abiotic Stress

Mukesh Meena, Sandeep Kumar Singh, Prashant Swapnil, and Pritee Kumari

Abstract Rapid and gradual change in environment causes abiotic stress in medicinal plants and ultimately reduces their yield. To tolerate abiotic stress such as salinity, drought, heavy metal, temperature, etc. (causes the production of reactive oxygen species including superoxide radical, hydroxyl radical and hydrogen peroxide) plants have developed various mechanisms. Plant growth promoting rhizobacteria (PGPRs) also play an important role in abiotic stress and trigger the tolerance mechanism in plants. Harmful pesticides and agrochemicals reduce the development of pathogens and threat to global food security and environment. PGPR emerged as biologically, cost-effective and eco-friendly substitutes to help plant growth. Medicinal plants have developed a set of different mechanisms for adaptation and survival under severe environmental conditions. This chapter discusses the effect of abiotic stress in medicinal plants and their interaction with PGPRs to facilitate the growth by synthesis of beneficial metabolites through various mechanisms.

Keywords PGPR · Medicinal plants · Abiotic stress · *Rhizobium* sp.

1 Introduction

Environmental stresses, abiotic and biotic, affect the most of the plant species, limit their growth and reduce the crop productivity worldwide (Yang et al. 2009; Kearl et al. 2019; Khan et al. 2019; Leontidou et al. 2020). Plants have ability to engage

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A. Husen, M. Iqbal (eds.), *Medicinal Plants*,
https://doi.org/10.1007/978-981-19-5611-9_10

beneficial microorganisms in their surroundings, and a wide range of bacterial population in the plant rhizosphere may exert positive effects on performance of plants to counterbalance the stress (Aroca and Ruiz-Lozano 2009; Bruto et al. 2014; Compant et al. 2019). Secondary metabolites or compounds of medicinal plants have long been used to cure various health disorders (Table 1). Several medicinal plants have been reported to be associated with bacterial entophytes and rhizobacteria (Sekar and Kandavel 2010). Bacterial entophytes are associated with the plant tissues internally without damaging the host plants, while the rhizosphere region of plants is in contact with rhizobacteria (Wilson 1995; Simarmata et al. 2019; Saryanah et al. 2021). Plant growth-promoting rhizobacteria (PGPR) induce the growth and development of plants by direct and indirect mechanisms. In the direct mechanism PGPRs expedite the procurement of nutrients from the environment or alter plant growth by altering the hormone levels, whereas indirectly they control plant pathogens (Glick 2012; Gouda et al. 2018; Kumari et al. 2018a, Kumari et al. 2020; Meena et al. 2020). Various abiotic stress factors like low temperature, salt, flooding, heat, drought, heavy metal stress and oxidative stress reduce the growth and productivity of medicinal plants (Ahmad et al. 2011; Flora et al. 2013; Wang et al. 2014; Barupal et al. 2020a, b; Meena et al. 2021). These abiotic stresses alter the morphological and physiological behavior of plants which leads to several changes in cellular and molecular processes (Chaudhry and Sidhu 2021) (Fig. 1). This chapter discusses the role of PGPRs in medicinal plants under different abiotic stresses.

2 Abiotic Stresses

Abiotic stress such as extreme temperatures, high intensity light, salinity, drought, gaseous pollutants, heavy metals adversely affect the crop productivity (Husen et al. 2014, 2016, 2017, 2018, 2019; Husen 2021a, b, c, 2022). Drought stress causes enormous loss in agricultural practices in different parts of the world (Umar et al. 2019). According to FAO (2008), accumulation of salts in the soil causes salinity (higher concentration of sodium binds with the clay particles) and affects 800 million hectares of land. Salinity resulting from natural means (primary salinity) and human activities (secondary salinity) creates oxidative stress in plant tissues and hampers plant growth, photosynthesis and crop yield (Hussein et al. 2017; Alharby et al. 2019a, b). Variations in climate such as heat, rainfall intensity, concentration of CO_2 and temperature affect the biotic and abiotic components of the world and lead to develop new weeds, pathogens and pests (Chakraborty and Newton 2011). Changes in climatic conditions impair plant growth, photosynthesis and metabolite production, and cause physio-chemical and molecular alterations in plants (Chaudhry and Sidhu 2021). Toxic trace metals or heavy metals (nickel, cadmium, chromium, mercury, zinc, silver, copper, cobalt and lead, etc.) also act as abiotic stress agents due to their bioaccumulation and toxicity, which ultimately cause oxidative stress in plant cells and affect plant physiology and metabolism (Ansari et al. 2019; Anjitha

Table 1 Some important medicinal plants and their medicinal properties

Medicinal Plants	Common name	Family	Stress factor	Impact on plant	Medicinal use	References
Aloe vera (L.) Burm.f	Ghritkumari	Liliaceae	Salt stress	Anthraquinones and lignins content increased, minerals content decreased, upregulation expression of salicylic acid	Analgesic, immune modulating, antimicrobial	Thu et al. (2013); Egbuna et al. (2020)
Aquilaria sinensis (lour.) Spreng.	Agar wood	Thymelaeaceae	Heat stress	Upregulation in the expression of jasmonic acid	Used against cancer, abdominal pains, asthma, colic and diarrhea	Xu et al. (2016)
Arabidopsis thaliana (L.) Heynh.	Mouse-ear cress	Brassicaceae	Cold stress	Formation of sterol glycosides as well as a higher enzymatic activity	Cure sores in the mouth	Mishra et al. (2013)
Arachis hypogaea L.	Peanut	Fabaceae	Salt stress	Shoot dry weight, chlorophyll and K^+ decreased	Reducing heart diseases	Bojadla (2020)
Azadirachta indica A.Juss.	Neem	Meliaceae	Heavy metal stress	Enhanced the content of nimbandiol and n-hexacosanol, increased amino acid content, upregulation of ascorbic acid	Analgesic, immune modulating, antimicrobial	Landy et al. (2011); Emran et al. (2015); Srivastava et al. (2020)
Beta vulgaris L.	Beet root	Amaranthaceae	Heat stress	Release of anthocyanin pigment from hairy root	Lower blood pressure, improve blood flow	Thimmaraju et al. (2003)
Bunium persicumi (Boiss.) B. Fedtsch.	Black Cumin	Apiaceae	Drought stress	Reduced water content stress, essential oil yield as significantly decreased essential oil yield, increased proline contents of and limonene contents increased	Antibacterial and antifungal activity	Saeidnejad et al. (2013); Shahzad et al. (2015)
Catharanthus roseus (L.) G. Don	Sadabahar	Apocynaceae	Salt stress	Decrease in protein content	Used to treat ulcer	Osman et al. (2007)
	Sadabahar	Apocynaceae	Water stress	Increased indole alkaloid content	In malaria, diabetes, Hodgkin's lymphoma and leukemia	Tolambiya and Mathur (2016)

(continued)

Table 1 (continued)

Medicinal Plants	Common name	Family	Stress factor	Impact on plant	Medicinal use	References
Cucumis sativus L.	Cucumber	Cucurbitaceae	Heat stress	Decrease in 5-aminolevulinate dehydratase, the first enzyme of pyrrole biosynthetic pathway	Help to stay hydrated, support heart health, protect brain from neurological diseases, reduce risk of cancer	Mohanty et al. (2006)
Curcuma longa L.	Turmeric	Zingiberaceae	Salt stress	Enhanced leaves number, stem height, stem and rhizome fresh biomass	Anti-HIV agent, antimicrobial, anti-inflammatory, anti-cancerous and anti-mutagenic	Kumar et al. (2016)
Datura stramonium L.	Datura	Solanaceae	Drought stress	Activation of phenylalanine ammonia-lyase	For used in analgesic, asthma, urinary and respiratory tract stimulation	Pretorius and Marx (2006)
Emblica officinalis Gaertn.	Amla	Phyllanthaceae	Salt stress	Phenolics and ascorbic acid increased, 1-diphenyl-2-picryl hydrazyl decreased	In cough, diabetes, cold, etc., in hyperacidity, and prevention of cancer	Kumar et al. (2012)
Hemidesmus indicus (L.) R. Br.	Indian sarap sarilla	Asclepiadaceae	–	–	Appetizer, carminative, aphrodisiac, astringent, wound healing	Ganesan et al. (2012); Deeb et al. (2010)
Hypericum brasiliense Choisy	Milfurada	Hypericaceae	Drought stress	Concentration and total phenolic compounds enhanced	Antiseptic, digestive, expectorant, ant depressive	de Abreu and Mazzafera (2005)
Labisia pumila (blume) Fern.-Vill.	Kacip fatimah	Primulaceae	Drought stress	Concentration and also overall production of total phenolics and flavonoids increased	Cardiovascular protection and osteoporosis	Jaafar et al. (2012)
Matricaria chamomilla L.	Chamomile	Asteraceae	Salt stress	Increase of phenolic compounds like chlorogenic and caffeic acids	Skin disease and rheumatism	Kováčik et al. (2009)
Mentha piperita L.	Peppermint	Lamiaceae	Salt stress	Lower number of leaves, leaf area and leaf biomass	Treatment of indigestion, pain in joints, diarrhea, cough	Tabatabaie and Nazari (2007)

Moringa oleifera lam.	Drumstick tree	Moringaceae	Both salt and drought stress	Limits reduced plant growth	In fevers, headaches, as cardiac stimulants and antimicrobial	Koul and Chase (2015)
Panax quinquefolius L.	American ginseng	Araliaceae	Heat stress	Enhancement of ginsenoside and reduction in photosynthesis	Boost energy, lower blood sugar level, reduce stress, treat diabetes	Jochum et al. (2007)
Perilla frutescens (L.) Britton	Beefsteak plant	Lamiaceae	Heat stress	Reduction in productivity of anthocyanins	Used for cure of asthma and cough	Zhong and Yoshida (1993)
Petroselinum crispum (Mill.) Fuss	Parsley	Apiaceae	Drought stress	Enhancement of monoterpenes	Plague and malaria	Petropoulos et al. (2008)
Salvia officinalis L.	Sage	Lamiaceae	Drought stress	Increase in the concentration of monoterpenes	Digestive problems, stomach pain	Nowak et al. (2010)
Salvia officinalis L.	Sage	Lamiaceae	Ozone stress	Increase in phenolic content, notably in gallic acid, caffeic acid	Digestive problems, stomach pain, diarrhea, bloating, heartburn	Pellegrini et al. (2015)
Salvia sclarea L.	Clary sage	Lamiaceae	Cold stress	Reduction in individual leaf area but an increased length and number of spikes and a longer inflorescence with higher content of essential oils	Stomach and digestive problems, kidney complaints and for insomnia	Kaur et al. (2015)
Santalum album L.	Indian sandalwood	Santalaceae	Salt stress	Decrease in growth, increase some in proline and amino acids and contents	Antiulcer, fever, infection of the urinary tract and skin disorders	Misra and Dey (2013)
Teucrium polium L.	Felty germander	Lamiaceae	Cold stress	Leaves are smaller and thicker, have more stomata and glandular hairs, photosynthetic rate higher	Gastrointestinal disorders, inflammations, diabetes and rheumatism	Lianopoulou et al. (2014a)

(continued)

Table 1 (continued)

Medicinal Plants	Common name	Family	Stress factor	Impact on plant	Medicinal use	References
Thymus sibthorpii Benth.	–	Lamiaceae	Cold stress	Leaves become smaller and thicker, higher photosynthetic rate	Ulcer and digestive problem	Lianopoulou et al. (2014b)
Triticum aestivum L.	Wheat	Poaceae	Heat stress	Decrease in 5-aminolevulinate dehydratase	Immune-modulator, antioxidant, astringent, laxative, diuretic, antibacterial and used in the acidity, kidney malfunction, swelling wounds	Mohanty et al. (2006)
Withania somnifera (L.) Dunal	Ashwagandha	Solanaceae	NaCl and $CaCl_2$	Increased total chlorophyll content and activity of proline oxidase	Neural nerves disorder, regulation of reproductive hormones	Sengupta et al. (2018); Chakraborty et al. (2021)

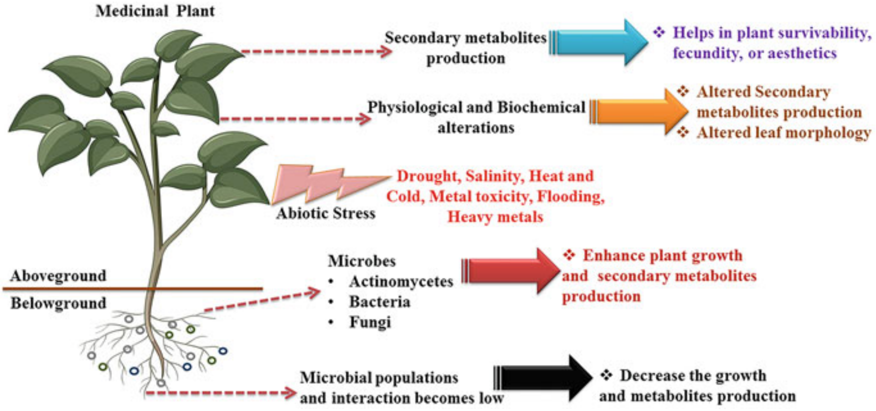

Fig. 1 Morphological and physiological changes in medicinal plants under abiotic stress

et al. 2021). In certain cases, these metals ions are also taken up by plants as nutrients and form the basis for determining the phytoremediation potential of the species concerned (Dutta and Khurana 2015; Iqbal et al. 2015; Jabeen et al. 2009).

3 Plant Performance under Abiotic Stresses

Plants thrive well under optimum environmental conditions. Roots are primitive organs which absorb nutrients from the soil and supply them throughout the plant body, thus maintaining homeostatic conditions. When the plant is subjected to abiotic stress, it undergoes structural and physiological deformation, such as thinning of cell wall, reduction in root length, loss of membrane integrity, and alteration in morphogenic as well as physiological phenomena (Zehra et al. 2021). Even the secondary growth patterns in woody plants, including the meristematic activity of vascular cambium, differentiation of secondary vascular tissues, composition of secondary phloem and secondary xylem, and production of secondary metabolites are altered under the impact of heat, cold, drought, flooding, salinity, heavy metals and a variety of environmental pollutants (Aquil et al. 2003; Gupta and Iqbal 2005; Ali et al. 2008; Aref et al. 2014; Bagheri et al. 2015; Verma et al. 2006).

Drought condition wherein plants are subjected to lack of water supply, reduction in root and stem height, stomatal size, photosynthesis rate, and transpiration, etc., is of common occurrence. However, plants have physiological mechanisms to resist drought condition, e.g. they accumulate biomolecules like abscisic acid, LEA proteins, dehydrins proteins, proline, betaine, sugars, glycine etc. which help plants to store water to resist drought. On the contrary, accumulation of excessive water around the growing plants leads to anoxia like conditions where plants lack energy, which affects the plant root system consequently leading to physiological disturbances, structural alteration and growth inhibition.

Heat stress results from exposure of plant to light and with rise in atmospheric temperature often due to emission of greenhouse gases and a variety of pollutants in gaseous and particulate forms. Plants exhibit reduction in biological yield and photosynthetic efficiency, stomatal closure, drop down in relative water content, suppressed enzymatic activity, failure in seed germination, stunted growth, necrosis, wilting, and a delayed reproductive phase. Molecular chaperons, also referred to as heat shock proteins and transcription factors help plants to endure the stress.

Plants which are unable to withstand high salt stress are known as glucophytes. In general, plants exhibit reduction in biomass production, photosynthesis, leaf-surface area, relative water content, etc. Moreover, physiological disturbances cause osmotic imbalance in plants.

4 Plant Growth-Promoting Rhizobacteria

Rhizosphere is the zone in which plant, the soil microorganisms and the soil establish complex relations and show a lot of diversity and activity of microorganisms (Kennedy 1998; Nihorimbere et al. 2011; Apastambh et al. 2016; Meena et al. 2017a, b; Chandran et al. 2021). Microorganisms of the rhizosphere show beneficial as well as deleterious effect plant growth and crop yield (Schippers et al. 1987; Persello-Cartieaux et al. 2003; Compant et al. 2005; Chandran et al. 2020). Diversified genera of bacteria in rhizosphere considered as PGPRs such as *Bacillus* and *Pseudomonas* spp. (Kremer 2006; Böhm et al. 2007). PGPRs have shown different distinct characters such as colonization, multiplication, competition, and plant protection (Benizri et al. 2001; Ahemada and Kibret 2014). PGPR either directly (through production of hormones, phosphorus solubilization and iron availability) or indirectly (by altering growth-restricting conditions) can encourage the growth of plants. To eliminate specific harmful microbes, PGPRs undergo induction of systemic resistance (ISR) and production of antagonistic substances, and protect plants against pathogens (Riaz et al. 2021; Weller et al. 2002). Approximately 2–5% of rhizobacteria, known as plant growth-promoting rhizobacteria, establishes inoculation with plants in a soil that contains competitive microorganisms and causes a beneficial effect on plant growth (Antoun and Kloepper 2001). PGPRs can be classified on the basis of their functional activities such as biofertilisers, phytostimulators, rhizoremediators, biopesticides and biological control (Nadeem et al. 2013; Kumari et al. 2018b; Mustafa et al. 2019). On the basis of range of associations with plant roots and association intimacy, PGPRs can be either ePGPR (extracellular plant growth-promoting rhizobacteria) e.g., *Arthrobacter*, *Agrobacterium*, *Azospirillum*, *Azotobacter*, *Burkholderia*, *Bacillus*, *Pseudomonas* and *Serratia*, or iPGPR (intracellular plant growth-promoting rhizobacteria) e.g., *Azorhizobium*, *Mesorhizobium*, *Bradyrhizobium* and *Rhizobium* of the family Rhizobiaceae (Gray and Smith 2005; Bhattacharyya and Jha 2012; Sundaramoorthy and Balabaskar 2012).

5 Mechanisms of PGPR

PGPRs facilitate the growth of plants either in the form of ePGPRs or iPGPRs through several mechanisms such as lowering of ethylene levels, nitrogen fixation, siderophores and phytohormones production, toxicity removal, nutrients solubilization, and mycorrhizal association advancement, etc. (Glick et al. 1999). Mechanism of PGPRs can be direct or indirect depending on traits followed by the bacteria (Ortíz-Castro et al. 2009). Direct mechanism includes biological nitrogen fixation, phytohormones (such as auxins, cytokinins and gibberellins) production, phosphorus and iron solubilization, production of siderophores and ISR, while indirect mechanism follows production of antibiotics, Fe chelation in the rhizosphere, extracellular enzymes synthesis to hydrolyze cell wall of fungal (Zahir et al. 2004; Patel et al. 2019). *Bacillus subtilis* and *Pseudomonas fluorescens* are involved in indirect mechanism of plant growth (Damayanti et al. 2007). PGPR show multiple mechanisms to promote the growth of developing plants such as the solubilization of minerals, production of ACC deaminase, plant growth regulators (indole-acetic acid, gibberellic acid, cytokinins and ethylene), biological nitrogen fixation, production of antibiotics, siderophores, chitinases, β-1,3-glucanase, cyanide and fluorescent pigment to show antagonistic activity against phytopathogens.

6 PGPR Associated with Abiotic Stress Tolerance

Pseudomonas putida MTCC5279 is a beneficial microbe, which can reduce the drought stress in chickpea (*Cicer arietinum*) plants by moderating the integrity of membrane, accumulating the osmolytes (glycine betaine, sucrose, trehalose and proline) and activating the ROS-scavenging enzymes (Rajkumar et al. 2017). In response to stress, *Pseudomonas putida* MTCC5279 positively modulated the differential expression of genes such as ACO and ACS, PR1, MYC2 for biosynthesis of ethylene, salicylic acid and jasmonate, respectively. It can enhance the transcription activity and activate the antioxidative responsive genes such as SOD (super oxide dismutase), CAT (catalase) and APX (ascorbate peroxidase). PGPRs activate the dehydration responsive element binding protein (DREB1A), NAC1 (transcription factors), LEA and DHN (late embryogenesis-abundant and dehydrin) (Tiwari et al. 2017). *Bacillus thuringiensis* NEB17 reduces the water-deficit conditions by producing thuricin 17 in soybean (*Glycine max*). It modifies the root structures and helps to increase their length, ABA (abscisic acid) content, nitrogen content and biomass of nodule (Prudent et al. 2015). Under constantly flooded conditions, *Pseudomonas fluorescens* REN1 can be used as a beneficial microbe, which produces ACC deaminase which can promote root elongation in rice (*Oryza sativa*) plants under flooded condition (Saghafi et al. 2020). ACC deaminase can also reduce salinity effects (Nadeem et al. 2010). *Variovorax paradoxus* 5C-2 also produces ACC deaminase, enhances the electron transport and photosynthetic rate, decreases xylem balance pressure and stomatal resistance, and balances the ion homeostasis (increment of K^+ flow towards shoot and deposition of Na^+ on roots) (Wang et al. 2016). ACC deaminase producing PGPRs increase the salt tolerance and antioxidant enzymes activity in okra (Habib et al. 2016). *Bacillus amyloliquefaciens* SQR9 helps to increase chlorophyll content and salt tolerance ability, as in PGPR-inoculated maize seedlings, compared to the uninoculated ones. Salinisation of irrigation water limits the crop yield in semiarid and arid regions, as salinity reduces the photosynthetic efficiency of plants, which alters the metabolic and physiological processes (Krasensky and Jonak 2012; Hussein et al. 2017; El Sabagh et al. 2020). However, several beneficial microorganisms such as *Azospirillum* sp. can tolerate salinity by producing osmoprotectants or compatible solutes like proline, glutamate, trehalose, sucrose and glycine betaine (Tripathi et al. 2002; Han and Lee 2005; Paliwal et al. 2021; Yadav and Meena 2021). Sorghum plants had more water content when inoculated with *Azospirillum* sp. compared to the control. ACC deaminase produced by PGPRs could protect the plants from phytopathogens, drought and salt stress (Saghafi et al. 2020). ACC deaminase hydrolyzes the ACC to ά-ketobutyrate and ammonia in plant growth promotion by regulating the production of ethylene in plants (Shahzad et al. 2014). Plant–water relation at the cell level can be affected by drought stress, which damages intracellular reactions. PGPRs reduced the drought stress in barley and *Asparagus officinalis* and enhanced the plant growth (Liddycoat et al. 2009). PGPRs secrete exopolysaccharides to form sheath of organo-mineral that helps the microorganism to survive in drought stress (Sandhya et al. 2009; Khan et al. 2020). Fujishige et al. (2006) reported that biofilm of microbes' community reduced the environmental stress in cells covered in an extracellular polymeric matrix. Phosphorus is an essential nutrient for the survival of all living cells and organisms, and its deficiency affects the food production and global agriculture (Song et al. 2014). PGPRs help in phosphorus solubilisation in the soil by producing metabolites, which reduce the soil pH and enhance the growth of plants (Ehteshami et al. 2007; Rajkumar and Freitas 2008). PGPRs play important role in heavy metals immobilization by reducing their translocation in plants through complex formation, precipitation and adsorption (Diwan et al. 2010; Iqbal et al. 2015). *Pseudomonas aeruginosa* and *Burkholderia gladioli* reduced the Cd stress in *Lycopersicum esculentum* seedlings mitigating its effect on photosynthesis and the growth of root and shoot in terms of length and fresh weight (Khanna et al. 2019). PGPRs, P-solubilising bacteria, soil microbes, arbuscular mycorrhizal fungi (AMF) and mycorrhizal-helping bacteria (MHB) play important roles in phytoremediation under metal stress (Khan 2005), by secreting various acids, phyto-antibiotic proteins, and other chemicals (Denton 2007). Extreme variation in temperature reduces the crop yield and ultimately causes crop failure (Nelson et al. 2009). *Serratia nematodiphila* produces gibberellin, which induces growth of *Capsicum annum* under low temperature conditions. The PGPR-inoculated plants produce less salicylate and jasmonate and more ABA and GA_4 (Kang et al. 2015). *Burkholderia phytofirmans* reduces the chilling stress in *Vitis vinifera* by modulating carbohydrate metabolism (Fernandez et al. 2012). PGPRs (*Pseudomonas frederiksbergensis*

OS261, *P. vancouverensis* OB155) induce expression of cold acclimation genes in *Solanum lycopersicum* (Subramanian et al. 2015).

7 Advantage of PGPR to Relieve Abiotic Stress

Plant microbe interaction can be both beneficial and harmful. This interaction takes place both above and below the ground. Endophytes live inside the tissue of the host plant and can be neutral, beneficial or even harmful to the plant health (Mei and Flinn 2010). During their interaction with microbe's plants synthesize secondary metabolites and root exudate. These metabolite and exudates, such as organic acids and sugars, stimulate plant metabolism and biogeochemical reactions (Canarini et al. 2019). Synthesis of strigolactone, a signaling molecule which helps in germination of plants, can be induced by mycorrhizal association with plants (Besserer et al. 2006). Leguminous roots enhance or support the growth of nitrogen-fixing microorganism such as rhizobacteria, and during these interaction, leguminous roots secrete flavonoids, which induce the biological nitrogen-fixation (Swarnalakshmi et al. 2020). Flavanoids also protect plants from harmful microbes such as oomycetes (Nishad et al. 2020). Plant hormones such as auxin/cytokinin help in plants growth in the presence of endophytic fungi or root-associated microflora (Mathur and Roy 2021). These endophytic fungi or root-associated microbes also help plants by providing nutrients like nitrogen, phosphorus and iron (Dudeja et al. 2012). PGPR enhances the growth of medicinal plants like *Catharanthus roseus*, *Withania somnifera*, *Aloe vera*, *Coleus forskohlii* and *Ocimum sanctum* (Karthikeyan et al. 2010; Attia et al. 2020). Several PGPRs belonging to the genera *Azotobacter*, *Azospirillum*, *Bacillus* and *Pseudomonas* have been isolated and applied to improve growth in plants. PGPR formulation including *Azotobacter chroococcum*, *Azospirillum lipoferum*, *Bacillus megaterium* and *Pseudomonas fluorescens* enhanced the germination rate, chlorophyll content and vigour index significantly, as in the case of *Catharanthus roseus* (Lenin and Jayanthi 2012).

8 Conclusion

The beneficial microorganisms or PGPRs accomplish the task of supporting plant growth and improving plant health. PGPRs function is purely based on the amended nutrient acquisition and stimulation of hormones. Use of PGPRs can be highly beneficial in agricultural biotechnology. Use of PGPRs offers an ecofriendly approach and is considered as an impending tool for enhancing the growth yield of medicinally important plants.

Further, hormonal and nutritional imbalances caused by stressful environments can be minimized or alleviated by naturally occurring PGPRs. Use of proteomics-based techniques to study the responsive protein and their genes, is likely to provide a new way to convert stress-sensitive plants to stress-tolerant plants by implying genetic engineering. Therefore, we need to focus on metabolic engineering of PGPRs under abiotic stress in order to identify PGPR strains and target genes.

Acknowledgements The authors are grateful to their respective Universities for providing supports during the work.

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Effect of Mineral Nutrition and PGRs on Biosynthesis and Distribution of Secondary Plant Metabolites under Abiotic Stress

Rahmatullah Jan, Lubna, Sajjad Asaf, Muhammad Aaqil Khan, and Kyung-Min Kim

Abstract Secondary plant metabolites are necessary for the survival of plants and help to establish interactions between plants and environment. They constitute an important part of the plant's defense mechanism against pathogenic attacks and environmental stresses, in addition to providing a valuable range of natural products. Plants being sessile in nature safeguard themselves from severe conditions around them by means of bioactive compounds produced as secondary metabolites. These metabolites serve as a deterrent to herbivores, provide a barrier against pathogen invasion, and reduce oxidative stress during stressful situations. The accumulation of these metabolites increases under environmental stress, such as soil water, minerals, temperature, salinity, light, humidity, air pollutants, heavy metals and frost, among others. Changes in the nutritional and hormonal status regulate the secondary metabolites production in plants. This article examines how the mineral nutrient elements and plant hormones influence the accumulation of secondary metabolites in plants under abiotic stress conditions.

Keywords Abiotic stress · Metabolites · Plant hormones · Elicitors · Plant defense

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A. Husen, M. Iqbal (eds.), *Medicinal Plants*,
https://doi.org/10.1007/978-981-19-5611-9_11

1 Introduction

Plants confront a variety of adversaries in natural systems; therefore, they have developed several defensive mechanisms and diverse resistance mechanisms to survive with numerous types of abiotic and biotic stresses (Ballhorn et al. 2009). They protect themselves by generating secondary metabolites (SMs), the chemicals that are required for acclimation and defense under environmental stress, but do not play a critical function in the plant's life processes. The vast majority of secondary metabolites (about 100,000) are thought to be contributing in plant defense mechanisms that have developed over millions of years of plant-invader interaction (Wink 1999). The SMs that play an important role in plants' environmental adaptability, are also an important source of medicines (Rao and Ravishankar 2002). They render plants their medicinal potential, although their distribution is very limited in comparison to primary metabolites. The importance of SMs in daily life, especially in health care sector, has prompted a surge in medicinal plant research during the last 50 years (Beigh et al. 2002; Mulabagal and Tsay 2004; Ahmad et al. 2011; Iqbal and Ahmad 2014; Bachheti et al. 2021; Husen 2021, 2022). Even in the case of herbal drugs with controversial botanical identity, where several different plant species have long been in use under the same drug name, attempts have been made to identify and authenticate the real and genuine plant species on the basis of the quantity of active ingredients of therapeutic importance (Iqbal et al. 2011, 2018). Because most of these phytochemicals are found in nature at very minute levels, massive harvesting is required to obtain sufficient quantities for therapeutic purposes. Thus, in order to utilize the SM on commercial scale, it is required to accomplish successfully the synthesis of these complex chemical compounds even by the rare or threatened plant species, which are often resistant to domestication (Beigh et al. 2005, 2008).

Plants exposed to a range of signal molecules or elicitors commonly accumulate secondary metabolites. The chemical components of medicinal plants have biological activity that can benefit human health and cattle health through the food and pharmaceutical sectors, and may also have additional value in the agrochemical, cosmetic and perfume industries (Beigh et al. 2003a, b; Hassan et al. 2012). The term "metabolome" is currently used to represent all of the tiny molecules in a cell, and metabolomics is now the fourth component of a "systems" approach to biology, which also includes proteomics, transcriptomics (RNA) and genomics (DNA). In this context, SMs are a subset of the plant metabolome, a collection of small molecules different from the numerous common metabolites necessary for life in different organisms.

SM biosynthesis begins with fundamental routes like glycolysis or shikimic acid, and then diversifies based on cell type, environmental stimuli and developmental stage (Patra et al. 2013). These chemicals can be found in a variety of plant cells, tissues, and organs, giving them distinct medicinal properties at different stages of plant development (Ahmad et al. 2004; Bartwal et al. 2013). The commencement and successive differentiation of cellular structures involved in the production and accumulation of SMs are influenced by developmental factors (Broun et al. 2006), which in turn are modulated by many environmental variables that can either stimulate or hinder plant growth (Ali et al. 2008; Ma et al. 2010). There are over 100,000 SMs that are exclusive to certain taxonomic groups. Based on their biochemical pathways, nitrogen-containing chemicals (cyanogenic glycosides, glucosinolates and alkaloids), phenolic compounds (phenylpropanoids and flavonoids), and terpenes are the three primary categories of plants SMs (isoprenoids) (Fang et al. 2011). Despite the rigorous research on SMs biosynthesis and accumulation, information on developmental and environmental variables that impact the synthesis and accumulation of SMs in medicinal plants is still rare. Plants exposed to various elicitors or signal molecules typically accumulate metabolites that have a key role in adaptation to environmental conditions and in medicinal, nutritional, and aesthetic functions (Seigler 1998; Saba et al. 2000). Various elicitors have been used by researchers to increase secondary metabolite synthesis in plant cell, tissue, and organ cultures (Ali et al. 1998, 1999; Sudha and Ravishankar 2003; Karuppusamy 2009). Pathogen invasion, UV irradiation, strong light, wounding, nutritional inadequacies, temperature, and herbicide application are all known to cause an increase in phenylpropanoids accumulation (Chalker-Scott and Fuchigami 2018). Phytochemical levels in plant tissues are also affected by nutrient stress (Chalker-Scott and Fuchigami 2018). Growing conditions alter the concentrations of various secondary plant products, which in turn affect the metabolic pathways that lead to formation of associated natural products. In this chapter we have discussed the impact of mineral nutrient elements and plant growth regulators on biosynthesis and distribution of secondary metabolites of medicinal plants (Fig. 1).

2 Response of Plant SMs to Soil Fertility

The nutrients in the soil are important for the development and establishment of medicinal plants and can have a big impact on their secondary metabolism. (Yang et al. 2018). Plants have an almost unlimited ability to synthesize medicinal and aromatic compounds that have been studied for their medicinal properties. Alkaloids, coumarins, saponins, and flavonoids are examples of these substances (Watson et al. 2001). Due to their antioxidant effects, flavonoids are perhaps the most well-known of these compounds (Havsteen 2002; Rice-Evans 2004). Supplemental plant mineral nutrition has the potential to affect the content of SMs as well as promote the seedling growth (Chandna et al. 2012). Cultivation success is based not merely on growth characteristics, but also on evaluations of medicinal potential and SM content of the plant. Unlike in the wild type plants, adequate fertilizer supply results in a substantial dose-dependent increase in antioxidant activity and SMs production in cultivated plants (Azaizeh et al. 2005). The link between the nutrient supply and SMs production in medicinal plants has been studied extensively. However, these studies were designed mostly to see how the addition of more nutrients, such as nitrogen, could affect alkaloid levels, rather than to see how the nitrogen deficiency affected alkaloid levels (Shengqi et al. 2012).

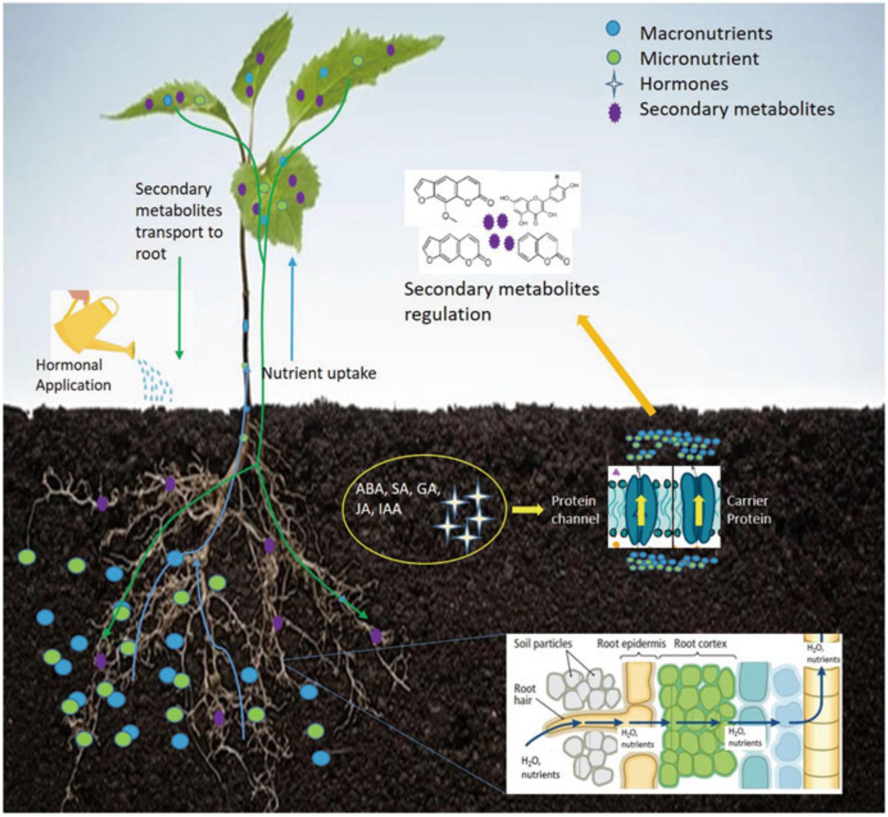

Fig. 1 Schematic presentation of plant growth regulator enhancing plant secondary metabolites through regulation of macro and micronutrients uptake. Exogenous application of plant growth regulators facilitates the absorption of soil nutrients through upregulation of carrier proteins. Due to enhanced availability of nutrients to the arial parts of the plant, the plant enhances the SM biosynthesis. The plant vascular system translocates the rizhospheric nutrients to the arial parts, and the secondary metabolites from shoots to roots

3 Effect of Macronutrients on Secondary Metabolites

3.1 Nitrogen and Phosphate

Many studies have suggested that the plant nutritional alteration regulates a wide range of plant secondary metabolites (Table 1). The inverse connection between nitrogen and phosphate accessibility and flavanol contents in various tissues of plant seedling was shown to be extremely substantial; quercetin, kaempferol, and isorhamnetin concentrations were raised in response to nitrogen or phosphate stress (Stewart et al. 2001; Yang et al. 2018). Studies on the effects of nitrogen fertilization on alkaloid content have yielded mixed findings, although in the majority of cases,

Table 1 Effect of macronutrients on regulation of plant secondary metabolites

Macronutrients	Plants	Modulated secondary metabolites	References
Nitrogen	*Brassica oleracea* L. *var. italica, Brassica oleracea* L. *var. capitata, Lactuca sativa* L.	Flavonoids	Jones et al. (2007); Zhu et al. (2009)
Nitrogen	*Olea europaea* L.	Flavonoids, Mannitol	Boussadia et al. (2010); Simonne et al. (2007)
Nitrogen	*Solanum lycopersicum* L.	Flavonoids, Carotenoids	Simonne et al. (2007)
Phosphorus	*Lens culinaris* Medik.	Anthocyanins	Sarker and Karmoker (2011)
Phosphorus	*Solanum lycopersicum* L.	Xanthophyll	Khavari-Nejad et al. (2013)
Potassium	*Solanum lycopersicum* L.	Carotenoids	Schwarz et al. (2013)
Potassium	*Ocimum basilicum* L.	Anthocyanins	Nguyen et al. (2010)
Calcium	*Prunus avium* L.	Flavonoids, Anthocyanins	Aghdam et al. (2013)
Calcium	*Solanum melongena* L.	Total Phenols	Sinha et al. (2002)
Calcium	*Nicotiana tabacum* L.	Total phenols	Ruiz et al. (2003)
Magnesium	*Rubus* L. sp.	Carotenoids	Tewari et al. (2006)
Magnesium	*Lactuca sativa* L.	Lactucopicrin	Seo et al. (2009)
Sulphur	*Arabidopsis thaliana* (L.) Heynh.	Raffinose, Tocopherol, Sitosterol	Zhang et al. (2011)
Sulphur	*Phaseolus vulgaris* L.	Carotenoids	Juszczuk and Ostaszewska (2011)
Sulphur	*Pisum sativum* L.	Saccharide	Scherer et al. (2006)

more nitrogen was found to boost alkaloid concentrations by 25–300%. Nitrogen deficiency, in turn, was thought to lower the alkaloid concentration. Ibrahim et al. (2011) on the other hand, reported that nitrogen levels influenced the synthesis of total phenolics and flavonoids in *Labisia pumila*. The amount of phenolics and flavonoids production decreased as more nitrogen was applied. Anthocyanins and one of the flavonols regularly increased by two- to three-fold in the leaves of different plants with nitrogen-deficient rhizosphere conditions, but the total non-anthocyanin flavonoids grew by just 14%, equivalent to the wild type plants. Furthermore, the deficiency of nitrogen causes regulation in the expression of genes involved in the biosynthesis of flavonoids, showing the mRNA levels for dihydroflavonol-4-reductase (DFR) and chalcone synthase (CHS) increasing and the mRNA levels for a chalcone isomeraseh omologous band (CHI) decreasing in response to N stress (Bongue-Bartelsman and Phillips 1995).

Many researches have examined the relationship between light and nitrogen and carbon availability in soil by correlating the light-incidence intensity with the nutrients available in the soil (Fajer et al. 1992; Bryant et al. 1983). These investigations do not yield conclusive results, but they do attempt to clarify hypotheses about the carbon/nutrient (C/N) relationship (Koricheva 2002; Coruzzi and Bush 2001). With the exception of nitrogenic substances, secondary metabolism is often increased as the proportion (C/N) increases. As a result, plants growing in nutrient-deficient soils may have a slower growth rate and produce more secondary metabolites, particularly phenolic derivatives (Cesco et al. 2012; Waterman and Mole 2019). Excessive nitrogen in the soil enhances the production of nitrogenous metabolites such as alkaloids, glycosides cyanogenic, and glucosinolates (Gouvea et al. 2012; Verma and Shukla 2015), which leads to a greater vegetative biomass production.

It's also worth mentioning that the amount of nitrogen incorporated in plant tissues has an impact on the formation of secondary metabolites (Mustafavi et al. 2018; Hussein and El-Anssary 2019). Nitrogen and phosphate deficiencies have a direct impact on the accumulation of phenylpropanoids (Solecka 1997). In acid soils, for example, there is less conversion of ammonia to nitrate, which hinders nitrogen uptake, explaining why the plants that thrive in this type of soil have higher levels of secondary metabolites, particularly of phenolic compounds. Low amounts of nitrogen, phosphorus, sulfur, and potassium have been shown to promote the formation of derivatives of ordinary chiquimic acid, hydrolyzable and condensed tannins. Nonetheless, the availability of nutrients was unaffected by metabolites derived from mevalonate (Kopaczyk et al. 2020).

3.2 *Potassium*

Potassium (K) insufficiency in the soil can impact the alkaloid content in *Lupinus angustifolius* (Lupin) seeds, as it showed up to 205% increase in the seeds of sweet varieties of lupins (Carvajal-Larenas et al. 2016). Lupanine was the most common alkaloid in sweet cultivars with K levels ranging from 0 to 240 mg/kg soil, while 13-hydroxylupanine was the most common alkaloid in bitter kinds (Gremigni et al. 2001). Nitrogenous fertilizers, on the other hand, raised the concentration of alkaloids by 9–17%, with NH_4NO_3 showing the greatest impact (Barłóg 2002). The content of alkaloids in seeds was reduced when magnesium, potassium, and nitrogen were applied simultaneously (Barłóg 2002). Similarly, the application of Sangral compound fertilizer increased the alkaloid content in *Datura innoxia* plants with increase in fertilization rate up to a point, beyond which the content showed a decline (Al-Humaid 2005).

3.3 Calcium

Calcium ions are vital in the construction and permeability of cell membranes, cell elongation and cell division, N-metabolism and carbohydrate translocation, among others (White 2000; El-Beltagi and Mohamed 2013). Signal transmission and nutrient absorption across cell membranes are also regulated by calcium cations (El-Beltagi and Mohamed 2013; Talukdar 2012). Ca^{2+} plays a key part in signaling and aids in the activation of polyphenol biosynthesis genes (Xu et al. 2014). In the Ca^{2+} treated plants, Ca^{2+} binds to the phospholipid membrane which reduces the level of malondialdehyde and stabilizes the lipid bilayer, which provides the structural integrity (Hirschi 2004; Yousuf et al. 2015). Ca^{2+} is commonly present in soil, however, in its most common form (e.g., $CaCO_3$), it is relatively insoluble. Some fungal species have been shown to be able to solubilize phosphates, micronutrients, and mineral cations in their external environment by secreting organic acids (Grondona et al. 1997). On the other hand, adding calcium cations at the same time as biocontrol agents boosts biocontrol agent action, i.e., through a synergistic effect (El-Mougy et al. 2012).

3.4 Magnesium

Magnesium is involved in a number of processes in plants, including ATP production, CO_2 fixation, chlorophyll creation and photosynthetic product integration, generation of reactive oxygen species (ROS) and leaf tissue oxidants, and chlorophyll structure. Its scarcity can lead to an increase in the formation and storage of ROS in plant cells, which can affect carotenoids production (Luciano et al. 2017; Cakmak and Yazici 2010). It is also a component of chlorophyll's molecular structure, and its lack causes serious plant stress, resulting in increased antioxidant enzymes and secondary metabolite production. Mg deficiency lowers carotenes while increasing the antioxidant enzymes including superoxide dismutase (SOD), peroxidase (POD), and ascorbate peroxidase (APX) (Tewari et al. 2006). Very little is known about the role of magnesium in biosynthesis of plant secondary metabolites. However, a study shows that leaves of plants grown in pots treated with magnesium increases anthocyanin biosynthesis (Nissim-Levi et al. 2007). Magnesium treatment increased pigment concentrations in aster plant without inducing the activity of key enzymes of the anthocyanin biosynthetic pathway in flower buds (Nissim-Levi et al. 2007).

3.5 Sulfur

The impact of sulfur (S) supplies on the content of alliin, cysteine, and glutathione, which are considered as potential precursors, was examined under field conditions

(Bloem et al. 2011). Sulfur fertilizer boosted cysteine, glutathione, and alliin levels substantially in leaves and bulbs, whereas nitrogen fertilization had little effect. In leaves, cysteine content increased by a factor of 1.3–1.5, while in bulbs, it increased by a factor of 1.0–2.0. S fertilization significantly increased alliin accumulation in bulbs at all harvesting dates and also at maturity. During the vegetative growth until the beginning of ripening, high S treatment in association with low N fertilization enhanced the alliin accumulation in garlic substantially. Because of increased amounts of alliin pre-cursors, garlic bulbs fertilized with a high level of S may have a better capacity to synthesize alliin after harvest (Bloem et al. 2011). High level of sulfur is important to produce good quality of garlic bulbs. The activities of nitrate reductase and ATP-sulphurylase enzymes were strongly correlated to seed and xanthotoxin yield in *Ammi majus*. The highest activity of these enzymes and xanthotoxin yield were achieved with S40: N50: K25: P25 treatment, which could be due to optimization of leaf soluble protein and photosynthetic rate, as these parameters are influenced by S and N assimilation (Ahmad et al. 2007). The potassium, sulfur, and magnesium deficiencies have also been linked to higher phenolic levels (Akula and Ravishankar 2011).

4 Effect of Micronutrient on Secondary Metabolites

Micronutrients play an important role in the synthesis of secondary metabolites in plants. Their effects on SM biosynthesis have been determined in various studies (Table 2). Plants require micronutrients in minute amounts, therefore improper concentrations can cause crop damage due to toxicity. However, the results of some studies on the relationship of micronutrients with SM production are contradictory, as we see in the case of cardenolide accumulation in *Digitalis* species (Gavidia and Pérez-Bermúdez 1997). The cardioactive heterosides concentration was enhanced more than twice in a study with *Digitalis grandiflora* leaves that were sprayed with manganese and molybdenum solution (Letchamo 1986). Spathulenol and caryophyllene oxide were found to have a strong relationship with nutritional balance (S, Ca, Fe) and foliar phenolics in a study of essential oils from *Eugenia uniflora* leaves, whereas production of selenin epoxide-1,3,7 (11) trien-8-one was linked to the levels of K, Cu, and Mn (Gershenzon 1984). It has been documented that micronutrients operate as enzymatic factors in the manufacture of secondary metabolites, e. g. in sesquiterpene synthase, which uses divalent metallic ions as cofactors (Gershenzon 1984). With the exception of sulfur and nitrogen deficiencies, nutritional stress induces a rise in the accumulation of secondary metabolites (Akula and Ravishankar 2011).

Copper ions have a stimulatory influence on plant SM synthesis, showing a positive impact on alkaloid production, and synthesis of shikonin (Mizukami et al. 1977), digitalin (Ohlsson and Berglund 1989) and betalains (Trejo-Tapia et al. 2001). In growth media, Cu^{2+} is essential for regulation of various physiological and biochemical pathways but in higher concentration it shows toxic effect (Narula

Table 2 Effect of micronutrients on plant secondary metabolites

Micronutrients	Plants	Modulated secondary metabolites	Key references
Iron	*Brassica napus* L.	Ascorbic acid	Tewari et al. (2013)
Iron	*Prunus domestica* L.	Asparagine, glutamine	Jiménez et al. (2011)
Cupper	*Papaver* L., *Vitis* L.	Carotenoids	Cambrollé et al. (2011)
Cupper	*Brassica nigra* L. *Oryza sativa* L.	Ascorbate	Feigl et al. (2013)
Zinc	*Solanum lycopersicum* L.	Carotenoids	Cherif et al. (2011)
Zinc	*Beta vulgaris* L.	Isocitrate dehydrogenase, Malate dehydrogenase	Sagardoy et al. (2011)
Boron	*Nicotiana tabacum* L.	Glucose, Fructose, Organic acids, Phenols	Beato et al. (2011)
Boron	*Citrus sinensis* (L.) Osbeck.	Carotenoids, Saccharose	Han et al. (2008)
Boron	*Daucus carota* L.	Ascorbic acid	Eraslan et al. (2007)
Molybdenum	*Glycyrrhiza uralensis* Fisch. Ex DC.	Glycyrrhizic acid, Squalene	Wang et al. (2013)
Manganese	*Trifolium* L.	Glutathione peroxidase	Dorling et al. (2011)
Manganese	*Vitis* L.	Polyphenol oxidase, Catalase	Mou et al. (2011)

et al. 2005). As a result, it's critical to keep the dosage of copper ions consistent throughout the plant's maturation period and to keep the amount of copper ions released into the environment to a minimum, which can be accomplished through encapsulation, which is an appropriate way of delivering nutrients for ecological balance and sustainable plant production (Vinceković et al. 2016; Vinceković et al. 2017; Jurić et al. 2019).

5 Ionic Balance Is a Tool to Increase Secondary Metabolites in Plants

The effects of ion balance on plant biomass and alkaloid yield in various plants, such as *Catharanthus roseus*, has revealed that ionic interactions between macro-elements impacted biomass production and alkaloid yield in differing aspects (Naeem et al. 2017). The culture medium rich in NO_3^- and K^+ had the highest biomass production, while the culture medium rich in SO_4^{-2} and K^+ gave the highest hyoscyamine yield (Sikuli and Demeyer 1997). K^+ had a positive role the biomass and secondary metabolites in *Arabidopsis* (Armengaud et al. 2004). P deficiency reduces secondary metabolites accumulation in *Salvia officinalis* (Nell et al. 2009). Application of N: P: K (1: 0.6: 1.2) fertilizer reduced flavonoid concentration while increasing the polyphenol content in *Berberis microphylla* (Arena et al. 2017). With increase in the amount of fertilizer, the antioxidant activities increased slightly (Arena et al. 2017). However, while N fertilization enhanced yield, particularly in *Hyoscyamus* sp., imbalanced fertilization with other nutrients decreased yield, or if there was a rise, it was small (Mahajan and Gupta 2009). A balanced nutrition in terms of N, P and K is critical for medicinal plants' growth and development; overfertilization with mineral nutrients (except for N in the form of nitrate) might be damaging to plants. Depending on the plant species, mineral nutrition may have specific impact on alkaloid production (Poutaraud and Girardin 2005). High Co application rates reduced the yield of all plant parts and the alkaloid content of leaves in *Datura stramonium*, while the alkaloid content of roots increased (Alinejad et al. 2020). The maximum plant galanthamine concentration was found in *Leucojum aestivum*, when the soil was dressed with a strong supply of N, K, Mg, B, Mo, Zn, Fe, and Cu (Gorinova et al. 1993). Petrishek et al. (1983) showed a link between Co supply, Co content in the plant, and the tropane alkaloid synthesis and accumulation in *Atropa belladonna*. In *Lupinus hartwegii*, the addition of Fe and Mn, as well as their combination, enhanced the overall concentration of seed alkaloids (Hassan 2012). Seed content (SEC) of meadow saffron (*Colchicum autumnale*) was examined for different alkaloids and a highly significant relation was observed between (Ca + Co) in seeds and alkaloid content, showing for the first time a positive influence of these mineral elements on the alkaloid synthesis in *Colchicum autumnale* (Poutaraud and Girardin 2005). In a study of four mineral elements (Ca, Fe, Co, and Mn) potential on alkaloid production, the contents of some mineral elements in the soil and the seed were found to be linear, especially in the case of Co and Ca (Poutaraud and Girardin 2005). Further, the seed content of Co + Ca and the seed content of alkaloids were highly correlated. Seeds are involved in the biosynthesis of colchicine and colchicoside alkaloids, with Co and Ca playing a key part in the control of these alkaloids.

6 Influence of Plant Growth Regulators on Secondary Metabolites

Plant growth regulators (PGRs) have been utilized as effective inducers in the stimulation of SM biosynthesis in plants (Table 3). Plant growth regulators include both naturally occurring (phytohormones) and synthesized analogues of these hormones (Basra 2000). Julian von Sachs proposed the idea of phytohormones at the late nineteenth century, describing them as mobile endogenous chemicals (Spartz and Gray 2008). Plant growth regulators are small substances that have a particular impact on the development of plant and can work at small concentrations (Nambara

Table 3 Regulation of plant secondary metabolites through plant growth regulators

Plant growth regulators	Plants	Modulated secondary metabolites	Key references
Salicylic acid	*Panax ginseng* CA Mey., *Glycyrrhiza glabra* L.	Saponin	Ali et al. (2006)
Salicylic acid	*Artemisia annua* L., *Trachyspermum ammi* (L.) Sprague ex Turrill	Carotenoids	Aftab et al. (2010)
Salicylic acid	*Centella asiatica* (L.) Urban	Centellosides	Bonfill et al. (2011)
Salicylic acid	*Taxus chinensis* (Rehder & EHWilson) Rehder	Paclitaxel	Wang et al. (2007)
Gibberellic acid	*Salvia officinalis* L.	Cineole	Schmiderer et al. (2010)
Gibberellic acid	*Artemisia annua* L.	Artemisinin	Aftab et al. (2010)
Gibberellic acid	*Salvia miltiorrhiza* Bunge	Tanshinone	Yuan et al. (2008a)
Gibberellic acid	*Cinchona scciruba* Pav. ex Klotzsch	Resveratrol, Anthraquinone	Khouri et al. (1986)
Gibberellic acid	*Hypericum perforatum* L.	Hypericin	Hohtola et al. (2005)
Gibberellic acid	*Echinacea purpurea* (L.) Moench	Caftaric acid	Jones et al. (2009)
Gibberellic acid	*Digitalis lanata* Ehrh.	Anthocyanin	Ohlsson and Björk (1988)
Gibberellic acid	*Vitis rotundifolia* Michx.	Anthocyanin	Ohlsson and Björk (1988)
Gibberellic acid	*Fagopyrum esculentum* Moench	Flavonol	Trotin et al. (1993)
Gibberellic acid	*Capsicum annuum* L.	Capsaicin	Varindra et al. (2000)
Methyl jasmonate	*Gypsophila paniculate* L., *Medicago truncatula* Gaertn.	Saponin	Fulcheri et al. (1998)
Methyl jasmonate	*Silybum marianum* (L.) Gaertn.	Silymarins	Gabr et al. (2016)
Methyl jasmonate	*Vaccinium pahalae* Skottsb.	Anthocyanin	Fang et al. (1999)
Methyl jasmonate	*Panax ginseng* CA Mey.	Ginsenosides	Thanh et al. (2005)
Methyl jasmonate	*Taxus cuspidata* Siebold & Zucc.	Paclitaxel	Patil et al. (2014)
Methyl jasmonate	*Artemisia annua* L.	Artemisinin	Baldi and Dixit (2008)
Cytokinins	*Mentha spicata* L.	Cineole	Stoeva and Iliev (1997)
Cytokinins	*Eucomis autumnalis* (Mill.) Chitt.	Iridoids	Stoeva and Iliev (1997)

(continued)

Table 3 (continued)

Plant growth regulators	Plants	Modulated secondary metabolites	Key references
Cytokinins	*Artemisia annua* L.	Artemisinin	Sa et al. (2001)
Cytokinins	*Rubia akane* Nakai	Anthraquinone	Jin et al. (1999)
Indole acetic acid	*Rauvolfia serpentina* (L.) Benth. ex Kurz	Serpentine	Salma et al. (2008a)
Indole acetic acid	*Datura stramonium* L.	Hyocyamine	Hilton and Rhodes (1993)
Indole acetic acid	*Withania somnifera* (L.) Dunal	Withanoloid	Murthy et al. (2008)
Indole acetic acid	*Fagopyrum esculentum* Moench	Flavonol	Trotin et al. (1993)
Indole acetic acid	*Salvia officinalis* L.	Carnosic acid	Grzegorczyk and Wysokinska (2010)
Indole acetic acid	*Centranthes ruber* (L.) DC.	Valepotriates	Gränicher et al. (1995)
Abscisic acid	*Salvia miltiorrhiza* Bunge	Tanshinone	Yuan et al. (2008a)
Abscisic acid	*Lactuca sativa* L.	Carotenoids	Li et al. (2010)
Abscisic acid	*Lactuca sativa* L.	Anthocyanin	Li et al. (2010)
6-Benzylaminopurine, benzyl adenine	*Centella asiatica* (L) Urban	Asiaticoside	Kim et al. (2004)
6-Benzylaminopurine, benzyl adenine	*Ammi majus* L.	Umbelliferone	Królicka et al. (2006)
6-Benzylaminopurine, benzyl adenine	*Vitis vinifera* L.	Anthocyanin	Qu et al. (2006)
6-Benzylaminopurine, benzyl adenine	*Pluchea lanceolata* (DC.) CB Clarke	Quercetin	Arya et al. (2008)
6-Benzylaminopurine, benzyl adenine	*Coscinium fenustratum* (Goetgh.) Colebr	Berberin	Khan et al. (2008)
6-Benzylaminopurine, benzyl adenine	*Vinca major* L.	Vincamine	Tanaka et al. (1995)
6-Benzylaminopurine, benzyl adenine	*Rauvolfia serpentina* (L.) Benth. ex Kurz	Serpentine	Salma et al. (2008b)
Naphthalene acetic acid	*Astragalus mongholicus* Bunge	Cycloartane	Ionkova et al. (1997)
Naphthalene acetic acid	*Hypericum perforatum* L.	Hypericin	Hohtola et al. (2005)
Naphthalene acetic acid	*Frangula alnus* Mill.	Anthraquinone	Kovačević and Grubišić (2005)
Naphthalene acetic acid	*Cruciata glabra* (L.) Opiz	Anthraquinone	Dörnenburg and Knorr (1996)
Naphthalene acetic acid	*Crataegus×sinaica* Boiss.	Anthocyanin	Maharik et al. (2009)

(continued)

Table 3 (continued)

Plant growth regulators	Plants	Modulated secondary metabolites	Key references
Naphthalene acetic acid	*Camellia chinensis* (L.) Kuntze	Flavonol	Nikolaeva et al. (2009)
Naphthalene acetic acid	*Fabiana imbricata* Ruiz & Pav.	Rutin	Schmeda-Hirschmann et al. (2004)
Naphthalene acetic acid	*Pluchea lanceolata* (D.C.) CB Clarke	Quercetin	Arya et al. (2008)
Naphthalene acetic acid	*Nicotiana tabacum* L.	Nicotine	Mantell et al. (1983)
2,4-Dichlorophenoxyacetic acid	*Citrus* L. species	Limonin	Barthe et al. (1987)
2,4-Dichlorophenoxyacetic acid	*Taxus* L. species	Taxol	Wang et al. (2001)
2,4-Dichlorophenoxyacetic acid	*Citrullus colocynthis* (L.) Schrad.	Cucurbitacin-E, Cucurbitacins	Hegazy et al. (2010)
2,4-Dichlorophenoxyacetic acid	*Dioscorea deltoidei* Wall. ex Griseb. *Diocorea doryophora* Hance	Diosgenin	Huang et al. (1993)
2,4-Dichlorophenoxyacetic acid	*Gymnema sylvestre* R. Br.	Gymnemic acid	Ahmed et al. (2009)
2,4-Dichlorophenoxyacetic acid	*Centella asiatica* (L.) Urban	Asiaticoside	Kim et al. (2007)
2,4-Dichlorophenoxyacetic acid	*Agastache rugosa* (Fisch. & CA Mey.) Kuntze	Rosmarinic acid	Lee et al. (2007)

and Marion-Poll 2005; Teale et al. 2006). Synthetic PGRs have long been recognized as valuable research tools for elucidating plant physiological responses and delving into the biochemical regulatory mechanisms. Agriculture has been amply utilizing, since the 1940s, the natural and synthetic PGRs to influence the plant developmental processes such as seed germination, vegetative reproduction, growth, maturity, senescence, etc. (Basra 2000). PGRs are known to control plant secondary metabolite synthesis in plant tissue culture, in addition to controlling the antioxidant capacity, and plant growth (Dörnenburg and Knorr 1995). The plant's response to PGRs might differ according to species, plant age, variety, environment factors, developmental stage, nutritional and physiological condition, and the endogenous hormone levels of the plant (Thomas and Bohnert 1993; Gaspar et al. 1996; Aftab et al. 2011; Li et al. 2020).

6.1 Auxin and Cytokinin

The effects of several plant growth regulators on secondary metabolite synthesis have been studied and verified in large number of plants (Shilpashree and Rai 2009; Bienaimé et al. 2015). The PGRs introduced to the medium for the initiation of callus of *Hypericum triquetrifolium*, were shown to be responsible for the existence of active compounds in cultured cells at greater levels through optimization of cultural conditions (Azeez and Ibrahim 2014). PGRs induce cell proliferation and division, which leads to an increase in SM synthesis (Karalija and Paric 2011). PGRs are effective in stimulating SM synthesis in *Saintpaulia ionantha* and *Hypericum mysorense* and have a substantial influence on SM metabolism (Karakas 2020; Shilpashree and Rai 2009). Benzyl adenine (BA) and naphthalene acetic acid (NAA) were reported to enhance secondary product synthesis in *Phyllanthus acidus* callus cultures (Duangporn and Siripong 2009). Similarly, in *Coscinium fenestratum*, naphthalene acetic acid (NAA) and indole acetic acid (IAA) were found to improve secondary metabolite production in tissue culture, while 2,4-dichlorophenoxyacetic acid (2, 4-D) improved the SM synthesis in *Nicotiana tabacum* (Kuo et al. 2012; Jamwal et al. 2018; Gatz et al. 2017).

Baque et al. (2010) reported that the biomass decreased in *Morinda citrifolia* when IBA (indole 3 butyric acid) and KIN (kinetin) were applied to adventitious roots in combination with thidiazuron (TDZ), but the SM content enhanced; being greater in TDZ (thidiazuron)-treated roots than in kinetin-treated roots (Baque et al. 2010). Similarly, variation in cytokinin accumulation, especially when combined with NAA (equimolar quantities), had a significant impact on SM synthesis in *Aloe arborescens* in *in vitro* plant regeneration (Amoo et al. 2012). Sakakibara et al. (2006) observed that on the one hand, cytokinins inhibit certain macronutrient transporters such as nitrate, sulphate, ammonium, and phosphate, on the other hand, nitrate normalizes the regulation of genes related to SM networks. Grzegorczyk-Karolak et al. (2015) found that 6-benzyl amino purine (BAP) was more effective than thidiazuron in improving shoot rejuvenation and SM synthesis in *Aconitum violaceum*, and in some cases differences in cytokinin concentrations considerably increased SM biosynthesis. Secondary metabolites in *Hypericum perforatum* and *H. sampsonii* seedlings are enhanced by cytokinins (Liu et al. 2007). According to Ibrahim and Jaafar (2013), ABA (abscisic acid) treatment produced oxidative stress in various plants and also stimulated the synthesis of secondary metabolites.

6.2 Jasmonates

The jasmonates, which include MeJA and JA, are a class of cyclopentanone-containing chemicals that play a significant part in plant defense and enhance SM synthesis in *in vitro* cultures. They are an important family of elicitors for numerous secondary plant metabolic pathways, generally demonstrated by the elicitation of SM production when plants are exposed to unusual environmental factors. (Pauwels et al. 2009). When a plant suffers an ironic wound or is attacked by a pathogen, JA is thought to be a signal molecule. JA and its more active derivative MeJA can lead to the formation of various secondary metabolites in cell cultures, including rosmarinic acid, terpenoid indole alkaloid, and plumbagin (Krzyzanowska et al. 2012; Almagro et al. 2014). The synthesis of rosmarinic acid in *Mentha piperita* plants is influenced by the elicitation of JA (Krzyzanowska et al. 2012). JA also affects the synthesis of anthocyanin in *Vitis vinifera* plants and plumbagin chemical in the hairy roots of *Plumbago indica* (Gangopadhyay et al. 2011). MeJA and JA are used as a inducer for the production of stilbene in the *V. vinifera* foliar cultures (Belhadj et al. 2006), *V. vinifera* cell cuttings (Tassoni et al. 2005), and *Vitis rotundifolia* root hair cutting cultures (Nopo-Olazabal et al. 2014). The accumulation of MeJA in *V. vinifera* cell cultures encouraged the production of anthocyanin compound (Tassoni et al. 2012). The use of transgenic expertise of MeJA has an extremely high production of compounds of tanshinones in the *Salvia miltiorrhiza* hairy roots (Hao et al. 2015). Production of withanone, and withanolide-A was also promoted by MeJA in root hair cultures of *Withania somnifera* (Sivanandhan et al. 2013). MeJA increases bacoside A synthesis, an important triterpenoid saponin, which increases activity of nootropic healing in *Bacopa monnieri* in-vitro stem culture (Sharma et al. 2013). The cell culture of *Andrographis paniculate*, showed a high production rate of andrographolide compounds due to MeJA (Sharma et al. 2015). Methyl jasmonate also increases the production of saponin and soyasaponin in *Glycyrrhiza glabra* plants (Hayashi et al. 2003). MeJA also increased the production of paclitaxel in *Taxus canadensis* and *T. cuspidata* plants (Ketchum et al. 1999), and of raspberry ketone benzalacetone in *Rubus idaeus* seedling (Pedapudi et al. 2000). The MeJA treatment enhanced NO generation in *Taxus chinensis* cells, suggesting that MeJA activation is dose-dependent (Wang and Wu 2005). Exogenous application of MeJa to plant cell cultures of a number of species promoted secondary biosynthetic pathways, resulting in increased synthesis of diverse plant secondary metabolites such as alkaloids, terpenoids, flavonoids, and phenylpropanoids (Wasternack and Hause 2013; Narayani and Srivastava 2017). In *Rubia tinctorum* root cultures, CA (caffeic acid) and MeJA treatments significantly increased the SM synthesis (Biçer et al. 2017).

6.3 Salicylic Acid

Salicylic acid (SA), which is widely recognized for inducing systemic acquired resistance in plant responses to a variety of biotic and abiotic stresses (Husen et al. 2018, 2019), may also stimulate the synthesis of secondary metabolites in plants. Using the transgenic technology, SA has considerably increased the synthesis of tanshinone in *Salvia miltiorrhiza* hairy roots (Hao et al. 2015). The higher production of the compounds with anolide A, withaferin A and withanone, was observed in the provoked hairy roots culture of *Withania somnifera* plants (Sivanandhan et al. 2013). In the cell suspension culture of *Vitis vinifera*, SA increases the synthesis of stilbene (Xu et al. 2015). SA also triggers the alkaloids, which include vinblastine in periwinkle and vincristine (Idrees et al. 2011), the tropane alkaloid scopolamine production in the *Brugmansia candida* plant hairy root cultures (Pitta-Alvarez et al. 2000), and production of pilocarpine compound in jaborandi plant leaves (Avancini et al. 2003). SA treatment in *Rubia cordifolia* plants enhances the manufacturing of anthraquinone (Bulgakov et al. 2002). Terpenoid base secondary metabolism is also affected due to SA treatment. SA initiates the gathering of the triterpenoids ginsenosides compounds in ginseng plant and produces glycyrrhizin in licorice plant (Chumley et al. 2006; Shabani et al. 2009). SA also enhances monoterpene production during suitable conditions (Xu et al. 2012). Despite the fact that salicylic acid, methyl jasmonate, and jasmonate may all stimulate plant secondary metabolite biosynthesis via different signaling pathways (Zhao et al. 2005), they all work in conjunction with NO (nitric oxide) to facilitate the SM biosynthesis. In tissue and cell cultures of medicinal plants, NO has been shown to play an important role in the SM synthesis elicited by inducers (Jamwal et al. 2018).

6.4 Gibberellic Acid

A phytohormone Gibberellin (GA), is also used as an active enhancer in the secondary metabolites production (Liang et al. 2013). Gibberellic acid (GA_3) is a diterpenoid that is produced in the same route as artemisinin (Bertea et al. 2005; Liu et al. 2005). The use of GA_3 as an elicitor has been studied in a number of ways. In hairy root cultures of *Echinacea pupurea* and *Salvia miltiorrhiza*, GA_3 was utilized to boost the synthesis of caffeic acid derivatives and tanshinones (Abbasi et al. 2012; Yuan et al. 2008b). In another work, gibberellic acid was employed as an elicitor to increase the production of polyphenolics (flavonoids and phenolics) in suspension cells of *Artemisia annua* (Banyai et al. 2011). Furthermore, in *Chicorium intybus*, GA_3 has been found to induce hairy root development in a variety of species with varying levels of SM synthesis (Bais et al. 2001).

7 Conclusion

Plants are constantly exposed to adverse environmental condition which has impacted their growth, diversity and survival. In order to reduce the effect of certain environmental stresses, plants produce varied types of secondary metabolites, which act as a defensive candidate during the environmental stress. Plants exposed to various elicitors or signal molecules typically accumulate metabolites and have a key role in adaptation, as well as for medicinal, nutritional, and aesthetic functions. Various elicitors have been used by researchers to increase the SM synthesis in plant cell, tissue, and organ cultures. In this chapter, we have discussed the impact of PGRs, micronutrients (Fe, Cu, Mn, B, Cl) and macronutrients (N, P, K, Ca, Mg, S) mineral elements on the SM biosynthesis and distribution in plants. Supplementation of plant mineral nutrients has the potential effect on the SM content and promotes seedling growth. Plant growth regulators are great inducers and stimulators of plant secondary metabolites. They are known to control the SM synthesis in plant tissue cultures, in addition to controlling antioxidant capacity, and plant growth. The effect of growth regulators on SM induction varies from species to species, and with plant-growth stage and environmental factor. Thus, it is evident that the application of PGRs and nutrients during stress condition induces the SM biosynthesis of in plants and thus enable the plants to cope with the environmental insult.

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Impact of Phytoprotectants on Growth and Yield of Medicinal Plants Under Abiotic Stress

Muhittin Kulak and Canan Gulmez

Abstract Due to their sessile nature, land plants cannot move and remain continuously exposed to biotic and abiotic stress factors, which, in turn, cause critical changes in the primary and secondary metabolisms of plants. Disturbances in the metabolism are, in general, manifested in retarded and reduced plant growth and development. In order to reduce the adverse effects of the stress, phytoprotectants are widely employed. In this context, phytoprotectants such as polyamines (putrescine, spermidine, and spermine), amino acids, sugars, proline, glycine betaine, trehalose, sorbitol and ectoine, salicylic acid, melatonin, abscisic acid, auxin, cytokinin, brassinosteroid, jasmonate and gibberellin, nitric oxide, hydrogen peroxide, and trace elements have been frequently used. However, this chapter focuses on the effects of merely polyamines, melatonin, salicylic acid, beta-carotene and tryptophan on the secondary metabolites and other parameters of medicinal plants. Exogenous applications of phyto-protectants cause substantial improvements in physiological and biochemical attributes of the plants, which leads to enhanced crop productivity. In medicinal plants, secondary metabolites are the main point of interest since they are related to the pharmacological and health-promoting potential of medicinal plants. Application of phytoprotectants causes critical changes in metabolites, and the extracts of the treated plants are then tested in vitro, for their pharmacological and health-promoting potential. Surprisingly, in this context, no in vivo trials have hitherto been carried out; this deserves serious attention in view of the significant differences between in vitro and in vivo approaches.

Keywords Phenolic compounds · Terpenoids · Priming · Secondary metabolites

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A. Husen, M. Iqbal (eds.), *Medicinal Plants*,
https://doi.org/10.1007/978-981-19-5611-9_12

1 Introduction

Plants are sessile organisms and, therefore, cannot escape from the environmental conditions of their habitat, which might cause *stress* for plants, either biotic or abiotic in nature. Abiotic stressors such as salinity, water stress (drought), cold, high temperature or high/low light, and pathogen attack, either individual or combined, substantially limit the plant growth and yield (Pardo-Hernández et al. 2020). Lichtenthaler (1998) divided 'stress' into (a) *eu-stress*, which is an activating and stimulating stress that has positive effects on plant growth and productivity, and (b) *dis-stress*, which critically retards the plant growth and performance. Plants have evolved a great elaborate system to cope with the dis-stress (Seki et al. 2007).

The abiotic stress is a prominent and increasing problem in agricultural lands. After a basic search on Web of Science using the term "abiotic stress" with reference to "plant sciences" 19,809 results from Web of Science Core Collection were recorded on 24th April, 2022. In spite of very many researches, the mechanisms or regulatory networks in plant systems are yet to be fully elucidated. Effects of stress on different crop and non-crop plants are stress-dose (severity), stress frequency, stress duration, stress-timing as well as plant species dependent (Ashraf et al. 2018). Because of the large number of dependent and independent variables, plant responses against the relevant stress factors are not uniform, suggesting that the stress studies are likely continue for some time yet.

Morphological and anatomical modifications of plants under abiotic stress may be clearly observed in plant phenotypes (Iqbal et al. 1987a, b). Also, the manifested/observed modifications in phenotypes may be linked to the tolerance capacity of the respective plants against the given stress. Mechanisms of plant responses to stress involve physiological, biochemical and metabolic processes at genetic and molecular levels (Anjum et al. 2017; Husen et al. 1999; Iqbal et al. 1996; Husen 2021a, b, c). Quite a large number of studies have reported the damage and tolerance levels in different plant types, including the species of medicinal importance (Beck et al. 2007; Iqbal et al. 2000a, b; Jaleel et al. 2009; Husen 2022a).

Critical modifications in plant metabolism such as changes in water relations, inhibited cellular activities, excessive accumulation of free radicals, impaired photosynthesis activities, and increased membrane leakage, etc., have been reported to occur due to stress in quite a large number of plant species. As a response to the stress, plants have evolved and adopted vital strategies for their survival. Inducing the biosynthesis of compatible solutes (i.e., proline and glycine betaine), adjusting the osmotic pressure, activating the enzymatic (superoxide dismutase, peroxidase, catalase, and ascorbate peroxidase, etc.) and non-enzymatic antioxidant system (vitamin C, vitamin E, phenolics, carotenoids, and glutathione) are some of the well-known strategies employed by plants for mitigating the adverse impacts of the stress (Aref et al. 2016; Hatami et al. 2017; Imran et al. 2021; Koyro et al. 2012; Mohammadi et al. 2018; Zhang et al. 2021).

2 Medicinal Plants as Specialised and Reputed Species

The plant kingdom is presented with an estimated number of 400,000 species and plants have been used for various purposes since pre-historic times. Considering the pharmacological and health-promoting interests, 20% of the plant kingdom (equivalent to 50,000–80,000) is deemed as specialised and reputed plant species due to their secondary rather than primary metabolites. Primary and secondary metabolites exhibit significant diversities according to their chemical structure, function and compartmentation in plants (Jan et al. 2021). Regarding their functions, the primary metabolites, such as carbohydrates, lipids, and proteins are strictly required and actively involved in plant growth and development, whilst the secondary metabolites are multifaceted compounds, which are involved in plant defence and its communication with the environment. Specifically, the chemical diversity of the secondary metabolism clearly confers an array of adaptive advantages to plants. Some secondary metabolites are confined to specific plant taxa and, for that reason, could be recognised as a chemical fingerprint or signature for those taxa (Ncube and Van Staden 2015; Noel et al. 2005; Gulmez and Kulak 2021). The secondary metabolites are basically classified into the sub-groups such as nitrogen-containing metabolites (alkaloids) and nitrogen-deficient metabolites (terpenoids and phenolics) (Fig. 1) (Ncube and Van Staden (2015). The yield and composition of relevant metabolites are the significant indicators for their potential uses in pharmacological and health-promoting practices (Gulmez and Kulak 2021). Relevant genes and enzymes involved in plant secondary metabolisms have been identified and subsequently contributed to our understanding of metabolic pathways involved in biosynthesis of secondary metabolites. As presented in Fig. 1, the secondary metabolites are biosynthesised though a series of coupled enzymatic conversions (Ncube and Van Staden 2015). The precursors required for secondary metabolites are produced in Krebs cycle and shikimate pathways and the primary metabolites are the critical initiator in biosynthesis of secondary metabolites. However, along with the tailoring of enzymes and the responsive molecular changes, alterations in the synthesised secondary metabolites as consequences of glycosylation, methylation, hydroxylation, acylation, oxidation, phosphorylation, and prenylation might come true (Jan et al. 2021).

3 What Is Preferable: Breeding or Use of Phytoprotectants?

In order to enhance the performance and crop productivity of plants against stress conditions, two main strategies viz. breeding and use of exogenous chemicals have been widely employed. However, tolerance mechanisms against biotic and abiotic stress are markedly distinct. The tolerance against biotic stress is associated with monogenic traits, whereas responses against abiotic stress factors are multigenic and more sophisticated. For that reason, breeding regarding plant tolerance against a

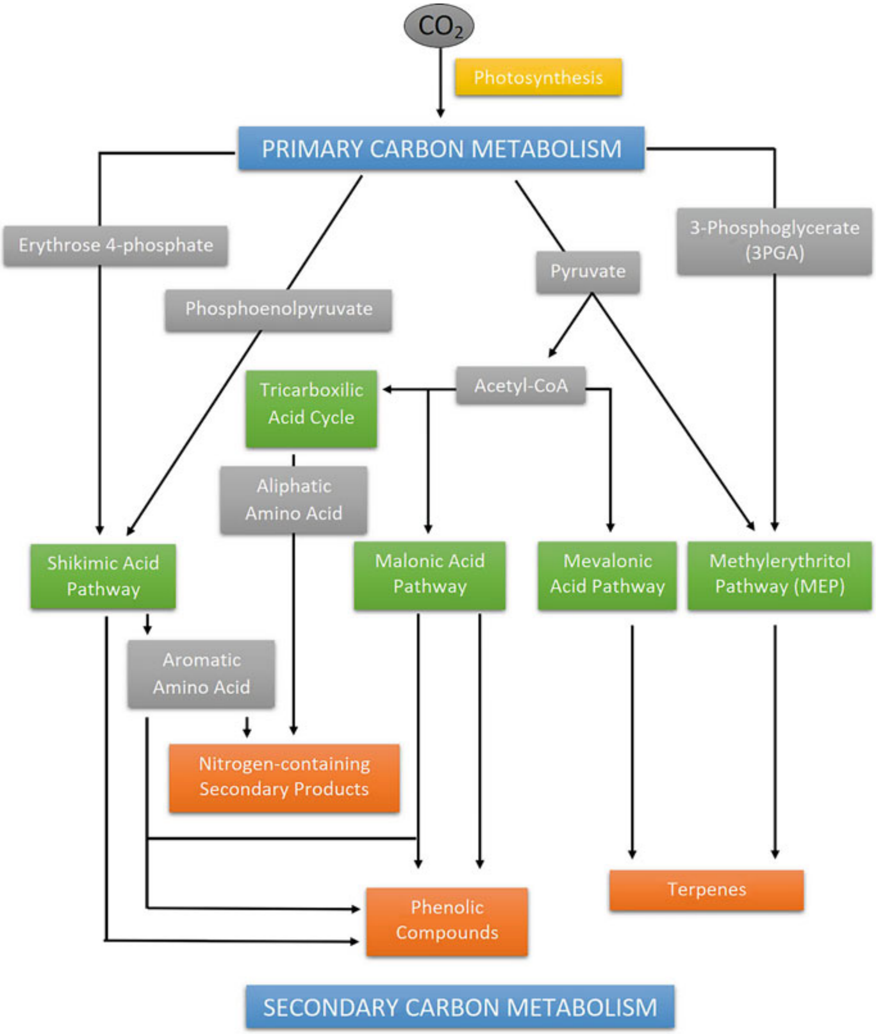

Fig. 1 The biosynthetic pathways of secondary metabolites [based Ncube and Van Staden (2015), with slight modification]

pathogen might be feasible but the same approach might not be preferable for enhancing tolerance against an abiotic stress factor (Wang et al. 2003; Wyenandt et al. 2010; Borsai et al. 2018).

Of the adopted strategies to combat with stress, plants synthesize compatible soluble phyto-protectants for adjusting their osmotic pressure. Critical fluctuations in biosynthesis of metabolites such as polyamines (putrescine, spermidine, and spermine), amino acids, sugars, proline, glycine betaine, trehalose, sorbitol and ectoine, salicylic acid, melatonin, abscisic acid, auxin, cytokinins, brassinosteroids, jasmonates and gibberellins, nitric oxide, and hydrogen peroxide have been observed as a response to the environmental stimuli. Finally, the adjusted osmotic pressure via phyto-protectants has been hypothetically linked to plant tolerance. In this context, production of metabolites (phyto-protectants) might likely contribute to flexibility and tolerance against stress factors. The protective roles of the phyto-protectants have been clearly confirmed but their modus operandi against stresses is not still completely clear possibly because of differences in plant species used, type of phyto-protectants, and their concentration, timing and mode of treatments.

4 Impact of Phytoprotectant Application on Medicinal Plants

4.1 Polyamines (Putrescine, Spermidine, and Spermine)

Polyamines are low-molecular weight poly cations found ubiquitously in all organisms. They have variable hydrocarbon-chains and two or more primary amino groups. A plethora of functions have been attributed to polyamines in plants. Of them, diamine putrescine (*Put*), triamine spermidine (*Spd*) and tetramine spermine (*Spm*) are the most common forms distributed in plant cells. Polyamines are presumed to modulate and interact with other biomolecules such as hormones, signalling molecule and amino acids, which in turn help plants in adjusting the physiological and biochemical responses for regular development and stress adaptation processes (Takahashi and Kakehi 2010; Rahman et al. 2017; Antoniou et al. 2021).

In several crop plants, increased endogenous levels of polyamines were observed by either exogenous treatments of polyamines or transgenic modifications under abiotic stress conditions (Kaur-Sawhney et al. 2003; Martin-Tanguy 2001). Out of the major polyamines, putrescine and water stress interaction caused significant modifications in biochemical and physiological attributes of *Salvia officinalis* L. (Mohammadi-Cheraghabadi et al. 2021). In this study, different concentrations of putrescine and water deficit stress levels were assayed and compared. Accordingly, applications of putrescine substantially decreased the adverse effects of water stress through modulations including increases in relative water content and leaf area index, decreases in compatible osmolytes and H_2O_2 contents and enhanced activities of antioxidant enzymes, such as CAT and APX, in *Salvia officinalis*. In particular, independently of water irrigation levels, endogenous concentration of putrescine increased with the increases in concentration of exogenous application of putrescine. Thus, putrescine acted as a stress-protective agent. Of the secondary metabolites, the highest contents of 1,8-cineole, camphor, α-thujone, β-thujone, cineole synthase, sabinene synthase, and bornyl diphosphate synthase were observed at the interaction of 80% depletion of available soil water and 0.75 mM putrescine treatment (Mohammadi-Cheraghabadi et al. 2021).

In *Stevia rebaudiana*, Bertoni subjected to salinity and drought stress, application of putrescine decreased the adverse effects of drought stress, as manifested by improvements in morphological and yield parameters (Pradhan et al. 2020). These improvements might be consequences of higher contents of photosynthetic pigments and anthocyanin as well as the regulation of stress indexes and related antioxidant enzymes (Gerami et al. 2019). In addition to the notable improvements in agro-morphological traits, high levels of stevioside and rebaudioside were also observed (Pradhan et al. 2020).

In sweet basil (*Ocimum basilicum* L.), the effects of three major polyamines (spermine, spermidine and putrescine) on essential oil composition were examined. Accordingly, spermine caused significant increases of linalool contents (between 40.4 and 56.8%) but depending on the concentrations, higher contents of 1,8-cineole content (between 9.6 and 17.8%) were recorded with spermidine treatments (Karaman et al. 2008).

Drought stress adversely affected biochemical attributes of *Valeriana officinalis* L., but the adverse effects were alleviated with foliar application of polyamines (putrescine, spermidine, and spermine) which provoked the antioxidant enzymes and triggered accumulation of proline. The essential oil yield was increased under drought stress and the exogenous application of polyamines did not exhibit any significant effects on the essential oil content (Mustafavi et al. 2016). The enhanced agronomic productivity of other medicinal plants due to polyamine application is also on record (Bakhtari et al. 2016; Mohammadi et al. 2017; Soroori et al. 2021).

4.2 Melatonin and Tryptophan

Melatonin (N-acetyl-5-methoxy-tryptamine) is a pleiotropic molecule found ubiquitously in living organisms. It was first discovered in bovine pineal gland in 1958 (Lerner et al. 1958) and then in plants in 1995 (Dubbels et al. 1995) (cited in Pardo-Hernández et al. 2020). The molecule is an indolic compound derived from tryptophan (Ke et al. 2018), exhibiting innumerable links with germination, circadian rhythms and senescence in plants (Arnao and Hernández-Ruiz 2015; Hernández-Ruiz and Arnao 2018). In particular, significant roles of melatonin in coping with abiotic stress conditions have been reported for quite a large number of crop plants (Zhang et al. 2015; Wei et al. 2015; Kaur et al. 2015; Zhao et al. 2022; Rehaman et al. 2021; Arnao et al. 2021; Shafi et al. 2021; Jahan et al. 2021). Priming with melatonin decreased the adverse impacts of high level of salinity in various medicinal plants. Also, melatonin-treated samples exhibited high antioxidant activities in sweet basil. The enhanced antioxidant activity was attributed to the modifications of essential oil and phenolic composition. The highest level of the secondary metabolites was observed at melatonin and salt stress interaction (Bahcesular et al. 2020). Foliar application of melatonin substantially improved glutathione content, redox state and essential oil production of *Salvia nemorosa* L. and *Salvia reuterana* Boiss under limited water supply (Bidabadi et al. 2020). Melatonin treatment enhanced the activities of antioxidant enzymes (GT, CAT, POD, SOD and GR) and reduced the oxidized glutathione (GSSG). As regards the essential oil compounds, β-caryophyllene, germacrene-B, spathulenol, and cis-β-farnesene, while (E) β-ocimene, α-gurjnnene, germacrene-D, hexyl acetate, and aromadendrene were the major compounds found in *Salvia* species. The composition and concentration of the relevant essential oil compounds were increased by melatonin treatment (Bidabadi et al. 2020). Similarly, melatonin increased the tolerance of *Mentha*× *piperita* and *Mentha arvensis* L. against heat stress by regulating the activities of antioxidant enzymes. Specifically, heat stress reduced the essential oil yield in both species but melatonin treatment increased it. Melatonin treatment increased the oxygenated monoterpenes also. In particular, heat stress decreased the menthol content, a dominant metabolite, whereas melatonin treatment significantly increased it (Haydari et al. 2019).

Likewise, melatonin treatment contributed significantly to the physiological and biochemical performance of lemon verbena (*Lippia citriodora* Kunth), an important medicinal shrub, under drought stress (Hosseini et al. 2021). Improvements were observed with the accumulation of proline, soluble sugars and abscisic acid, which then contributed to the adjustments in leaf water status. Similarly, the oxidative damage was alleviated with the enhanced antioxidant activities of enzymes and production of other non-enzymatic compounds. Among the specific phenolic compounds, higher content of chicoric acid, caffeic acid and chlorogenic acid were recorded with melatonin treatment. The authors postulated that accumulation of secondary metabolites might contribute to the antioxidant defence of the plants under drought stress (Hosseini et al. 2021).

Tryptophan is the precursor of melatonin and is an essential amino acid ubiquitously found in plants, animals and bacteria. Tryptophan has been relatively little studied, but its stimulatory roles on germination and growth are well known (Hanci 2019; Kahveci et al. 2021). In the case of sweet basil subjected to the salt stress, tryptophan significantly improved the photosynthetic gas exchange parameters and secondary metabolism (production of phenolics, flavonoids) in plants. In a study of tryptophan, salicylic acid and β-carotene tryptophan has been found to be the more effective molecule than salicylic acid and β-carotene (Kahveci et al. 2021).

4.3 Salicylic Acid and β-Carotene

Salicylic acid (SA) or ortho-hydroxybenzoic acid belonging to the phenolic groups of plants has been widely applied on plants under stress (Kulak et al. 2019). Depending on concentration, timing and mode of application of salicylic acid, innumerable functions related to ion accumulation, photosynthesis-related parameters, stomatal adjustments, enzymatic and non-enzymatic antioxidant system, flowering, senescence, etc. in plants have been reported to be affected (Hayat et al. 2008; Dianat et al. 2016; Husen et al. 2018, 2019). In the case of lemon verbena (*Lippa citriodora*), low water supply increased the sugar and proline contents, as well as antioxidant enzymes (superoxide dismutase, catalase and peroxidase) activities and content of essential oil. Foliar application of salicylic acid increased the plant acclimation and performance under water deficit condition and improved the physiological parameters and essential oil yield. These alterations were hypothesised to be linked to the preservation of membrane structure of the cell and proteins against the potential effects of reactive oxygen species (Dianat et al. 2016).

For sweet basil exposed to water stress, priming with salicylic acid improved the plant growth, development and productivity through modulation of relative water content, water potential, quantum yield, shoot height, stem diameters, leaf dry weight, branch number as well as leaf number. In addition, significant modifications were noted in macro/micro element contents of leaves in SA-primed basil plants. No significant changes were noted in essential oil composition (1,8-cineol, linalool, eugenol and α-bergamotene), but both water deficit and salicylic acid yielded high levels of phenolics, which were then reflected into the higher antioxidant activities (Kulak et al. 2021).

Kahveci et al. (2021) examined the impact of salicylic acid in basil plants exposed to salt stress. Contrary to the former report, priming with salicylic acid did not contribute significantly to the physiological parameters such as photosynthetic pigments, assimilation rate and transpiration rate in sweet basil subjected to the salt stress. However, stomatal conductance was positively modulated with SA priming. Of the essential oil compounds, linalool, methyl eugenol and eugenol were significantly modified by SA treatment (Kahveci et al. 2021).

The protective potential of salicylic acid against stress conditions has been tested in numerous plant species, such as *Thymus daenensis* Celak subsp. *daenensis* Celak (Pirbalouti et al. 2014), *Thymus kotschyanus* Boiss. & Hohen (Mohammadi et al. 2019), *Thymus vulgaris* L. (Khalil et al. 2018), *Mentha*× *piperita* and *Mentha arvensis* L. (Haydari et al. 2019), *Salvia officinalis* L. (Es-sbihi et al. 2020a, b), *Nigella sativa* L. (Rezaei Chiyaneh and Pirzad 2014), *Brassica carinata* A. Br. (Husen et al. 2018) and *Guizotia abyssinica* (L. f.) Cass. (Husen et al. 2019). In all these studies, the protective role of salicylic acid was found to be associated with the regulation/modulation of photosynthesis apparatus, antioxidant system and/or secondary metabolism of medicinal plants.

As aforementioned, salicylic acid is the most employed molecule against stress. Kulak (2018) performed a bibliometric analysis to reveal the core content of salicylic acid uses in agricultural and biological sciences. Accordingly, the exogenous use of salicylic acid was divided into two major categories (Fig. 2). The first one, dealing primarily with abiotic stress and antioxidant system, can be described as biochemistry of plants under abiotic stress. The second one, related to biotic stress and plant immunity with identification of molecular targets, may be termed as molecular biology under biotic stress.

β-carotene exerts a critical and direct impact on photosynthesis machinery and oxidative stress tolerance. However, its role on secondary metabolism is not fully understood and for that reason, Kahveci et al. (2021) investigated its priming effects on phenolics and essential oil components in sweet basil exposed to salt stress. Accordingly, agronomic traits of basil plants were positively affected. As to the

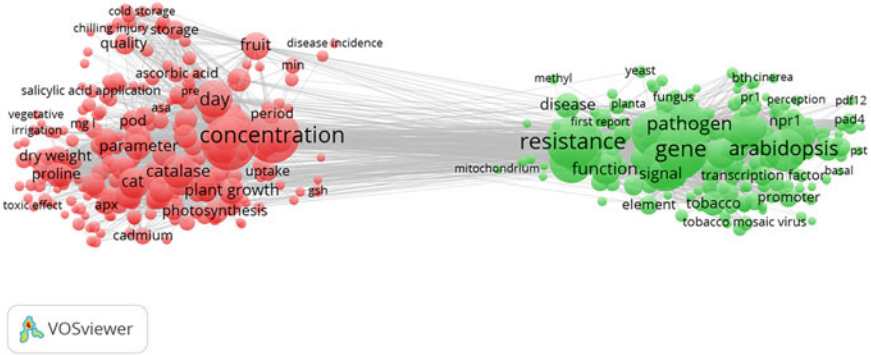

Fig. 2 Common terms retrieved from salicylic acid studies in agricultural and biological fields [according to Kulak (2018), with slight modification]. As uttered in main text of the current study and according to VOS viewer-mediated powerful analysis by Kulak et al. (2019), salicylic acid is the most preferred signal molecule employed for fighting against abiotic stressors. For that reason, due to the higher number of documents, relative to other phytoprotectants, salicylic acid reports were visualized as in the current figure

secondary metabolites, β-carotene significantly modified the essential patterns of the predominant compounds of basil leaves. Major compounds such as caffeic acid, chicoric acid and rosmarinic acid were adversely affected with β-carotene priming. However, those modifications had no significant effect on the DPPH activities of the basil extracts.

Thus, several protective molecules have been tested against drought and salinity impacts on some medicinal plants and their protective roles identified. In general, the relevant molecules were effective in improving the physiological attributes, triggering the antioxidant enzyme activities, as well as reducing the membrane leakage and lipid peroxidation. These alterations must contribute towards improvement in growth and productivity of medicinal plants under abiotic stress (Table 1).

5 Pharmaceutical Value Versus Agronomic Yield of Medicinal Plants

Efficacy of the medicinal plants is directly dependent on the secondary metabolites (SMs) they produce (Li et al. 2020; Soni et al. 2015). In addition, SMs are also considered as the critical indicators of stress conditions (Aftab 2019; Borges et al. 2017; Gouvea et al. 2012). Studies have revealed that the content and composition of these metabolites are relatively sensitive to stressful conditions. In many cases, SM production improves while several growth parameters are hampered by so many stresses, which the plants are exposed to (Singh et al. 2000). The secondary metabolites (phenolics, flavonoids, essential oils) have been examined and quantified with special attention in the stress-exposed medicinal plants. For instance, salt

Table 1 Some phytoprotectants and their effects on medicinal plants under drought and salinity stress

Treatments	Stress	Species	Findings	Authors
Proline	Drought	*Foeniculum vulgare* Mill.	Exogenous proline enhanced the tolerance of fennel plants against drought stress by modulating the osmoregulation, physiological functions and essential oil content	Zali and Ehsanzadeh (2018)
Proline and γ-aminobutyric acid (GABA)	Drought	*Coix lacryma-jobi* L.	Proline was reported to be more efficient than GABA in alleviating the effects of drought stress through maintenance of dynamic dissipation of the photosynthetic energy and accumulation of proline	Ferreira et al. (2020)
Trehalose	Drought	*Trigonella foenum-graecum* L.	Exogenous trehalose led to significant increase in photosynthetic pigments, yield components, carbohydrate, protein, total phenolic, flavonoids, growth parameters and seed yield	Sadak (2016)
Trehalose and 5-aminolevulinic acid	Drought	*Alpinia zerumbet* Pers.	Exogenous applications of trehalose or 5-aminolevulinic acid to stress-submitted plants improved agronomic traits and photosynthetic parameters, triggered the accumulation of glycine betaine and proline, and enhanced the activity of superoxide dismutase and peroxidase	Zulfiqar et al. (2021a)
Trehalose and salicylic acid	Drought	*Ocimum basilicum* L.	Co-application of trehalose and salicylic acid alleviated the adverse impacts of drought stress by reducing the oxidative stress through enhancing the activity of antioxidant enzymes. Also increased the proline and glycine betaine content but decreased malondialdehyde in drought-exposed plants	Zulfiqar et al. (2021b)
Abscisic acid	Drought	*Bupleurum chinense* DC.	Exogenous application of abscisic acid to leaves enhanced the plant tolerance against drought via increasing the soluble protein and sugar contents, and the superoxide dismutase and peroxidase activities	Yang et al. (2020)

(continued)

Table 1 (continued)

Treatments	Stress	Species	Findings	Authors
Brassinosteroid	Salinity	*Mentha piperita* L.	Exogenous brassinosteroid applications alleviated the negative effects of salt stress by enhancing activities of antioxidant enzymes and decreasing the membrane leakage and lipid peroxidation	Çoban and Baydar (2016)
Salicylic acid	Drought	*Verbascum sinuatum* L.	Treatment with salicylic acid reduced the negative effects of drought stress by enhancing the antioxidative potential of the plant	Karamian et al. (2020)
Gibberellic acid	Salinity	*Hibiscus sabdariffa* L.	Application of gibberellic acid enhanced the growth parameters *viz.* length, fresh and dry weights, and leaf area. Also, improved the physiological attributes including relative water content, anthocyanin, and photosynthetic pigments	Ali et al. (2012)
Nitric oxide and salicylic acid	Salinity	*Crocus sativus* L.	Applications of nitric oxide improved the plant growth more than salicylic acid did. It reduced the hazardous effects of stress by regulating the solute accumulation, antioxidant enzyme status and biosynthesis of secondary metabolites	Babaei et al. (2021)
Nitric oxide	Salinity	*Ocimum basilicum* L.	Application of nitric oxide alleviated the adverse impacts of salinity by modulating the physiological attributes and antioxidant system of basil plants	Saeidnejad et al. (2013)
Salicylic acid	Salinity	*Brassica carinata* A.Br.	Foliar application of SA improved the performance of Ethiopian mustard cultivars and mitigated the damage caused by salt stress by modulating the cell redox balance and protecting the plants from oxidative damage.	Husen et al. (2018)
Salicylic acid	Salinity	*Guizotia abyssinica* (L.f.) Cass.	SA application fortified salt-tolerance capacity by alleviating the membrane injury and improving the assimilatory activities, plant water status and defence arsenal, thus reducing the adverse effects of salt stress on foliar functions and plant growth	Husen et al. (2019)

stress modifies the patterns of bioactive compounds that constitute the nutraceuticals, functional foods and cosmetics (Bistgani et al. 2019). It has been noticed that whereas the stresses clearly diminish the agronomic traits of plants (*dis-stress*), the potential metabolites to be used in various industrial applications, especially in the pharmaceutical industry, often receive a positive effect (*eu-stress*) (Ni et al. 2021). The impacts of *dis-stress* on agronomic traits have been studied more intensively in comparison to the *eu-stress* impacts on metabolites. The extracts of stress-submitted plants have been frequently assayed for in vitro antioxidant or anti-microbial activities but in vivo studies on stress-submitted plants (e. g., Arshi et al. 2004, 2006; Ahmad et al. 2007; Ali et al. 2008) are relatively few. It is suggested that the positive relationship between salt stress/salinity and some secondary metabolites may be exploited in the pharmaceutical industry. However, this requires clinical trials to confirm the efficacy of metabolites so produced. Salt stress, especially at 4 g/L $CaCl_2$ salt concentration, increased the total alkaloid and galanthamine level in bulb and leaves of *Leucojum aestivum* L. (Ates et al. 2021). In another study, salt stress increased the phenolic compounds and antioxidant capacity in *Thymus vulgaris* and *T. daenensis.* The cinnamic acid and thymol levels increased in both species under salinity stress. The high salt tolerance exhibited by both species has been associated with the exclusion of sodium ions from the vascular system and its high phenolic content and radical scavenging capacity to protect tissues from salt toxicity (Bistgani et al. 2019). The effects of salt stress have been analysed on the accumulations of 11 chemical components in *Limonium bicolor* (Bag.). Among them, myricitrin (myricetin-3-O-α-L-rhamnoside) showed the highest abundance. It was noted that the CHS gene is sensitive to salt stress and is related to the chemical response induced by NaCl (Wang et al. 2016).

Based on the previous reports, salt stress may also be tested and utilised to increase the production of beneficial compounds in essential oils. Irrigation with saline water increased the essential oil contents and its main components α -cadinol, γ-and Δ-cadinene in *Calendula officinalis* L. (Khalid and da Silva 2010). In our previous study, we investigated the effects of different salt compounds and salinity ratios on *Salvia officinalis* and found that α-pinene and camphor percentages increased under all salt stress but depending on the salt concentration (Kulak et al. 2020). In other study, salinity reduced the percentage of linalool and eugenol, but increased methyl eugenol. It also decreased total phenolics and major individual fractions in *Ocimum basilicum* (Kahveci et al. 2021). Based on the positive increases in the beneficial components or antioxidant system parameters, salinity can be recommended as the trigger to enhance the potential of the stress-submitted medicinal plants but its effect on human health needs further confirmatory studies. The same applies to phytoprotectants-applied medicinal plants.

In the recent years, nano-engineered particles or fertilisers have been considered as promising approaches to combat the impact of stress on plants' performance (Iqbal et al. 2019; Kumar et al. 2021). Because of the upcoming changes in the global climatic conditions, the stress factors are likely to increase their severity, frequency and duration. Given this, nanoparticles can preferably be used to lessen the adverse impacts of various stresses (Farsaraei et al. 2020; Husen and Iqbal 2019;

Zulfiqar and Ashraf 2021; Husen 2022b). Moreover, as a new approach, the aforementioned various molecules can possibly be conjugated with nanoparticles in order to enhance their impact to fortify the plant defence system.

6 Conclusions

The abiotic stressors are critical and detrimental factors for medicinal as well as other crop plants. In order to cope with the stressors, exogenous treatments of plant-originated signal molecules or primary metabolites have been successfully employed with remarkable outcomes with reference to enhanced activities of enzymatic and non-enzymatic defence system of plants, over-accumulation of solutes, and improvements in photosynthetic gas exchange parameters, nutrient uptake and secondary metabolism. Even though a great number of phyto-protectants have been reported in the literature, we have focused here on polyamines, melatonin and salicylic acid, which are quite common and the most preferred molecules. Since such studies dealing with medicinal plants have hardly applied molecular approaches, the exact *modus operandi* of phytoprotectants is not fully known. Behaviour of secondary metabolism is more important than agronomic traits in the case of medicinal plants; further studies involving the proteomic and metabolomic integrated approaches are the need of the hour.

Also, the exogenously-treated plant samples have been screened and assayed for their biological activities “in vitro”. These activities, mostly linked to antioxidant assays, are hypothetically correlated to the secondary metabolites available (phenolics and flavonoids in particular). Since the living system (in vivo) is more dynamic in comparison to the non-living media for “in vitro” experiments, the exact action mechanisms of the plant as the potential drug for human consumption must be investigated in the “in vivo” conditions, i.e. through pharmacological trials.

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Biostimulants and Phytohormones Improve Productivity and Quality of Medicinal Plants Under Abiotic Stress

Kirubel Teshome Tadele and Gebeyanesh Worku Zerssa

Abstract Abiotic stresses such as drought, salinity, low/high temperature, and other environmental extremes can limit the productivity and quality of medicinal plants via disturbing their metabolic and physiological processes. The rising need for medicinal plants worldwide necessitates appropriate management of efficient mineral nutrient supply so as to ensure optimum production of high quality material of therapeutic importance. The nutrient uptake-related problems of medicinal plants due to abiotic stress can be solved through application of plant growth promoters and biostimulants such as plant-growth-promoting rhizobium, phytohormones, biochar and nanomaterials. These treatments improve stress tolerance ability of medicinal plants by various mechanisms such as stimulating the secondary metabolites biosynthesis, enhancing the synthesis and transportation of phytohormones, increasing the antioxidant potential, enhancing the membrane stability index, increasing the primary-metabolites content and nutrient uptake, reducing the oxidative stress, and increasing the chlorophyll content. Stresses in combination cause greater damage to plants, and integrated treatments do better in mitigating the detrimental effects of stresses. This chapter discusses the treatments that are supposed to reduce the damaging effects of abiotic stresses and may promote quality production of herbal medicine under abiotic stress.

Keywords Secondary metabolites · Oxidative stress · Plant growth promoters · Nanomaterials · Combined treatments

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A. Husen, M. Iqbal (eds.), *Medicinal Plants*,
https://doi.org/10.1007/978-981-19-5611-9_13

Abbreviations

ALA	5-Aminolevulinic acid
AMF	Arbuscular mycorrhizal fungus
CeO_2-NPs	Cerium oxide nanoparticles
GB	Glycine betaine
GO	Graphene oxide
IC	Irradiated carrageenan
MDA	Malondialdehyde
NO	Nitric oxide
PGP	Plant growth promoters
PGPR	plant growth-promoting rhizobium
ROS	Reactive oxygen species
SA	Salicylic acid
SRI	Systematic resistance induction
UV	Ultraviolet visible
WHO	World health organization

1 Introduction

Human beings survived before the development of medical science through getting remedy from medicinal plants either via eating them as a food or using as traditional medicines. Primitive people in some African, Latin American and Asian countries still depend largely on traditional herbs (Agbor and Naidoo 2015; Anis and Iqbal 1994; Anis et al. 2000; Beigh et al. 2002, 2003a, b; Husen 2021, 2022), and plant-based drugs are playing a key role in maintaining health of many people in developing countries (Parveen et al. 2020b; Asfaw et al. 2022). The dependence of animals, especially wild animals, on medicinal plants is by far greater than that of humans. The remedy animals get from medicinal plants by simply eating them as a food is not that much wondering, but their natural ability to identify specific medicinal plants for different accidents like snake bites, or for killing their preys, is quite amazing. Living organisms receive lifesaving services from medicinal plants either by a direct consumption or indirectly through food chain and food web. Furthermore, medicinal plants also provide the base for modern drugs and, in short, the modern drugs are almost the processed medicinal plants. Recent reports indicate that about 30% of the plants available are already used as a medicine (Agbor and Naidoo 2015). The world health organization (WHO) also confirms that major chunks of the world population use medicinal plants for their health maintenance.

Medicinal plants constitute the traditional medicines in many countries, playing a critical role in maintaining human health via preventing, diagnosing, and treating diseases (Firenzuoli and Gori 2007; Parveen et al. 2020a, 2022). The natural products obtained from plants are classified into primary and secondary metabolites depending on their role in the biological system of plants. There is no significant difference among plants regarding the primary metabolites production and their role in the plant's life. However, the bioactive secondary metabolites synthesized from primary metabolites are unique for different plant families, and ecologically important for plant survival and reproduction in diverse environments (Abbas et al. 2017). There are around 100,000 secondary metabolites in the whole plant kingdom and they are classified into three main groups, viz. nitrogen-containing compounds, phenolic compounds and terpenes, based on their biosynthesis mechanism (Fang et al. 2011; Parveen et al. 2020b). These highly valuable compounds for the welfare and species continuity of the plants provide pollinators, attractants, repellants and antimicrobials. Also, they regulate the interaction of plants with the environment through successive adaptation reactions (Ahmad et al. 2018; Bennett and Wallsgrove 1994; Yang et al. 2018). Apart from protecting the plants from various harms including pathogenic attacks, the bioactive secondary metabolites, such as alkaloids, terpenes, polyphenols and others, also protect animals and human beings from different pathogenic and other attacks via stimulating their immune system (Gutzeit and Ludwig-Müller 2014; Bustos-Segura and Foley 2018).

Secondary metabolites content in plants depends on diverse factors such as physiological identity, geographic difference, environmental circumstances and genetic variety; whereas biotic and abiotic stresses enhance their accumulation (Figueiredo et al. 2008; Iqbal et al. 2018). Medicinal plants need favorable environment to produce adequate quantity of secondary metabolites with their therapeutic quality intact. In view of the immensely increasing need for these bioactive compounds of medicinal plants, their production in terms of both quantity and quality aspects requires to be enhanced. However, the global warming and increased accumulation of environmental pollutants containing various hazardous chemicals are bound to limit their productivity and quality (Iqbal et al. 2018; Rhaman et al. 2021). Abiotic stress has detrimental effect on living organisms and hampers their performance significantly. There are various abiotic stresses such as temperature (high/low), salinity (salt/alkalinity), water (drought/flooding), chemicals (heavy metals, toxic gases, particulate matters, pesticide residues) and oxidative stress, that affect plant growth and productivity (Aishwath and Lal 2016; Ali et al. 2008; Aref et al. 2016; Hussein et al. 2017; Iqbal et al. 2000a, b).

Abiotic stress causes excessive production of cytotoxic (at high concentration) free radicals or reactive oxygen species in plants, thereby changing the metabolic pathways to influence the metabolite yield and quality negatively (Ali et al. 2008; Anjum et al. 2012; Iqbal et al. 2011; Zehra et al. 2019) (Fig. 1). It is the main crop productivity and quality minimizing factor, because of which more than 50% crop yield is lost (Bray et al. 2000). Abiotic stresses have an integrating behavior in affecting plants and the reaction of plants involves up-regulation of a variety of genes in the process of developing their tolerance against the stresses (Mahajan and Tuteja 2005). All plants, including the medicinal plants, prepare secondary metabolites for themselves, depending on the type and degree of the stress they face; these bioactive compounds enable plants to respond to the abiotic-stress influences (Qureshi et al. 2007). As plants are sessile, their only option to cope with diverse

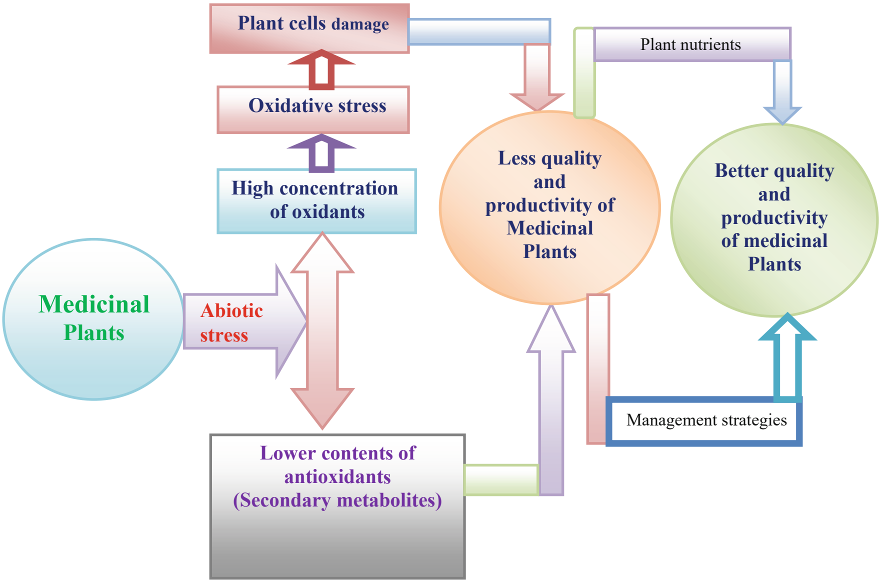

Fig. 1 Effect of abiotic stress on quality and productivity of medicinal plants and their negative effects mitigation approaches

abiotic stress is to develop quick adaptation strategies although the adapting ability varies with species (Lexer and Fay 2005; Aleel and Grennan 2006). The first step in plants responding process to abiotic stress is sensing the stress signals with multiple sensors, but the process is not simple and straight forward due to composite nature of abiotic stresses. The effective recognition of the stress makes secondary messengers activate the stress-reactive genes and start up the response (Joseph et al. 2010). The damaging effect of the combined stresses on plants may range from decrease in productivity to death of the plant (Parida and Das 2005). The dangerous effects are due to high accumulation of reactive oxygen species (ROS) in plants, which causes oxidative stress by creating a physiological disproportion between antioxidants and oxidants (Aref et al. 2016; Khan et al. 2009) (Fig. 1). The high accumulation of ROS oxidizes plant key biomolecules such as photosynthetic pigments, enzymes, DNA and lipids, leading to cell death and finally to plant damage (Diwan et al. 2010a, b; Yordanov et al. 2000) (Fig. 1). However, increased production of secondary metabolites has been seen in many plant species (Singh et al. 2000; Selmar and Kleinwächter 2013). high production of phenylpropanoid phytochemicals such as flavonoids, sinapoyl aldehydes, scopolin and sinapic acid can decrease the damage due to free radicals by enhancing the antioxidant potential of the plants under abiotic stresses (Zandalinas et al. 2018; Alhaithloul 2019).

The life-saving role of medicinal plants is based on their secondary metabolites, whose synthetic pathways are greatly influenced by environmental changes since their biosynthesis activating genes are changed by various stresses (Borges et al. 2017; Sanchita and Sharma 2018). The combined effects of abiotic stresses also complicate the synthesis and accumulation of the secondary metabolites in medicinal plants (Yang et al. 2018; Elansary et al. 2018; El-Esawi et al. 2018; Elkelish et al. 2019), decreasing their remedial potential. The rapid population growth leading to increased demand for medicinal plants and the climate change that is reducing their quality and productivity are the two opposite factors that our present world is faced with. A promising option to meet this challenging situation is to use effective nutrients with adequate management strategies. Some integrated soil management approaches, including incorporation of plant-growth-promoting rhizobium (PGPR), and application of plant phytohormones, nanomaterials and biochar, etc. are showing promising results. This review discusses the sole and integrated treatments and strategies for improving the stress-tolerance capacity of medicinal plants and ensuring their high quality and productivity under abiotic stress.

2 Strategies for Tolerance Against Abiotic Stress

The negative impacts of abiotic stresses such as drought, salinity, low/high temperature, and other environmental extremes can limit the productivity and quality of medicinal plants via disturbing their metabolic and physiological pathways. Hence, efficient methods and strategies that can enhance stress tolerance ability of medicinal plants are the need of the hour. The core issue in this context is to minimize oxidative stress by reducing the excessive production of ROS (Semwal and Khanna-Chopra 2020). Popular approaches to achieve this goal include the use of beneficial microbes, micronutrients, nanomaterials, biochar, phytohormones, and integration of these to generate the optimum secondary metabolite content in various organs and tissues of the medicinal plants.

2.1 Plant-Growth-Promoting Rhizobium (PGPR)

Microbes have versatile functions in plant's rhizosphere comprising of plentiful fungi, nematodes, bacteria and protozoa (Narula et al. 2009). There is a mutual benefit between plants and microbes, because the various organic compounds secreted by roots of the plants are the food for the microorganisms that keep accumulating in the rhizosphere and enhance the solubilization of inorganic phosphorus (P) and mineralization of the organic phosphorus sources (Dutta and Khurana 2015). Microorganisms thus enhance the availability of nutrients for plants (Patel et al. 2015; Hamilton et al. 2016).

Plant growth-promoting rhizobacteria (PGPRs) are a special class of soil bacteria with great rhizosphere and rhizoplane potential to enhance plant growth and productivity via direct and indirect mechanisms (Rizvi et al. 2022). The direct mechanism includes facilitating the supply of available phosphorus and nitrogen, plant hormones production and ethylene concentration reduction (Glick 1995; Glick et al.

1999; Mayak et al. 2004). The indirect mechanisms by which the PGPRs promote plant growth and productivity include making iron unavailable for phytopathogens, competing with damaging microorganisms for plant root sites and activating resistance against various pests and pathogens via production of antibiotics and induced systematic resistance (ISR) (Ramamoorthy et al. 2001; Aloo et al. 2019; Parewa et al. 2018). Bacteria acting as PGPR, include *Agrobacterium, Arthrobacter, Azospirillum, Azotobacter, Bacillus, Burkholderia, Caulobacter, Chromobacterium, Erwinia, Flavobacterium, Micrococcus, Pseudomonas* and *Serratia* (Gray and Smith 2005). These bacteria enhance the productivity and quality of plants under both biotic and abiotic stresses by activating nitrogen fixation as well as the phytohormones and amino acids production (AlKahtani et al. 2020a, b).

Microbes under PGPR enable plants to effectively resist abiotic stresses (Dutta and Khurana 2015) and also enhance plant resistance against diseases caused by various phytopathogens (Shrivastava et al. 2015; Meena et al. 2020). Thus, PGPR are one of the most efficient alternatives to maintain the productivity and quality of medicinal plants affected by various stresses, and inoculation with PGPR has gained considerable attention because of its capacity to ameliorate the effects of stresses (Enebe and Babalola 2018; Rizvi et al. 2022). Seeds pre-treated with a variety of PGPR showed better germination and seedling growth (Rahmoune et al. 2017; Bakhshandeh et al. 2020), possibly because PGPR manage to maintain hormones balance, like auxin to cytokinin levels, during seed germination at the beginning of plant growth (Chu et al. 2019; Qessaoui et al. 2019).

2.1.1 Bacteria Application

Bacillus pumilus enhanced drought tolerance of *Glycyrrhiza uralensis* by increasing the contents of secondary metabolites via rising C- and N- metabolisms leading to generation of primary metabolites (Table 1). It also increased the glycyrrhizic acid quantity (Zhang et al. 2021). Biosynthesis of secondary metabolites increased in *Mentha piperita* under salinity stress after treatment with volatile compounds obtained from *Bacillus amyloliquefaciens* GB03, thereby enhancing the antioxidant potential of the plant. Basically, salt stress increases the accumulation of secondary metabolites (Table 1), as the phytochemicals are the main components in the defense pathway against the stressful condition. Concentration of secondary metabolites is higher in plants subjected to *B. amyloliquefaciens* GB03 compounds (Cappellari et al. 2020), which cause a positive influence by activating the closure of stomata (Cho et al. 2008), inducing the systematic resistance against various phytopathogens via secretion of strong antimicrobial terpenes (Ikram et al. 2020; Sangwan et al. 2001), and causing the formation of biofilm (Vaishnav et al. 2015; Chen et al. 2016). This indicates the potential the compounds possess for enhancing the quality and productivity of medicinal plants under abiotic stresses.

Bacillus cereus promoted the growth of tomato (*Solanum lycopersicum*) by mitigating the effects of heat stress. The applied bacterium produces ACC-deaminase (Table 1), which decreases the effect of ethylene on the growth of

Table 1 Specific roles of abiotic stress effects mitigating plant growth promoters

Key references	Medicinal plant	PGP	Stress type	Specific role
Zhang et al. (2021) and Cho et al. (2008)	*Glycyrrhiza uralensis*	*Bacillus pumilus*	Drought	Raised C- and N metabolisms Increased glycyrrhizic acid content
Mukhtar et al. (2020)	*Solanum lycopersicum*	*Bacillus cereus*	Heat	Produced ACC-deaminase
Hemida and Reyad (2019)	Safflower	*Bacillus cereus* and *aerius*	Salt	Stimulated secondary metabolites biosynthesis
Mariotti et al. (2021)	Purple basil	*Azospirillum baldaniorum* Sp245	Drought	Enhanced synthesis and transportation of phytohormones
Mirzaie et al. (2020)	*Cymbopogon citratus*	*Pseudomonas putida* and *azotobacter chroococcum*	Drought	Increased antioxidant potential of the plant
Rostami et al. (2021)	*Thymus vulgaris*	arbuscular mycorrhizal fungus	Heavy metal	Enhanced membrane stability index Increased primary metabolites content
Ismail et al. (2021)	*Glycine max* and *Helianthus annuus*	*Gliocladium cibotii*	Heat	Increased phenolic compounds content
Saia et al. (2021)	*Ocimum basilicum*, Lamiaceae	Arbuscular mycorrhiza and *Trichoderma koningii*	Salt	Increased antioxidant potential of the plant
Ait-El-Mokhtar et al. (2020)	*Phoenix dactylifera*	Arbuscular mycorrhizal fungus	Salt	Increased nutrient uptake Decreased oxidative stress
Kang et al. (2021)	*Glycine max*	*Rhodobacter sphaeroides* and biochar	Flooding	Increased phytohormones content
Jabborova et al. (2021)	*Abelmoschus esculentus*	*Arbuscular mycorrhizal* and biochar	Drought	Increased carbon, nitrogen and chlorophyll content
Dad et al. (2021)	radish plant	Iron-enriched biochar	Heavy metal	Reduced reactive oxygen species
Zafar-ul-Hye et al. (2021)	*Mentha piperita*	Biochar (rhizobacteria and compost)	Heavy metal	Reduced lead uptake Increased potassium, nitrogen and phosphorus content
Zafar et al. (2021)	*Syzygium cumini*	Salicyclic acid	Drought	Increased quantity of antioxidant enzymes
Hassan et al. (2021)	*Catharanthus roseus*	Chitosan nanoparticles	Salt	Stimulated the alkaloids biosynthesis

(continued)

Table 1 (continued)

Key references	Medicinal plant	PGP	Stress type	Specific role
Asgharipour and Mosapour (2016)	*Foeniculum vulgar*	Silicon	Drought	Increased concentration of the essential oil and soluble sugars
Chen et al. (2021)	*Cyclocarya paliurus*	Hydrogen sulfide	Salt	Enhanced the activity of NR enzyme Down regulated reactive oxygen species
Wang et al. (2021a, b)	*Capsicum annuum*	Hydrogen sulfide 5- and Aminolevulinic acid	Chilling	Enhanced chlorophyll content and photosynthetic rate
Tang et al. (2020)	Blueberry	Hydrogen sulfide	Chilling	Reduced membrane peroxidation
Jia et al. (2020)	*Isatis indigotica*	Hydrogen sulfide	Heavy metal	Decreased cadmium transportation from root to shoot
Antoniou et al. (2020)	*Medicago sativa*	NOSH and NOSH-aspirin	Drought	Increased the proline content
Attia et al. (2021)	Chitosan	*Solanum lycopersicum* L.	Drought	Reduced hydrogen peroxide and malondialdehyde
Islam et al. (2021)	*Panax ginseng*	Putrescine	Salt	Increased osmolytes content and antioxidant enzymes activities
Nazar et al. (2020)	*Carthamus tinctorius*	Glycine betaine	Drought	Increased ascorbic acid and phytochemicals contents
Alam et al. (2020)	*Brassica juncea*	24-Epibrassinolide	Heavy metal	Increased photosynthetic efficiency
Ahanger et al. (2021)	*Solanum lycopersicum*	Nano-organic fertilizer	Drought	Increased the rate of water uptake
Sheikhalipour et al. (2021)	*Stevia rebaudiana*	Selenium and titanium dioxide nanoparticles	Salt	Increased Stevioside and proline content
Azimi et al. (2021)	*Dracocephalum moldavica*	Chitosan-selenium nanoparticles	Heavy metal	Mitigated the activity of cadmium transporters related genes
Nejatzadeh (2021)	*Satureja hortensis*	Silver nanoparticles	Salt	Enhancing the seed germination and growth of the plant

the plant by mitigating its synthesis (Mukhtar et al. 2020). It also enhances nutrient uptake by increasing the root growth (Shahzad et al. 2013). The treatment increased the concentration of secondary metabolites (antioxidants) that inhibited the synthesis of ROS and reduced the damage due to oxidative stress. It also increased the concentration of proline in leaves, which protects membrane permeability to influence water movement in tissues (Ansari and Ahmad 2018). The growth promoting endophytic bacteria (*B. cereus* and *B.aerius*), which have excellent abiotic-stress-alleviating potential enhanced salinity tolerance of safflower plants by stimulating the biosynthesis of secondary metabolites (Hemida and Reyad 2019).

Drought stress negatively affects the quality and productivity of medicinal plants via inducing molecular, biochemical, morphological, physiological and ecological changes (Aref et al. 2013a, b; Umar et al. 2018). Plants defend the influence of drought stress via secondary metabolites accumulation, which induces biomass reduction (Isah 2019). Drought tolerance ability of purple basil (*Ocimum basilicum* cv. Red Rubin) was enhanced after treatment with *Azospirillum baldaniorum* Sp245, as the rhizosphere bacteria catalyzed the synthesis and transportation of the growth-promoting phytohormones (Mariotti et al. 2021).

Panax ginseng is a Korean medicinal plant, which is among the leaders for its medicinal significance. However, its quality and productivity are adversely affected by the environmental stress. Its resistance to heavy-metal stress was enhanced by the siderophore-producing rhizobacteria (SPR) isolated from rhizospheric soil samples (Huo et al. 2021). *Mesorhizobium panacihumi* DCY119^T was identified as the best promoter of HM-stress resistance. Siderophore-producing *M. panacihumi* DCY119^T isolated from ginseng rhizosphere enhanced the growth of this plant under iron stress by increasing the plants biomass, and secondary metabolites content via enhancing the antioxidant genes levels. The increase in concentration of secondary metabolites such as phenolic and flavonoid compounds enhanced the plant tolerance by reducing the concentration of the reactive oxygen species (ROS) accumulated in response to the stress, and minimizing the consequent tissue damage due to oxidative stress (Huo et al. 2021). The isolates reduced the metal ions negative influence via forming bond between the metals and siderophores (Etesami 2018; Dimkpa et al. 2009). The growth and productivity of plants grown in HM-contaminated soil is enhanced due to root proliferation induction and the improved nutrient and metal uptake (Iqbal et al. 2015; Sessitsch et al. 2013). Ion transport system has a role in deciding the efficiency of mineral/metal uptake (Ahmad et al. 2005).

Bacillus bacteria strains (EGY05, EGY21, and EGY25) and *Enterobacter* strains (EGY01, EGY16 and EGY31) enhanced salt stress tolerance of tomato (*Solanum lycopersicum* L.), as evident by the activity of antioxidant enzymes. The six strains minimized the disease severity index of the tested plant grown under stress, which might be associated with enough quantity of the plant's secondary metabolites for effective defense against the disease, but less accumulation of the secondary metabolites due to the salinity stress (Abdelshafy Mohamad et al. 2020).

Plant growth promoting rhizobacteria (*Pseudomonas putida* and *azotobacter chroococcum* strains) improved drought stress tolerance of lemongrass (*Cymbopogon citratus*). The functional potential of the rhizobacteria is associated with their ability to increase the antioxidant capacity of the plant by enhancing the concentration of secondary metabolites (Mirzaie et al. 2020).

2.1.2 Fungi Application

Thyme (*Thymus vulgaris* L.) medicinal plant, affected by cadmium stress, was treated with arbuscular mycorrhizal fungus (AMF). The treatment enhanced the growth of the plant, as evidenced by the increased leaves number, plant height, dry weight, etc. AMF also significantly enhanced the membrane stability index, the concentration of primary metabolites like carbohydrates, and also the concentration of secondary metabolites (Table 1) (Rostami et al. 2021).

Endophytic fungus *Gliocladium cibotii* isolated from *Verbena officinalis* L. promoted the growth of *Glycine max* and *Helianthus annuus* affected by heat stress. The fungal treatment enhanced the phenolic compounds and primary metabolites contents (Table 1) by reducing reactive oxygen species (ROS) and inhibiting proline (Ismail et al. 2021).

Secondary metabolites (both volatile and non-volatile) of basil (*Ocimum basilicum* L.) were retained by arbuscular mycorrhiza fungus (AMF) and *Trichoderma koningii strains* under salinity stress, sustaining the quality and productivity of the plant. The treatment increased the antioxidant potential of the plant (Saia et al. 2021) (Table 1).

Application of AMF and compost, either separately or in combination, mitigated the damaging effect of salinity stress on date palm (*Phoenix dactylifera* L.). The fungus promoted the growth of the plant via enhancing its nutrient uptake performance under the stress and decreasing its ROS content. The combined application of fungi and compost did the most efficient growth promotion in the plant, by causing a relatively high gas exchange, pigments accumulation, and antioxidant enzyme action in the treated plant (Ait-El-Mokhtar et al. 2020).

2.2 *Integrated Biochar Application*

Rhodobacter sphaeroides KE149 integrated with biochar improved the growth and fresh biomass of *Glycine max* L under flooding stress. The effects of the two treatments manifested in increased phytohormones like abscisic acid under the water logging stress, which was relatively low under normal conditions. On the other hand, the treatment stabilized the jasmonic acid level under the flooding stress (Kang et al. 2021).

Combined applications of biochar and *Arbuscular mycorrhizal* fungus on *Abelmoschus esculentus* under drought stress improved the growth of the plant. The integrated treatment increased the concentration of carbon, nitrogen and chlorophyll under the stress (Jabborova et al. 2021) (Table 1), which is responsible for enhanced synthesis of primary as well as secondary metabolites.

Iron-enriched biochar supported the normal homeostasis and ionic regulation of radish plant exposed to cadmium stress. The biochar treatment increased nutrient uptake, as well as chlorophyll content, and reduced reactive oxygen species

(Table 1) due to adsorption of cadmium ions on the pores of the biochar, reducing its concentration in the soil and finally minimizing the stress (Dad et al. 2021).

Biochar, derived from rhizobacteria, and compost improved the growth of mint (*Mentha piperita* L.) plants exposed to lead stress, by increasing the potassium, nitrogen and phosphorus contents in the leaves and by improving the biomass of roots and leaves. The biochar application played a positive role by reducing lead uptake from the soil (Zafar-ul-Hye et al. 2021). Addition of biochar to rhizobacteria improved its performance, possibly due to the pore spaces available on the biochar, because adsorption of lead on these pore spaces would minimize the concentration of lead and improve the bioavailability of nutrients in the soil (Table 1) (Zafar-ul-Hye et al. 2019).

2.3 Phytohormones Application

2.3.1 Salicylic Acid

Salicyclic acid (SA) is an important plant hormone, which helps in regulation of plant growth, production and various processes of abiotic stress tolerance (Husen et al. 2018). This phytohormone is a phenolic compound, which also acts as a signaling agent (Per et al. 2017). It is involved in regulating many physiological processes of plants like photosynthesis, free radicals scavenging (antioxidants) enzyme action and osmotic production by which it enhances plant tolerance to many abiotic stress (Husen et al. 2018, 2019). It also has an important role in seed germination and in the uptake, transport and transpiration of nutrients (Koo et al. 2020; Li et al. 2019). Its ability to improve abiotic stress tolerance of plants is attributed to its potential of maintaining the cell membrane stability via increasing the quantity of antioxidant enzymes and photosynthetic capacity of leaves (Belkadhi et al. 2015; Janda et al. 2014; Alsahli et al. 2019).

Salicylic acid improved drought stress resistance of *Syzygium cumini* plant via enhancing its chlorophyll a and b contents, leaf size (high gas exchange), and dry weight production. The high concentration of reactive oxygen species (ROS) due to the stress was reduced by the increased quantity of antioxidant enzymes after the treatment (Zafar et al. 2021) (Table 1).

Foliar SA enhanced the productivity and quality of fenugreek (*Trigonella foenum-graecum* L.) under salinity stress. Treatment of the plant with SA increased the concentrations of proline, secondary metabolites like phenolic compounds and flavonoids, and the antioxidant enzymes (Abdelhameed et al. 2021). The positive effect of SA application on medicinal properties of plants is most evident by the increased production of secondary metabolites.

SA application alleviated the adverse effects of salinity on Ethiopian mustard (*Brassica carinata*) and improved the overall performance of the plant. It mitigated proline production, which had been increased under salinity stress, but further increased the activity of antioxidant enzymes (Husen et al. 2018). Similarly, application of salicylic acid (SA) in combination with salt stress mitigated the salinity-induced adverse effects on growth attributes, chlorophyll contents and photosynthetic parameters of niger (*Guizotia abyssinica*), an oilseed plant, but the upregulation of antioxidant enzymes evoked by salinity was further enhanced. In short, SA application fortified the salt-tolerance capacity of the above-mentioned plants via alleviation of membrane injury, acceleration of assimilatory activities, rise of plant water status, and activation of defence arsenal (Husen et al. 2019).

Artemisia annua was exposed to arsenic stress and treated with irradiated carrageenan (IC) oligomers and salicylic acid (SA) combined. The integrated application enhanced the content of artemisinin, which is responsible for anti-malarial activity of the plant. This shows the significance of integrative applications of plant growth regulators in maintaining quality production of medicinal plants under abiotic stresses (Naeem et al. 2021).

2.3.2 Hydrogen Sulfide

Hydrogen sulfide (H_2S), a colorless gas with a pungent odor of rotten egg, is the third gastro-transmitter with nitric oxide and carbon monoxide. At low concentrations, it has a key signaling role in biological processes of plants (Chen et al. 2011), but proves detrimental at high concentration, as it inhibits mitochondrial electron-transport chain (Fakhari et al. 2019). It is produced in plants as a result of cysteine degradation catalyzed by L-cysteine desulfhydrase and D-cysteine desulfhydrase. Many key plant biological processes such as photosynthesis, organogenesis, and seed germination are regulated by hydrogen sulfide (Khan et al. 2020; Sonkar et al. 2021). It also promotes abiotic stresses tolerance in plants by regulating ionic homoeostasis and enzyme activities (Arif et al. 2020; Mukherjee and Bhatla 2020).

Cyclocarya paliurus is a medicinal plant, widely used for diabetes treatment in China. The therapeutic potential of the plant can be influenced by many environmental factors like abiotic stresses. However, H_2S application reduced the damaging effect of salinity stress on *Cyclocarya paliurus* and helped in enhancing the quality of the plant by raising the activity of NR enzyme, thus leading to a high nitric oxide content. It is important because nitric oxide has its own role in stress tolerating response via inducing physiological processes (Mur et al. 2013). It is confirmed that treatment of wheat seedlings under stress with NO donor increases the content of both NR key enzyme and NO, but application of NO scavengers decreases their concentrations (Khan et al. 2017). The potential of H_2S to reduce the salt-stress effect might be associated with down regulation of reactive oxygen species (ROS) via activation of antioxidant enzymes as well as with increase in the concentration of secondary metabolites in the medicinal plants (Chen et al. 2021) (Table 1).

Hydrogen sulfide, in combination with 5- Aminolevulinic acid (ALA), improved chilling-stress tolerance of pepper (*Capsicum annuum* L.). ALA, a precursor in chlorophyll synthesis required for photosynthesis (the critical plant process), is reduced in concentration under various abiotic stresses including the chilling/freezing stress, resulting in a reduced chlorophyll content and photosynthesis rate. ALA and H_2S improved the stomatal gas exchange of different medicinal plants under various abiotic stresses (Ali et al. 2014), and enhanced the chlorophyll content and photosynthetic rate of pepper seedlings (Table 1), leading to an improved quality and yield of the plant (Wang et al. 2021a, b).

Hydrogen sulfide improved the tolerance of Peach (*Prunus persica* L. Batsch) against water-logging stress by increasing the proline concentration (Table 1), reducing the oxidative stress via enhanced synthesis of secondary metabolites, and restraining the ethylene production (Xiao et al. 2020). Likewise, the decreased carotenoid concentration and chlorophyll quantity in the leaves of blueberry due to freezing stress was improved after treatment with H_2S obtained from NaHS. It reduced decomposition of chlorophyll and carotenoids, enhanced the concentration of proline, reduced membrane peroxidation (Table 1), thereby decreasing the oxidative damage by inhibiting the formation of oxidative stress (Tang et al. 2020).

Similarly, H_2S decreased the detrimental effect of cadmium stress on *Isatis indigotica* by minimizing its transportation from root to shoot. The leaves and roots of *Isatis indigotica* are widely used for medicinal purposes in China. The plant is affected badly by abiotic stressors including the heavy metals. Heavy metals block the photoelectric transport chain transmission that disturbs chlorophyll structure and reduces photosynthetic rate (Mustafa and Komatsu 2016). It was found to promote cadmium accumulation in *Isatis indigotica* root, but reduce its transport to the shoots (Jia et al. 2020) (Table 1).

Medicago sativa exposed to drought stress *was* treated with the nitric oxide (NO) and hydrogen sulfide (H_2S)-releasing compounds (NOSH and NOSH-aspirin). NOSH-aspirin can also release acetylsalicylic acid in addition to H_2S and NO. NO and H_2S are signaling molecules with a key role in stress-tolerance pathways of plants (Corpas et al. 2019). Simultaneous application of NOSH and NOSH-A improved drought tolerance of the plant better than sole treatments. The combined application improved the chlorophyll content in plants under stress, thus enhancing the photosynthetic rate (Table 1). It also increased the proline content by up-regulating its homeostasis (Antoniou et al. 2020). Proline is essential for plants affected by drought stress since it may maintain the required respiration under water shortage by acting as an energy sink (Sharma et al. 2011), sustaining the needed photosynthetic rate (Akbari et al. 2011). Higher efficiency of combined signaling molecules applications in mitigating the detrimental effects of abiotic stresses indicates the presence of a well-orchestrated interaction between their signal transduction pathways (Knight and Knight 2001). Furthermore, it indicates that the combined influence of abiotic stresses on plants can be tackled better by integrative applications.

2.3.3 Other Phytohormones

Melatonin improved the quality of *Moringa oleifera* L. affected by drought stress. As a result of the melatonin treatment, phenolic compounds quantity and antioxidant enzyme activities, which were reduced due to the drought stress, were improved

(Sadak et al. 2020). Biostimulating potential of melatonin even for plants under abiotic stresses might be associated with its natural antioxidant potential (Arnao and Hernandez-Ruiz 2014), which can save the secondary metabolites from being damaged by abiotic stresses. Similarly, exogenous application of glycine betaine (GB) on saffower (*Carthamus tinctorius* L.) affected by drought stress increased the tolerance capacity of the plant via enhancing stomatal gas exchange and the primary and secondary metabolites content (Table 1). The treatment improved the production of the potential antioxidant ascorbic acid and phenolic compounds, which could reduce the impact of oxidative stress (Nazar et al. 2020). Also, in a study of *Vicia faba* grown in water-deficient and water-sufficient conditions, the negative effects caused by water deficiency to the plant growth and leaf characteristics could be markedly minimized by the application of indole-acetic acid (Husen et al. 2017).

Salt stress was found to suppress dry mass production, relative water content and leaf characteristics such as pigment concentration, maximum quantum yield of PS II, stomatal conductance, net photosynthetic rate, transpiration rate and water-use efficiency in young pea plants (*Pisum sativum* L. cv Adi). However, a foliar spray of indole acetic acid (IAA) on affected plants markedly improved all these parameters, reducing the effect of salinity to a great extent (Husen et al. 2016). Likewise, chitosan application on tomato (*Solanum lycopersicum* L.) exposed to salinity stress reduced the damaging effect of the stress. The treatment improved the growth and productivity of the plant by reducing hydrogen peroxide (H_2O_2), malondialdehyde (MDA) and Na^+ levels. Growth promotion due to chitosan application is strongly related to enhancement of photosynthetic pigments, which increases the photosynthetic rate (Attia et al. 2021).

Exogenous putrescine enhanced the salt stress tolerance of *Panax ginseng* by increasing the contents of proline, total soluble proteins, carbohydrates and sugar. Enhanced production of primary metabolites could boost the activities of antioxidant enzymes and increased the contents of secondary metabolites of the adaptogenic plant, which raised its stress-tolerating potential. Furthermore, the treatment reduced the levels of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) by increasing osmolytes and antioxidant enzymes activities (Islam et al. 2021) (Table 1). Hence, putrescine application is useful for maintaining the quality and productivity of medicinal plants affected by salinity stress due to its extensive growth-promoting ability by maintaining the chlorophyll content.

Foliar application of 24-Epibrassinolide alleviated the damaging effect of cadmium stress on Indian mustard (*Brassica juncea* (L.) Czern.) by limiting the high concentrations of proline and glycinebetaine. The treatment improved the growth of the plant by enhancing its stress tolerance, which is related to increased photosynthetic efficiency and reduction of reactive oxygen species (ROS) concentration (Alam et al. 2020). Moreover, it reduced electrolyte leakage via modifying the electrical properties of biological membranes (Saeidnejad et al. 2012). Thus, 24-Epibrassinolide has a potential to stimulate the photosynthetic rate and the antioxidant capacity of medicinal plants by maintaining the contents of their secondary metabolites to the required level in the HM-stressed environment.

2.4 Nanomaterials Application

Nanomaterials, which have a suitable and high surface area, can be fabricated with different shapes and size (Husen and Siddiqi 2014; Nasrollahzadeh et al. 2019; Husen and Iqbal 2019a; Husen and Jawaid 2020). Shape and size are critical factors that determine the impact of nanoparticles, including the impact on flora and fauna. However, other factors like physico-chemical composition, stability and particle size distribution can also contribute to their impact on crops (Kanwar et al. 2019; Kumar et al. 2018, 2021, 2022; Sharma et al. 2021). Various nanoparticles (NPs) have shown promising ability to mitigate the abiotic stress effects by enhancing enzymatic action, nutrients uptake and linkage of plant roots with plant-growth-promoting bacteria (Husen and Iqbal 2019b; Singh and Husen 2019, 2020; Iqbal et al. 2020). The combined effect of the most serious abiotic stress, drought and salinity on soybean was reduced by integrated treatment of plant growth-promoting microbes (PGPMs) and Si-Zn nanocomposite. The combined treatment was found to be more effective than individual treatments and the effect is related to increased root length and leaf size, higher dry weight and enhanced antioxidant enzymes for extensive production of secondary metabolites (Osman et al. 2021). Application of nano-organic fertilizer on tomato (*Solanum lycopersicum* L.) plant exposed to drought stress enhanced stress tolerating capacity of the plant by minimizing ROS, which contributes for membrane stability. The nano-based organic fertilizer also increased the rate of water uptake, facilitating osmolytes such as proline and glycine betaine (Ahanger et al. 2014, 2021) (Table 1), which might be indicative of enhanced plant growth and productivity.

Two nanoparticles (Chitosan-functionalized selenium and anatase titanium dioxide) enhanced salinity stress tolerance of Stevia (*Stevia rebaudiana* Bertoni), a well-known medicinal plant, by increasing the photosynthetic rate and antioxidant enzymes performance, and decreasing the free radicals concentration under the stress. The main bioactive compound of *Stevia* is Stevioside. Treatment with NPs increased the stevioside content in plants under the stress (Sheikhalipour et al. 2021) (Table 1). The two NPs increased the concentration of proline via enhancing nitrate reductase activity (Sotoodehnia-Korani et al. 2020). Application of silver NPs improved salt stress tolerance of *Satureja hortensis* l. by enhancing the seed germination and growth of the plant (Table 1). It may also enhance the growth of other medicinal plants (Nejatzadeh 2021).

Selenium nanoparticles raise the concentration of secondary metabolites in pepper by enhancing the biosynthesis pathway-related genes expression (Li et al. 2020; Rajaee Behbahani et al. 2020). Hence, these NPs may be promising candidates for abiotic stresses treatment. Chitosan-selenium NPs, in which Chitosan was used as a carrier to release the selenium NPs at appropriate position, improved cadmium stress tolerance ability of *Dracocephalum moldavica* L (Azimi et al. 2021). Cadmium is a strong photosynthesis inhibitor (Haider et al. 2021) due to its stomata-closing potential (Zhang et al. 2020). The high mobility of cadmium creates its saturation in roots and shoots of a plant under cadmium stress, leading to competition of absorption with comparable nutrients (Haider et al. 2021). Selenium can be transported with cadmium via the same carrier and reduce the concentration of cadmium in transmembrane transporter active sites, thereby decreasing its uptake (Pereira et al. 2018). Besides, its NPs mitigate the activity of cadmium transporters related genes, minimizing its accumulation (Wang et al. 2021a, b). The potential of NPs makes them a promising candidate for ensuring the quality and productivity of plants under stressed environments.

Selenium NPs alleviated the negative effects of drought stress in pomegranates (*Punica granatum* L.). They promoted plant growth under stress via increasing the nutrients content, phenolic compounds concentration, osmolytes and antioxidant enzymes content. Thus, selenium NPs are capable of maintaining the contents of secondary metabolites of medicinal plants up to the required level under the influence of various abiotic stresses (Zahedi et al. 2021).

Graphene oxide (GO) synthesized from natural graphite, glycine betaine (GB) and glycine betaine functionalized graphene oxide (GO-GB) reduced the negative impacts of salinity stress on sweet basil (*Ocimum basilicum* L.) and improved the growth of the plant by increasing the contents of phenolic compounds, proline, photosynthetic pigments and essential oils as well as the activities of antioxidant enzymes (Ganjavi et al. 2021). Medicinal plants respond to abiotic stresses differently from other crops, as the synthesis and accumulation of secondary metabolites in medicinal plants differ under the stress (Jan et al. 2020). The NPs enhanced the concentration of essential oils possibly by acting as an elicitor for their fabrication (Gohari et al. 2020). The nano form combination of GO and GB did far better than the two could do separately. Application of NPs alleviated the negative effects of salinity stress even at a severe concentration, which indicates their great potential for ensuring quality production of medicinal plants under abiotic stresses (Ganjavi et al. 2021).

Moldavian dragonhead (*Dracocephalum moldavica* L.), an established medicinal plant, was exposed to salinity stress and the potential of cerium oxide nanoparticles (CeO_2-NPs) in mitigating the damaging effects of salinity stress was investigated. Application of NPs promisingly alleviated the stress and improved the growth of the plant (Mohammad et al. 2021). *Catharanthus roseus*, a potential source for anticancer drug development because of its high alkaloid content, was treated with chitosan NPs to mitigate the detrimental effects of salt stress. The treatment enhanced the antioxidant enzymes activity and stimulated the alkaloids biosynthesis (Table 1). These NPs may also be useful against salt stress in other medicinal plants and crops (Hassan et al. 2021).

Iron NPs and salicylic acid applied separately and in combination on strawberry under salinity stress improved the tolerance of the plant by increasing the antioxidant activity enzymes, reducing the concentration of ROS accumulated due to the stress (Dedejani et al. 2021). Iron has an important role in plant's biochemical and physiological processes such as photosynthesis, and its nano form is rapidly taken up by strawberry plant. Salicyclic acid, a phytohormone, has an important role in physiological pathways like biosynthesis of secondary metabolites, stomatal gas exchange, photosynthesis and respiration (Harun-Or-Rashid et al. 2017). Combined application of salicyclic acid and iron NPs resulted in higher content of proline, which induced a superior osmotic potential in the cells. This might be the main reason for the higher tolerance of stresses (Dedejani et al. 2021). Combined applications of stress relievers often prove more effective than separate applications.

2.5 Silicon Application

Silicon is the second most abundant element in the Earth's crust, which promotes plant growth under abiotic stresses via stimulating nutrient uptake by plants and preventing Na and Cl accumulation in different plant parts under salt stress (Abd El-Mageed et al. 2020). Silicon stimulated the growth of Coriander (*Coriandrum sativum* L.) affected by salinity stress. It increased the secondary metabolites (phenolic and flavonoid) contents and decreased the activity of catalase and superoxide dismutase enzymes (Amiripour et al. 2021). The potential of silicon in enhancing salinity stress tolerance is directly related with its ability to stimulate the secondary metabolites production (Fatemi et al. 2020).

Drought resisting ability of Fennel (*Foeniculum vulgare* Mill.), a widely used medicinal plant cultivated mainly for its essential oil, was enhanced by silicon (Table 1). The negative impact of the stress was was mitigated after silicon treatment, which increased the concentrations of essential oil and soluble sugars (Asgharipour and Mosapour 2016). Sugar beet affected by drought stress was treated with silicon and proline individually and in combination. The plant growth decreased under the stress due to reduction of soil moisture, chlorophyll content and nutrients such phosphorus, nitrogen and potassium, and upregulation of antioxidant enzymes. The integrated treatment improved the plant performance by maintaining the secondary metabolites content and enhancing the nutrient uptake. The combined application of silicon and proline resulted better effect than the two alone (AlKahtani et al. 2021).

Silicon (Si) application improved the growth and productivity of Flax plants affected by copper stress. It reduced the copper uptake as well as transportation, and hence its accumulation in the plant tissues. Further, the treatment increased the secondary metabolites content, such as flavonoid and phenolic contents due to the strengthening of antioxidant system (El-Beltagi et al. 2020).

3 Conclusion

The need for herbal medicine is markedly increasing, especially due to their biocompatibility, wide distribution of infectious diseases, and drug resistance of pathogenic microbes. Medicinal plants promote the survival of animals including human beings by either fortifying their immune system or killing the pathogens. Various plant growth promoters and biostimulants such as PGRs, phytohormones, biochar and nanomaterials enhanced the stress tolerance of different plants and promote quality production of medicinal plants under stressful environments. Application of plant-growth promoters and biostimulants improve stress tolerance ability of medicinal plants by various mechanisms, e.g. by stimulating the secondary metabolites biosynthesis, enhancing the synthesis and transportation of phytohormones, increasing the antioxidant potential of plants, enhancing the membrane-stability index, increasing the primary metabolites content, increasing the nutrient uptake, decreasing the oxidative stress, and increasing the chlorophyll content and photosynthetic rate. Combined applications are more efficient in mitigating the damaging effect of the abiotic stresses, the possibly because the abiotic stress influences also have a highly integrative character. Further combined-treatment investigations may explore newer efficient strategies that enable quality production of medicinal plants under stressful environments.

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Light (High Light/UV Radiation) Modulates Adaptation Mechanisms and Secondary Metabolite Production in Medicinal Plants

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Abstract Traditional medicines have long been making the use of plants that produce the secondary metabolites of therapeutic importance. However, in order to meet the ever-growing and exponential demand of herbal medicine, alternative methods need to be explored to enhance the yield and quality of the pharmaceutically important secondary metabolites. Amid such a situation, Ultraviolet (UV) rays as elicitors have caught a lot of attention for increasing the production of therapeutically important secondary metabolites due to their promising nature. Medicinal plants develop a variety of secondary metabolites as one of their defense mechanisms. They mainly provide photoprotection by filtering UV rays and quenching reactive oxygen and nitrogen species through the elevation of enzymatic and non-enzymatic antioxidant agents created on exposure to UV irradiation. Each of the UV rays i.e., UV-A, UV-B and UV-C, has a distinct impact on the plant metabolism and holds the potential to make changes at a molecular level by up-regulating and down-regulating genes. This chapter aims to shed light on the outcome of exposing plants to different types of UV irradiation and on different adaptive mechanisms plants go in for producing the commercially and therapeutically important secondary metabolites.

Keywords UV-irradiation · Elicitors · Secondary metabolites · Adaptive mechanisms · Reactive oxygen species

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A. Husen, M. Iqbal (eds.), *Medicinal Plants*,
https://doi.org/10.1007/978-981-19-5611-9_14

Abbreviations

CLS	Chalcone synthase
H_2O_2	Hydrogen peroxide
LHC	Light harvesting complex
PAL	Phenylalanine ammonia lyase
ROS	Reactive oxygen species
SMs	Secondary Metabolites
TIAs	Terpenoid indole alkaloids
UV	Ultraviolet

1 Introduction

Medicinal plants have long been important in the human society's evolution. It is widely acknowledged how plants have always been at the forefront of more or less all civilizations and cultures as a source of medicine. Many tribal people all over the globe still rely heavily on ethnomedicine, and the Indian sub-continent has been a hub of traditional medicine since the ancient times (Atique et al. 1985; Anis et al. 2000; Beigh et al. 2002, 2003; Husen 2021, 2022; Asfaw et al. 2022). Medicinal plants are considered to be a highly rich and dependable source of traditional remedies, and many traditional as well as modern medications are derived from them (Atique and Iqbal 1992; Dar et al. 2017; Parveen et al. 2020a, b; Abate et al. 2021). This is mainly due to the presence of potentially active secondary metabolites (SMs) that are useful for either therapeutic purposes or are precursors for useful drugs (Sofowora et al. 2013). Plants synthesize many of the therapeutically valuable SMs as a result of their response to stresses. Natural stresses on medicinal plants that grow in the wild are removed when they are cultivated under "ideal" growth circumstances. This lowers the production of SMs and, consequently, the therapeutic efficacy of the plants (Gorelick and Bernstein 2014).

To overcome this difficulty, elicitation is one of the most relevant and successful approaches for triggering a plant's defensive systems, wherein particular pathways are turned on or off by introducing certain agents (elicitors) (Yue et al. 2016; Yang and Stöckigt 2010). Light is considered as an essential abiotic elicitor that controls the development, growth, and morphogenesis of the plants exposed (Tariq et al. 2014; Adil et al. 2019). Light is also essential in controlling primary and secondary metabolisms in plants (Abbasi et al. 2007; Shohael et al. 2006; Liu et al. 2006). Medicinally important plants have been subjected to light stress to boost the SM synthesis (Gorelick and Bernstein 2014; Namdeo 2007). By now, light sources like UV-A, UV-B and UV-C have been used and trusted as efficient elicitors of pharmacologically important metabolites. The signaling, regulatory, and metabolic systems that elicit SM synthesis and the mechanism of light precipitation, are still partly understood. This chapter critically examines the research studies that explore the role of UV radiation as potential elicitors of medicinally important SMs.

2 UV Radiation and Its Detrimental Impact on Plant Health

Ultraviolet radiation has long been a popular abiotic elicitor that has been employed for increasing the SM synthesis in a variety of plant cultures (Xuan et al. 2016). According to the International Commission on Illumination, the ultraviolet (UV) wavelength (400–200 nm) is a small part of the solar radiation reaching the Earth's surface, and it affects all living organisms negatively. UV-A (315–400 nm), UV-B (280–315 nm), and UV-C (200–280 nm) are the three types of UV radiation that reach the Earth's surface (Häder et al. 2007). Where sunlight is an essential component for plant growth and sustenance, a slightly large than normal amount of UV light in the rays can cause irreversible damage to plants. Fortunately enough, most of the harmful UV rays are filtered out by the Ozone Layer before they reach the Earth (Madronich and Flocke 1997). Owing to the continued depletion of ozone layer, exposure of plants to these rays is enhancing and damaging plants in multiple ways. The most detrimental to plant health are the UV rays that have the shortest wavelengths, i.e. UV-C (Piri et al. 2011). However, UV-C is absorbed by the ozone layer almost fully, whereas UV-B and UV-A reach the earth surface in parts. UV rays can be damaging for DNA molecules, membranes and even hormones, leading to hormonal disorders among plants. Changes that occur in a plant at the molecular level can have deep-rooted impacts and can alter the activity of genes involved in metabolic activities including the photosynthesis.

When the intensity of the photosynthesis process is negatively affected, this can hamper the growth of the entire plant (Brosché and Strid 2003). It needs to be understood how plants exposure to UV rays can lead to a long-lasting damage, which goes down to affect the entire ecosystem in direct and indirect ways. Literature reveals that UV holds the potential to damage photosynthetic apparatus at multiple sites, such as D1/D2 reaction center proteins, oxygen-evolving species, donor and acceptor sites of Photosystem II, cytochrome b, quinone molecules and also the content and activity of 1,5 ribulose bisphosphate carboxylase (Kataria et al. 2014). It has also been reported that UV B, a kind of UV rays, can lead to chlorophyll degradation in photosynthetic plants (Sztatelman et al. 2015).

UV rays, when able to penetrate deep into the genome can alter the genes by inducing oxidative damage and lead to the formation of cross-links between molecules of DNA or those of DNA and proteins. These complexes cause a retardation in the growth trajectory of plants and hinder their development. UV-B has been particularly known for nuclear, chloroplast and mitochondrial DNA damage in plants (Tuteja et al. 2001). Figure 1 presents a pictorial view of some of the damages caused to the plant by different types of UV rays.

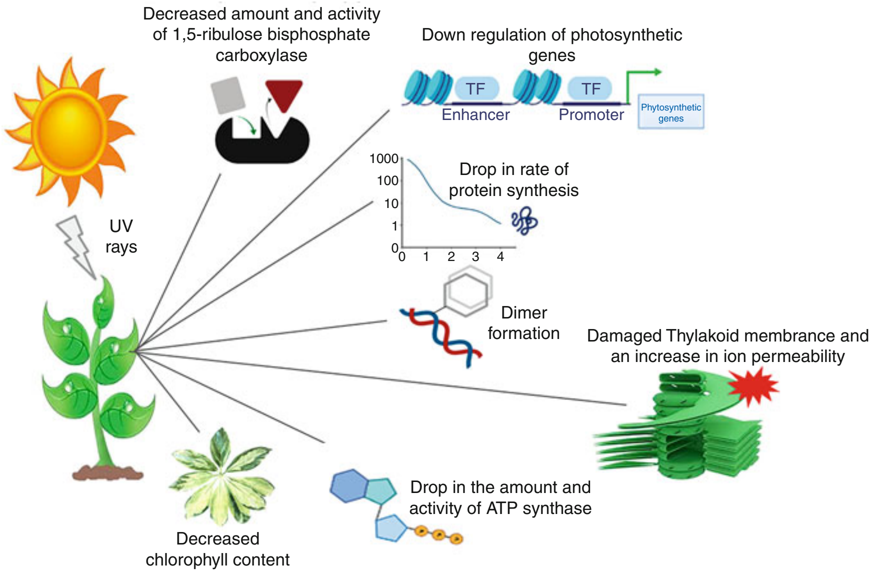

Fig. 1 An overview of the harmful effects of UV radiation on plant health. UV radiations when fall on plants, cause different damages at the molecular level that might lead to the disruption of natural plant mechanism. All kinds of UV lights (**a**–**c**) are capable of causing damage to plants by up or down regulating genes, decreasing protein-synthesis rate, forming DNA-DNA or DNA-protein dimers, damaging thylakoid membrane and decreasing the chlorophyll content

2.1 UV-A

Since UV radiation acts as an elicitor, it is primarily involved in the activation of plant defense systems, which in turn produce SMs that are valuable to humans for medicinal purposes (Yin et al. 2016). In medicinal plants cultivated under controlled parameters, UV-A light can operate as a possible elicitor to induce the formation of SMs (Table 1). The UV-A and UV-B radiations affect the phototropism and morphogenesis in plants (Vanhaelewyn et al. 2020). It has been reported that UV-A exposure in plants leads to the production of reactive oxygen species (ROS). The stress induced by UV-A seems to be less effective than UV-B because the latter causes damage to plants directly (Guo and Wang 2010). Plant has photoreceptors, cryptochromes and phototropins for UV-A absorption. Plants can produce phenolic compounds as a stress response to screen UV-A rays, which function as anti-oxidants when exposed to UV-A radiation (Verdaguer et al. 2017). UV-A has a greater intensity than UV-B but the difference is biologically insignificant (Hashim et al. 2021). Hence, it can be assumed that UV-A has a lower potential of acting as an elicitor for the enhanced production of SMs in the medicinal plants. A large number of studies have been reported demonstrating the role of UV-A on SMs biosynthesis; a few of them are described below (Table 1).

Table 1 Some major studies on the effects of UV-A light on the production of key SMs in medicinal plants

Light Source	Intensity	Exposure time	Medicinal plant	Secondary metabolite	References
UV-A	352 nm	5 days	*Ixeris dentata* Nakai	Phenolics and flavonoids	Lee et al. (2014)
UV-A	365 nm	–	*Lonicera japonica* Thunb.	Isochlorogenic acid and secoiridiod glycoside	Ning et al. (2012)
UV-A	360 nm	60 days	*Phyllanthus tenellus* Roxb.	Phenols, Ellagic acid, geraninn and carotenoids	Victorio et al. (2011)
UV-A	4–5 W/m^2	3 h per day for 16 days	*Capsicum annum* L.	Cynaroside	Ellenberger et al. (2020)
UV-A	10–30 μmol m^{-2} s^{-1}	15 days	*Lactuca sativa* L.	Total phenolic content, flavonoids and anthocyanins	Chen et al. (2019a)
UV-A	7 W/m^2	12 h	*Solanum lycopersicum* L.	Anthocyanins	Guo and Wang (2010)

Ixeris dentata, a medicinal plant used to treat indigestion, pneumonia and tumors, is also known to exhibit neuroprotective, anti-inflammatory, anti-allergic and anti-mutagenic properties (Shin et al. 2017). The phenolic content, flavonoids, and antioxidants were considerably raised after the UV-A exposure. Along with this, phenylalanine ammonia lyase (PAL), the main enzyme in the synthesis of phenolic compounds, was also enhanced by UV-A exposure in *Ixeris dentata* (Lee et al. 2014). Likewise, Ning et al. (2012) reported dramatic increase in the levels of isochlorogenic acid and secoiridiod glycoside SMs in *Lonicera japonica* on exposure of UV-A.

Polyphenols are SMs that provide protective shield against ultraviolet radiations and help in defense against pathogens. Ellagic acid and geraniin have protective effect against cancer and also possess analgesic, anti-microbial and cytoprotective properties (Pandey and Rizvi 2009; Cheng et al. 2017). Victorio et al. (2011) investigated the effect of UV-A on the SM production of a medicinal plant *Phyllanthus tenellus*. to UV-A radiations of 30 μmol m^{-2} s^{-1} intensity for a period of 60 days resulted in the increased production of phenol SMs, ellagic acid, geraninn and carotenoids.

Cynaroside, a flavone acting as a protective barrier for cardiomyocytes against apoptosis induced by ROS, also has anti-inflammatory property and reduces the kidney damage caused by anti-cancer drug cisplatin (Sun et al. 2011). Ellenberger et al. (2020) showed increase in the levels of cynaroside in *Capsicum annum* after elicitation by UV-A radiations for 3 h per day for 16 days. Anthocyanins, also known as flavylium, are a subgroup of flavonoids which are synthesized in response to UV stress. These are found in flowers and fruits of plants and have the ability of absorbing UV radiations; these are thus vital in eradicating the ROS (Khoo et al. 2017; Del Valle et al. 2020). Increased production of flavonoids, total phenolic content and anthocyanins was reported after the exposure of *Lactuca sativa* to UV-A (Chen et al. 2019a). Similarly, *Solanum lycopersicum* demonstrated significant enhancement of anthocyanins after exposure to UV-A (Guo and Wang 2010).

2.2 UV-B

The UV-B radiations reach the Earth due to depletion of the stratospheric layer of ozone and cause several changes in plants. Different strategies and adaptive methods are developed by the plants to resist such changes (Takshak and Agrawal 2019; Manukyan 2013). Plants have different types of photoreceptors to detect the incoming UV rays and generate an appropriate response to cope with UV stress. Five kinds of photoreceptor have been reported; UVR8, blue light sensing cryptochromes, phototropin, zeitlupe and red far-red light sensing photoreceptor (Heijde and Ulm 2012). Of these, the UV Resistance 8 (UVR8) is the most reported UV-B photoreceptor and mediates the UV-B signalling in plants. UVR-8 also regulates the expression of genes associated with UV-B protection mechanism and flavonoid biosynthesis, protection against oxidative stress, DNA repair and photo-inhibition (Yavaş et al. 2020).

UV B has several effects on plants (Deepti et al. 2022). It induces changes in photosynthetic activity, plant growth and morphology, DNA repair capacity, gene expression and secondary metabolism. It also has the potential to produce ROS. In order to cope with such hazards imposed by UV-B, the plants can either induce protective mechanisms or repair mechanisms. Production of UV-absorbing SMs flavonoids and other phenolics is the most common protective response by plants. These light-absorbing phenolics are synthesised by changes in the phenylpropanoid metabolism. The UV-B stress has been known to regulate the increase in levels of the main enzyme, i.e. phenylalanine ammonia-lyase (PAL) of phenylpropanoid metabolism, which in turn speeds up the synthesis of phenolic compounds. The PAL enzyme catalyses the conversion of L-phenylalanine to ammonia and trans-cinnamic acid, which in turn leads to the production of several phenolics. The compounds get accumulated in vacuoles and act as anti-oxidants and free-radical scavengers. These compounds act to protect DNA and also help in protection against pathogens (Interdonato et al. 2011; Sun et al. 2010; Kumari and Agrawal 2010; Mosadegh et al. 2018; Chen et al. 2019b). The UV-B exposure stimulates defense responses and production of different SMs in plants. Thus, UV-B has a great potential to act as an elicitor for gaining medicinally important SMs from plants (Vanhaelewyn et al. 2020; Takshak and Agrawal 2016) (Table 2).

The phenolic compounds produced by plants in response to UV-B stress have shown anti-cancer, anti-oxidant, anti-inflammation, anti-ageing and anti-proliferative effects (Kumar and Goel 2019; Lin et al. 2016; Schreiner et al. 2009). They also contribute to anti-oxidant reaction by donating electrons and exhibit free

Table 2 Some major studies on the effects of UV-B light on the production of key SMs in medicinal plants

Light source	Intensity	Exposure time	Medicinal plant	Secondary metabolite	References
UV-B	35 μW cm^{-2} nm^{-1}	30 min for 15 days	*Prunella vulgaris* L.	Phenolics, flavonoids, rosmarinic acid and caffeic acid	Zhang et al. (2017)
UV-B	0.075 W h m^{-2}	2 h	*Tropaeolum majus* L.	Phenols	Schreiner et al. (2009)
UV-B	4–15 W m^{-2}	5 h per day	*Kalanchoe pinnata* (Lam.) Pers.	Phenolics and flavonoids	Nascimento et al. (2015)
UV-B	10.97 kJ m^{-2} day^{-1}	1 h per day for up to 10 days	*Salvia verticillate* L.	Chlorogenic acid, caffeic acid, rosmarinic acid and salvianolic acid	Rizi et al. (2021)
UV-B	312 nm	2–4 h	*Echinacea purpurea* (L.) Moench	Phenolics and caffeic acid	Manaf et al. (2016)
UV-B	31 kJ m^{-2} day^{-1}	2 weeks	*Rosmarinus officinalis* L.	Flavonoids	Luis et al. (2007)
UV-B	Low: 7.14 kJ/m^2/day High: 21.42 kJ/m^2/day	7–14 days	*Ginkgo biloba* L.	Quercetin, isorhamnetin and kaempferol	Zhao et al. (2020)
UV-B	82.90 μW cm^{-2}	240 min	*Ginkgo biloba* L.	Flavonoids	Sun et al. (2010)
UV-B	1.13 W m^{-2} and 0.43 W m^{-2}	3 days and 15 days	*Glycyrrhiza uralensis* Fisch. ex DC.	Glycyrrhizin	Afreen et al. (2005)
UV-B	3.38 μw cm^{-2} nm^{-1}	–	*Taxus chinensis var. mairei* (Lemée et Lévl) Cheng et LK Fu	Flavonoids and taxol	Zu et al. (2010)
UV-B	–	13 days	*Nymphoides humboldtiana* (Kunth) Kuntze	Flavonoids	Nocchi et al. (2020)
UV-B	1.26 μW/cm^2	5 min	*Catharanthus roseus* (L.) G. Don	Catharanthine and vindoline	Ramani and Jayabaskaran (2008)
UV-B	9000 μW/cm^2	48 h	*Catharanthus roseus* (L.) G. Don.	Terpene indole alkaloids	Binder et al. (2009)
UV-B	224 μmol m^{-2} s^{-1}	−2 h for 2 days and 2 h for 5 days −1 h for 2 days and 2 h for 2 days	*Ocimum basilicum* L. (Purple Basil) *O. basilicum* L. (Green basil)	Anthocyanins, flavonoids and phenolics	Dou et al. (2019)

(continued)

Table 2 (continued)

Light source	Intensity	Exposure time	Medicinal plant	Secondary metabolite	References
UV-B	102 kJ m^{-2} day^{-1}	3 days	*Ocimum Basilicum* L.	Phenolics	Mosadegh et al. (2018)
UV-B	20 μW cm^{-2}	4 days	*Triticum aestivum* L.	Phenolics	Chen et al. (2019b)
UV-B	4.2 kJ m^{-2} day^{-1}	14 days	*Artemisia annua* L.	Artemisinin and carotenoids	Rai et al. (2011)
UV-B	+1.8 and +3.6 kJ m^{-2} d^{-1}	3 h per day	*Cymbopogon citratus* (D.C.) Staph	Carotenoids and phenolic compounds	Kumari and Agrawal (2010)
UV-B	40 J cm^{-2}	8 h	*Alternanthera brasiliana* (L.) Kuntze *A. sessilis* (L.) R. Br. ex DC.	Flavonoids	Klein et al. (2018)
UV-B	1.14 kJ m^{-2} day^{-1}	4 h per day for 14 days	*Capsicum annum* L.	Flavonoids	Rodriguez-Calzada et al. (2019)

radical inhibition (Aryal et al. 2019). *Prunella vulgaris*, a medicinal plant commonly used to treat thyroid gland disorders, hypertension, hepatitis, and mastitis, also acts as a sedative and anti-febrile agent and exhibit anti-viral, anti-bacterial, anti-diabetic and anti-inflammatory properties (Yu et al. 2021; Bai et al. 2016; Chen et al. 2018; Zhang et al. 2017). UV-B irradiation of 35 μW cm^{-2} nm^{-1} intensity for 15 days enhanced the production of total phenolic content of *P. vulgaris*. UV-B rays also increased the production of total flavonoids, rosmarinic acid and caffeic acid (Zhang et al. 2017). Likewise, Schreiner et al. (2009) showed that phenolic content of *Tropaeolum majus* was vastly enhanced after its exposure to UV-B radiation at an intensity of 0.075 W h m^{-2}. Similarly, elicitation with UV-B radiation for 5 h per day amplified the phenolics and flavonoids production in *Kalanchoe pinnata* (Nascimento et al. 2015). Moreover, elicitation of *Salvia verticillata* with UV-B radiation at an intensity of 10.97 kJ m^{-2} day^{-1} for up to 10 days increased the production of flavonoids and phenolic content such as rosmarinic acid, chlorogenic acid, caffeic acid, and salvianolic acid (Rizi et al. 2021).

Echinacea purpurea is a medicinal herb, popular for alleviating the symptoms of cold. It also has anti-depressant, anti-inflammatory and anti-anxiety properties (Manayi et al. 2015). The callus and cell suspension cultures of *E. purpurea* were irradiated with UV-B for varying periods. Exposure time of 2 h enhanced the production of total phenols and caffeic acid in callus culture while exposure of 4 h increased the levels of caffeic acid and phenolic content in cell suspension culture (Manaf et al. 2016). Likewise, Luis et al. (2007) reported enhanced production of flavonoids i.e., caffeic acid, naringin, cirsimaritin, carnosol, rosmarinic and carnosic acids, on exposure to UV-B irradiation in *Rosmarinus officinalis*. Similarly, *Ginkgo biloba* showed amplification in levels of quercetin, isorhamnetin and kaempferol on exposure to UV-B radiation (Zhao et al. 2020). *G. biloba* also showed raised concentrations of flavonoids when elicited with UV-B radiation at an intensity of 82.90 μW cm^{-2} for 240 min (Sun et al. 2010).

Production of SMs with anti-tumorous activity has also been elevated by elicitation with UV-B. Glycyrrhizin, a triterpenoid saponin, is reported to have anti-tumor, anti-inflammatory, anti-cancer, anti-ulcer, anti-allergic properties and is highly active against HIV-1 and SARS associated viruses (El-Saber Batiha et al. 2020; Srivastava et al. 2019). High-intensity UV-B light of 1.13 W m^{-2} for 3 days and low-intensity UV-B light of 0.43 W m^{-2} intensity for 15 days, both enhanced the production of glycyrrhizin in *Glycyrrhiza uralensis* (Afreen et al. 2005). Likewise, Zu et al. (2010) showed significant accumulation of flavonoids and taxol on UV-B exposure of *Taxus chinensis var. Mairei* at an intensity of 3.38 μw cm^{-2} nm^{-1}. Similarly, UV-B irradiation enhanced flavonoid content and antioxidant activity in *Nymphoides humboldtiana* (Nocchi et al. 2020). Moreover, UV-B radiation of 1.26 μW/cm^2 intensity increased the production of vindoline and catharanthine alkaloids in *Catharanthus roseus* (Ramani and Jayabaskaran 2008). High-intensity of UV-B (9000 μW/cm^2) up to 48 h also enhanced the production of terpene indole alkaloids in *C. roseus* (Binder et al. 2009).

Ocimum basilicum (sweet basil) is widely used in traditional treatments of various ailments like warts, diarrhea, headaches, coughs, and kidney disorders (Joshi 2014). Elicitation by UV-B radiation (224 μmol·m^{-2} s^{-1} intensity) significantly improved the production of anthocyanins, flavonoids, and phenolics in *O. basilicum* (Dou et al. 2019). Likewise, application of UV-B on *O. basilicum* at an intensity of 102 kJ m^{-2} day^{-1} increased the level of phenolic compound production (Mosadegh et al. 2018). The phenolic content of *Triticum aestivum* was also enhanced by UV-B exposure at 20 μW cm^{-2} intensity for 4 days (Chen et al. 2019b). Moreover, increased production of artemisinin and carotenoids could be obtained from *Artemisia annua* by applying 4.2 kJ m^{-2} day^{-1} UV-B radiations for 14 days (Rai et al. 2011).

Cymbopogon citratus (lemon grass) is widely used to treat digestive and nervous system disorders (Hacke et al. 2020). The phenolic content of *C. citratus* was increased with UV-B exposure for 3 h per day (Kumari and Agrawal 2010). The species of *Alternanthera* genus also showed increased production of flavonoids on elicitation with UV-B for 8 h. *Alternanthera sessilis* reported 51% and *Alternanthera brasiliana* showed 62% increase in flavonoid concentration (Klein et al. 2018). Enhanced production of flavonoid was also reported in *C. annum.* leaves on elicitation with UV-B radiation at 1.14 kJ m^{-2} day^{-1} intensity, 4 h per day for 14 days (Rodriguez-Calzada et al. 2019).

2.3 UV-C

Compared to other radiations, UV-C has a greater impact because of its shorter (200–280 nm) wavelengths, which have a higher absorbance. Therefore, most of UV-C radiations are absorbed by ozone layer and few that reaches earth are readily absorbed by mountain peaks (Häder et al. 2007).

UV-C has the potential to damage various chemical bonds in plant cells that lead to photochemical reactions (Kovacs and Keresztes 2002). As a result, oxidative stress develops and ROS are overproduced, which attack various macromolecules in cells that reduces cell viability and eventually leads to cell death (Jansen 2002; Zacchini and de Agazio 2004; Procházková and Wilhelmová 2007; Takeuchi et al. 2007; Toncheva-Panova et al. 2010; Schreiner et al. 2014). The main targets of these radiations are DNA, lipids, proteins, nucleic acids, amino acids, photosynthetic machinery, membranes, pigments and quininones (Jansen 2002; Vass et al. 2005; Edreva et al. 2007; Takeuchi et al. 2007). The damage caused also depends on the sensitivity of plants (Zu et al. 2011; Lavola et al. 2003).

However, when UV-C radiations are provided in low doses, it stimulates UV hormesis (positive effects on plant tissues stimulated by low doses of radiations) (Luckey 2006). Plant cells are able to recover their regular physiological functioning and trigger various acclimation reactions in plant cells such as activation of enzymatic and non-enzymatic defense systems (Loyall et al. 2000; Jansen 2002; Lavola et al. 2003; Katerova and Todorova 2009, 2011; Katerova et al. 2009; Rai et al. 2011). As a result, increased amount of plant SMs are produced (Kreft et al. 2002; Antognoni et al. 2007; Nadeau et al. 2011; Schreiner et al. 2014). Since the biosynthesis pathways are interlinked, the production of some plant SMs increases while for some it decreases. These metabolites possess ROS-scavenging ability that protects plant cells from damage caused by UV-C radiations (Jansen et al. 2008). Hence, elicitation by UV-C radiations can be used as an effective strategy for accumulating SMs in medicinal plants (Marti et al. 2014; Schreiner et al. 2014).

Phenolic compounds, i.e. derivatives of phenols, protect the plant against various stresses including UV light. A study conducted by Cetin (2014) showed increase in the total phenolic content, flavonol content, catechin accumulation, ferulic acid, trans-resveratrol and α-tocopherol in the callus culture of Okuzgozu grape cultivar, when exposed to UV-C for 5 min. Ferulic acid increased sixfolds in comparison with control. Similarly, in *Vitis vinifera* (grape), the antioxidant capacity, phenolic compounds and anthocyanin were greatly increased as a result of thermovinification and UV-C treatment, thus showing the potential to produce antioxidant and phenolic-rich red grapes for use in wine industry (Tahmaz and Söylemezoğlu 2017). Another study conducted by Douillet-Breuil et al. (1999) on *V. vinifera* showed the accumulation of two important classes of stilbenes i.e. ε-viniferin and resveratrol within a time period of 3 days. The concentration of both these compounds was greater than 100 μg/g fresh weight of cultivars. Moreover, a 20-min UV-C irradiation promoted the accumulation of piceids and resveratrols in grape callus, besides increasing the growth of callus. Increased production was observed over a time period of 72 h but the optimum harvest time was 48 h to obtain the highest stilbene accumulation (Liu et al. 2010). Likewise, UV-C treatment on table grapes increased the expression of ANS, STS, CHS and PAL genes, which collectively increased the phenolics, flavonoids and anthocyanins of table grapes (Sheng et al. 2018). In addition, Surjadinata et al. (2017) demonstrated that UV-C exposure to cut carrots increased its antioxidant capacity as well as the concentration of many phenolic compounds, viz. isocoumarins, ferulic acid and chlorogenic acid, while exposure to uncut carrots did not show any prominent effects. Resveratrol, a stilbene, accumulates strongly on exposure to UV-C irradiation at an intensity of 0.282 W m^{-2} (Bravo et al. 2013; Krasnow and Murphy 2004). Amounts of Scoparone and scopoletin (the phenolic coumarins) increased in *Citrus paradisi* due to UV-C exposure. They function as phytoalexins and help in resistance against pathogen (D'hallewin et al. 2000).

Flavonoids play important roles in cosmetic, medicinal and pharmaceutical fields. Their therapeutic importance is attributed to their anti-cancerous, anti-mutagenic, anti-oxidant and anti-inflammatory properties (Panche et al. 2016). Flavonoids possess high UV-screening capacity and mostly accumulate in the upper layer of plant tissues. Treatment with UV-C has been reported to trigger the synthesis of flavonoids in many plants. A study conducted by Rivera-Pastrana et al. (2014) on 'Maradol' papaya showed the accumulation of flavonoid and phenolic compounds in the peel of plant on UV-C treatment. Anthocyanins and compressed tannins, are another class of flavonoids that are produced by phenylpropenoid pathway through the action of chalcone synthase (CHS). This pathway converts p-cumaric acid into chalcones, a flavonoid compound. Tiecher et al. (2013) showed an increased production of chalcones in response to UV-C irradiation. Similarly, UV-C exposure of 10 min on callus culture increased the production of rosmarinic acid 2.3 folds, while a 50 min exposure increased peonidin concentration to 2.7 folds and anthocyanin and chichoric acid concentration to 4.1 folds in *Ocimum basilicum* (Nazir et al. 2020). Also, when *in-vitro* cultures of *Fagonia indica*, (a medicinal plant), were grown under the photoperiod (16L/8D h) after UV-C exposure (5.4 kJ/m^2), maximum accumulation of phenolic (11.8 μgGAE/mg) and flavonoid (4.05 μgGAE/mg) content was observed (Abbasi et al. (2021). Phytochemical production was increased to 6.967 μg/mg DW, as quantified by HPLC. These phytochemicals included isorhamnetin (1.022 μg/mg DW), myricetin (1.022 μg/mg DW), apigenin (1.057 μg/mg DW and kaempferol (1.377 μg/mg DW).

Triterpene are known for their remarkable anti-cancerous activities (Yesilada et al. 2005). Increased concentration of leaf triterpene saponins in the leaves of *Quilaja brasiliensis* were obtained in response to combined stress induced by ultrasound, salicyclic acid, jasmonic acid and UV-C radiations (de Costa et al. 2013). Likewise, when cell cultures of *Linum usitatissimum* were exposed to different photoperiods i.e., 16L/8D h, 24 h light and 24 h dark along with different doses of UV-C radiations (3.6 kJ/m^2), an increase in the production of neolignans, lignans and other bioactive compounds were observed (Anjum et al. (2017). Similarly, *Catharanthus roseus*, another medicinal plant widely known for its potential anti-cancerous activity, has shown increased accumulation of phenolic and flavonoid contents as well as the terpenoid indole alkaloids (TIAs) through the upregulation of genes involved in the biosynthetic pathway of TIAs on exposure to UV-C (Moon et al. 2017). *Lepidium sativum*, and a rich source of polyphenols, showed a 2.5 fold increase in the production (32.33 mg/g DW) of nine SMs in response to 60 min of UV-C exposure. These compounds include three important phytochemicals viz. quercetin, kaemferol and chlorogenic acid. This further enhanced antidiabetic, antioxidant and enzymatic capacity of *L. sativum* callus cultures (Ullah et al. 2019).

It appears from some studies that combined treatments are more effective than individual UV-C treatment with regard to SM accumulation. In berry skins and grape leaves exposed to UV-C in combination with calcium chloride, the accumulation of resveratrol was the highest (greater than fivefolds), compared to when only UV-C was applied (Wang et al. 2013). The expression of 3-O-β-glycosyltransferases, stilbene synthase, coumaroyl-CoA ligase, cinnamate-4-hydroxylase and phenylalanine ammonia lyase also increased, as all are related to resveratrol synthesis. The combination treatment was also involved in delaying the decline of resveratrol when berries were stored at low temperature (−1 ± 0.5 °C, RH 95%) for 27 days. Another study by Xu et al. (2015) discussed the combined effect of salicyclic acid, methyl jasmonate and UV-C on *Vitis vinifera* cv. Cabernet Sauvignon. Methyl jasmonate at 100 μM combined with 20 min of UV-C treatment caused the maximum stilbene concentration (2005.05 ± 63.03 μg g^{-1} DW), while the combined treatment of UV-C and salicyclic acid resulted in increased trans-resveratrol concentration (2.33 ± 0.15 mg l^{-1}) and the maximum stilbene concentration (1630.93 ± 44.17 μg g^{-1} DW) (Table 3).

3 Adaptive Mechanism

In response to UV light stress, pants produce SMs as a defense mechanism and many of these metabolites are used for medicinal purposes (Fig. 2). Since plants are sessile, they have to develop adaptive mechanisms to survive in the altered environment i.e., under exposure to UV radiations. Mild exposure can lead to acclimatization response; however, a severe stress condition can cause metabolic disorders. So, to adapt to such conditions plants alter their morphological traits, physiological characteristics (Yang et al. 2004, 2005) at the biochemical and genetic levels (Kumari et al. 2010; Tripathi et al. 2011). The response to UV radiation depends on multiple factors, which include mainly the duration of exposure and wavelength of radiation. In addition to this, different crop species and cultivars within the same species can have altered responses, depending on their prior exposure to such factors (Ulm and Nagy 2005; Hectors et al. 2007).

The protective measures include the formation of hair, deposition of waxes, and cellular modifications to provide photoprotection by inducing the synthesis of secondary metabolites (Hamid et al. 2019) and production of enzymatic and non-enzymatic antioxidants (Takshak and Agrawal 2019). Studies on various crops have shown that production of SMs increases from 10 to 300% under UV radiation (Kakani et al. 2003). These SMs tend to accumulate in the epidermal layer

Table 3 Some major studies on the effects of UV-C light on the production of key SMs in medicinal plants

Light source	Intensity	Exposure time	Medicinal plant	Secondary metabolite	References
UV-C	254 nm	5 min	Okuzgozu grape cultivar	Total Phenolic content, flavonol content, catechin, ferulic acid, trans-resveratrol and α-tocopherol	Cetin (2014)
UV-C	254 nm	1 h	*Vitis vinifera* L.	Phenolic compounds and anthocyanins	Tahmaz and Söylemezoğlu (2017)
UV-C	254 nm	3 days	*Vitis vinifera* L.	Resveratrol and ε-viniferin	Douillet-Breuil et al. (1999)
UV-C	3.6 kJ m^{-2}	–	Table grapes	Phenolics, flavonoids and anthocyanins	Sheng et al. (2018)
UV-C	11.8 W m^{-2}	15 min	Carrot (*Daucus carota* L.)	Phenolics	Surjadinata et al. (2017)
UV-C	0.282 W m^{-2}	1–12 h	*Solanum lycopersicum* L.	Resveratrol	Bravo et al. (2013)
UV-C	0.5 kJ m^{-2}	4 weeks	*Citrus paradisi* Macfad.	Scoparone and scopoletin	D'hallewin et al. (2000)
UV-C	8220 mWm^{-2}	3 min	Maradol papayas	Phenols and flavonoids	Rivera-Pastrana et al. (2014)
UV-C	3 W m^{-2}	10–50 min	*Ocimum basilicum* L.	Rosmarinic acid, peonidin, anthocyanin and chichoric acid	Nazir et al. (2020)
UV-C	5.4 kJ m^{-2}	30 min	*Fagonia indica* Burm. f.	Isorhamnetin, myricetin, apigenin and kaempferol	Abbasi et al. (2021)
UV-C	10.5 kJ cm^{-2}	–	*Quilaja brasiliensis* (A. St.-Hil. & Tul.) Mart.	Triterpene saponins	de Costa et al. (2013)
UV-C	3.6 kJ/m^2	–	*Linum usitatissimum* L.	Total phenolic content, total flavonoid content Neolignans and lignans	Anjum et al. (2017)
UV-C	720 J/cm^2	60 min	*Catharanthus roseus* (L.) G. Don	Phenolics. flavonoids and terpene indole alkaloids	Moon et al. (2017)

(continued)

Table 3 (continued)

Light source	Intensity	Exposure time	Medicinal plant	Secondary metabolite	References
UV-C	3 W/m^2	60 min	*Lepidium sativum* L.	Quercetin, kaemferol and chlorogenic acid	Ullah et al. (2019)
UV-C + Methyl Jasmonate + Salicyclic acid	10 W m^{-2}	20 min	*Vitis vinifera* L.	Stillbene and trans-resveratrol	Xu et al. (2015)

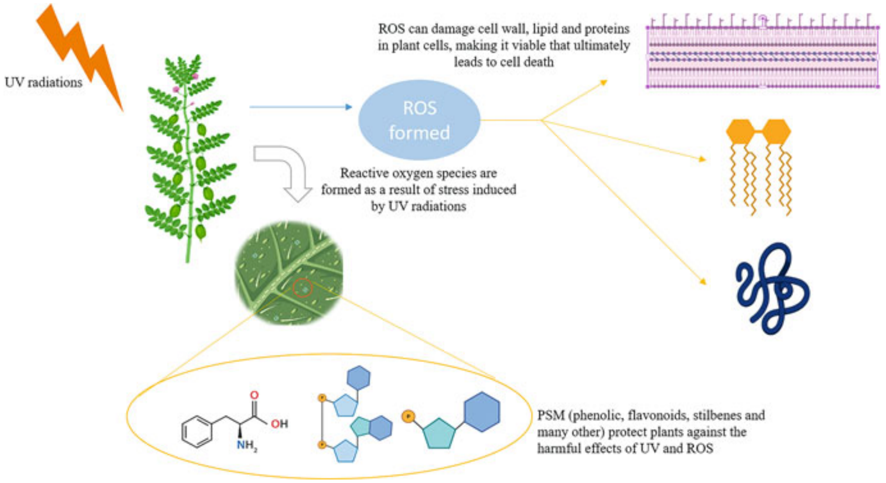

Fig. 2 Responses of plants to UV irradiation. Stress induced by UV produces ROS, which damages cell components leading to cell death. However, as a defensive mechanism plant produces various secondary metabolites to protect itself from the damaging effects caused by the elevation of ROS on exposure to UV

and act as sunscreens to protect the sensitive plant tissues from damage. However, prolonged exposure may lower the protective potential of SMs by reducing the overall photosynthate production. As of early 2013, over 200,000 SMs were identified in various plants, whereas the total number of these molecules in existence is estimated to be much higher (Zhao et al. 2013) (Fig. 3).

3.1 Photoprotection Mechanisms that Produce Secondary Metabolites

3.1.1 Photoprotection by Carotenoids

Carotenoids are crucial in offering photoprotection to chloroplasts and exist in thylakoids in two variants: (a) free carotenoids make only 15% of total carotenoids present, and act as antioxidant by scavenging the ROS formed by light-harvesting complex (LHCs) and reaction-centre complex (RC) (Havaux et al. 2004; Dall'Osto et al. 2010), and (b) carotenoids that are adhered to photosynthetic machinery to remain in close vicinity of Chl molecules (Pinnola and Bassi 2018).

Even though the evolutionary process has generated a lot of diversity over time, the chlorophyll content in land plants is still highly reserved with respect to general conformation and localization of chloroplast structures. Most of the carotenoids adhere to photosynthetic complexes and exhibit continuous dispersal among different components. For example, (1) beta carotene binds to reactive complexes, and

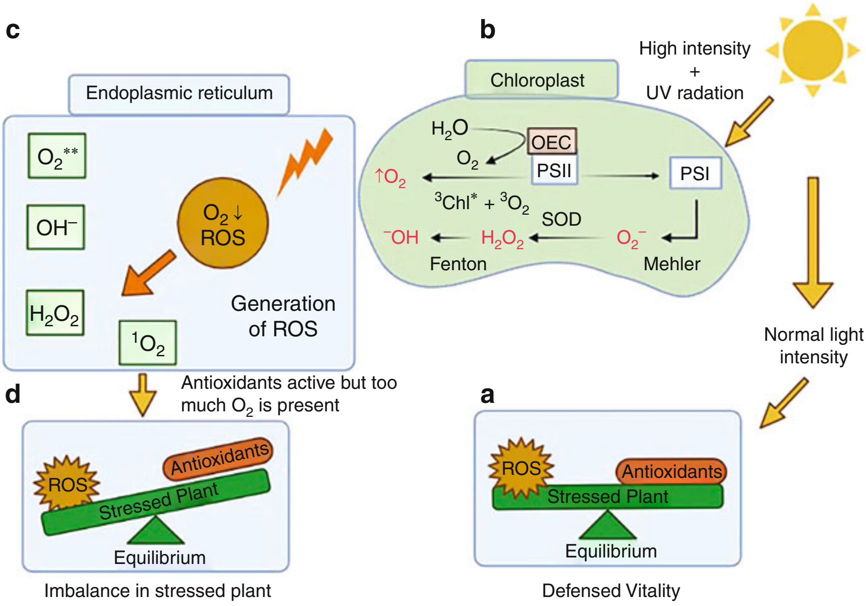

Fig. 3 Localization and generation of ROS in plants (**a**) Plants develop an equilibrium between ROS generation and antioxidants under normal light intensity; (**b**) On exposure of plants to UV radiation, ROS are produced at one of the prime sites, (chloroplasts), by electron transport chain of PSI and PSII. The chlorophyll (Chl) pigment absorbs light photons and gets excited to triplet state leading to the elevation of ROS; (**c**) Secondary site like endoplasmic reticulum also continues to produce ROS; (**d**) Generation of oxidative stress through ROS leads to the activation of antioxidants, which helps in quenching their production and enables plants to maintain their equilibrium state

(2) xanthophylls like Lutein (Lut), Violaxanthin (Viola), and Neoxanthin (Neo) bind to light-harvesting complexes and, if LHS is present in high quantity, then it binds to Zeaxanthin (Zea).

The composition of carotenoid with its binding site being conserved proposes that each molecular species has an exclusive and significant part to play. There are three different photoprotection mechanisms involved in the carotenoid mechanism.

3.1.1.1 Modulation of ^{3}Chl* Yield

This mechanism is used to transfer excitation energy from ^{3}Chl* to carotenoid through the coupling of Chls and carotenoids within Chl-protein complexes. As a result, a carotenoid triplet excited state (^{3}Car*) is produced. With increase in intensity of light, the concentration of ^{3}Car* in leaf (Witt 1971), thylakoids (Jávorfi et al. 2000), isolated photosystems (PS) (Mathis et al. 1979), and light-harvesting complexes (Mozzo et al. 2008; Peterman et al. 1995) also increases. Quenching of

^{3}Chl* helps in the prevention of $^1O_2$ formation. It has been found that Lutein is the amplest xanthophyll species in the photosynthetic apparatus of plant and green algae. It has a significant role in quenching ^{3}Chl*. In addition, it also acts as a scavenger of ROS and is produced through reaction of ^{3}Chl* with O_2. (Formaggio et al. 2001)

$$^3\mathrm{Chl} + \mathrm{Car} \rightarrow {}^3\mathrm{Car} + {}^1\mathrm{Ch1}$$

$$^3\mathrm{Car}^* \rightarrow \mathrm{Car} + \mathrm{heat}$$

3.1.1.2 Scavenging of ROS

In this mechanism, the excitation energy of ROS is transported to carotenoids and yields the ground triplet state of molecular oxygen ($^3O_2$) and ^{3}Car*. The carotenoid triplet decays radiationless into the ground state, whereas the triplet excitation energy is transformed effectively into heat (Edge et al. 1997).

$$\mathrm{ROS}^* + \mathrm{Car} \rightarrow \mathrm{Car}^* + \mathrm{O}_2$$

$$^3\mathrm{Car}^* \rightarrow \mathrm{Car} + \mathrm{heat}$$

3.1.1.3 Modulation of ^{1}Chl* Yield

$$^1\mathrm{Chl}^* + \mathrm{Car} \rightarrow {}^1\mathrm{Car}^* + {}^1\mathrm{Ch1}$$

$$^1\mathrm{Car}^* \rightarrow \mathrm{Car} + \mathrm{Heat}$$

This mechanism involves the binding of violaxanthin and zeaxanthin to regulate the concentration of ^{1}Chl* in isolated pigment-binding protein, as observed by fluorescence analysis. Studies have shown that the effect of modulation is stronger in monomeric light-harvesting complexes as compared to trimeric light-harvesting complexes II, which suggests that its effect is probably associated with the binding site L2, which is used for swapping of violaxanthin to zeaxanthin instead of V1 binding site (Morosinotto et al. 2002). This idea gets support from the finding that V1 binding site occurs only in light-harvesting complex II and light-harvesting complex stress-related (LHCSR). The effect of the quenching mechanism which involves replacement of violaxanthin with zeaxanthin was specifically studied in light-harvesting complex stress-related protein 1 and it was observed through fluorescence that the yield of zeaxanthin reduced by 50% (Pinnola et al. 2013, 2016, 2017). The reason behind this was that energy was transferred from ^{1}Chl* to the zeaxanthin S1 state, followed by the rapid decay of the zeaxanthin ground state (Pinnola et al. 2016).

3.1.2 Other Photoprotective Agents and Components

Avoiding the production of ROS in plants is impossible even though the above-mentioned mechanisms play an excellent protective role. Therefore, other mechanisms take place in chloroplast to deactivate ROS and consequently reduce photodamage. These mechanisms hover around the enzymatic as well as non-enzymatic antioxidant agents (Scandalios 2005).

3.1.2.1 Non-enzymatic Antioxidant Agents

(a) **Prenylquinols**

Prenylquinols are important organic molecules used for scavenging of $^1O_2$. They are fatty acid esters of hydroquinone rings including prenylquinol phosphates (Szymańska and Kruk 2018). Prenylquinols and carotenoids both are categorized as prenyllipids (lipid-soluble compounds). While carotenoids comprise of β-carotene and xanthophylls, the phenylquinols include plastoquinone and tocochromanols such as tocopherol, plastochromanol, and tocotrienols (Pospíšil and Prasad 2014). Tocopherols are biologically interesting molecules because of their capacity to quench $^1O_2$. Physical scavenging, in which the excited state of oxygen is deactivated without light emission, is related with tocopherol scavenging, whereas chemical quenching, which results in the formation of different oxidation products is connected with prenylquinols, which include plastoquinols. Physical scavenging by electron energy transfer almost predominates, with its rate depending upon the polarity of the solvent (Fahrenholtz et al. 1974). While carotenoids are attached to pigment-protein complexes, prenylquinols exist freely in the thylakoid lipid matrix.

There are two major oxidation mechanisms involved when it comes to tocopherols: (a) Oxidation of tocopherol radical through one-electron transfer reaction, and (b) Reaction with $^1O_2$ yielding hydroperoxide, which is equivalent to a two-electron transfer reaction (Neely et al. 1988).

Studies have shown that, in plants, tocopherol co-operates with ascorbate (Müller-Moulé et al. 2003, 2004). It has been seen that mutant plants with decreased concentrations of ascorbate are compensated with a higher concentration of tocopherol. *In vivo* studies show that tocopherols and carotenoids have interrelated functions. The mutant *Arabidopsis thaliana* npq1, that lacks zeaxanthin, accumulates a high concentration of α-tocopherol in the growing leaves, when exposed to excessive light. This suggests that high amounts of tocopherol might compensate for scavenging function of zeaxanthin (Havaux et al. 2000). In similar studies on mutant *A. thaliana vte1* where the plant lacks tocopherol, it was found that zeaxanthin was produced in higher quantities when exposed to excess light (Havaux et al. 2005). Accumulation of α-tocopherol was also found in a double mutant of *Chlamydomonas reiharditii* that lacked npq1 and lor1 genes, which translated for lutein and zeaxanthin, respectively (Baroli et al. 2003). Another interesting study showed a buildup of α-tocopherol in a double mutant, which lacked *npq1* and *lor1*, by expression of homogentisate phytyl-transferase vitamin E2 from *Synechocystis sp*. and induced resistance to other oxidative stresses in *C. reiharditii* (Li et al. 2012). Absence of α-tocopherol has also been associated with the absence of photosynthesis and D1 protein when exposed to excess light (Trebst et al. 2002).

(b) **Ascorbate (Vitamin C)**

It is the most commonly found as a soluble antioxidant in chloroplasts and can achieve concentration range as wide as 20–300 mM during the process of acclimation to excess light. Ascorbate has certain distinct functions such as: photoprotection through scavenging of $^1O_2$, O_2^- and OH, regeneration of α-tocopherol from α-tocopherol radicals, donation of electrons to photosystem II, acting as co-factor for violaxanthin (Viola) de-epoxidase, and acting as a scavenger of hydroxyl ions through ascorbate peroxidase (Smirnoff 2000). Studies on *Arabidopsis thaliana* mutant, which were deficient in ascorbate, have shown that the plants became hypersensitive to oxidative stresses like ozone, UV radiation, high light intensity, and salt stresses (Smirnoff 2000), whereas overproduction of ascorbate was linked with the prevention of photosystem II from heat stresses (Tóth et al. 2011).

(c) **Glutathione**

It plays a significant role in the detoxification of $^1O_2$ and OH^- along with rejuvenation of both α-tocopherol and ascorbate via glutathione-ascorbate cycle (Foyer et al. 1994).

3.1.2.2 Enzymatic Antioxidant System

It involves enzymes like superoxide dismutase, ascorbate peroxidase, catalase, glutathione peroxidase, and peroxiredoxin. It has been found that all these enzymes are localized in all subcellular components. An organelle tends to have multiple enzymes involved in the scavenging of ROS (Scandalios 2005; Mittler 2002; Mittler et al. 2004). The major oxidant produced by photosystem I is O_2^- which is scavenged by superoxide dismutase into H_2O_2. While the hydrogen peroxide (H_2O_2) detoxification system present in the chloroplast is run through the ascorbate glutathione cycle (Asada 1992). Since ascorbate peroxidase is the primary enzyme (Asada 1992), it acts as an electron donor and reduces hydrogen peroxide (H_2O_2) to water (H_2O). Thus, water-water cycle plays a crucial role in photoprotection of photosystem-I.

Under excess of light, photoreduction of O_2 occurs in photosystem-I by yielding O_2^- as the primary product (Mehler 1951), which can be transformed to hydrogen peroxide (H_2O_2) by superoxide dismutase. Followed by the reduction of hydrogen peroxide to water by ascorbate peroxidase, this reaction scavenges excess electrons and reduces excitonic pressure on photosystem-I but yields ΔpH with the consumption of ATP molecules (Pinnola and Bassi 2018).

4 Conclusions

Environmental stresses have been exploited widely for the overproduction of SMs in biological systems, particularly in plants. There lies a lot of scope and potential for screening of a wide range of pharmaceutically important metabolites with respect to the use of light as elicitor. Out of the three types of UV light, UV-B has been exploited the most, followed by UV-C. Photoprotection and up-regulation of enzymatic and non-enzymatic antioxidants involve plant signalling networks and their regulatory role towards the secondary metabolite synthesis. Thus, manipulating UV irradiation may pave the way for obtaining the maximum productivity or altering the metabolic flux of plants producing SMs of therapeutic value. Besides, UV radiations, possibly in combination with other biotic and abiotic elicitors, may be helpful in elicitation of SMs production.

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Recent Strategies to Engineer Alkaloid Biosynthesis in Medicinal Plants

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Abstract Plants are known to synthesize a plethora of secondary metabolites viz., terpenoids, terpenes, flavonoids and alkaloids. Of these, alkaloids belong to a major class of phytoconstituents, which are responsible for many properties of therapeutic interest such as analgesic, antimicrobial, antimalarial, antipyretic, antihypertensive, cardioprotective, anticancer and antiarrhythmic. Consequently, plants possess several biosynthetic pathways which are critical for alkaloid biosynthesis. The recent involvement of commercial interest has triggered the exploration of techniques for altering the production of alkaloids. Moreover, the amount of natural production of alkaloids is low and vulnerable due to the dependency on climatic conditions. Considering the above facts, different techniques such as transcription factors plant tissue culture, miRNA-mediated manipulation of these secondary metabolites, gene editing by CRISPR/Cas9, and nanoparticles (NPs)-based enhancement are being extensively used to stabilize and enhance alkaloid production for the large-scale use at the industrial level. Additionally, a detailed understanding of alkaloid biosynthetic pathway genes, transcription factors and their mode of action is essential for the improved production of important alkaloids. This chapter is focussed on reviewing a set of biotechnological tools that are the potential candidates for improved biosynthesis of different alkaloids, which can further revolutionize the pharmaceutical industry.

Keywords Secondary metabolites · Alkaloids · Plant tissue culture · CRISPR-Cas9 · Genes · Nanoparticles · Drug discovery

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A. Husen, M. Iqbal (eds.), *Medicinal Plants*,
https://doi.org/10.1007/978-981-19-5611-9_15

1 Introduction

Alkaloids are a class of naturally occurring nitrogen-containing organic compounds, having low molecular weight, usually found in about 20% of plant species (Kohnen-Johannsen and Kayser 2019). Research on plant alkaloids began in the nineteenth century. In 1804, Friedrich Wilhelm Adam Serturner, the German chemist, isolated a "soporific principle" from opium, which he called "morphium", referring to Morpheus. Joseph Louis Gay-Lussac first used the term "morphine". In the early stages of development, Pelletier and Caventou had given considerable contribution to the alkaloids chemistry by discovering strychnine and quinine. Various other alkaloids were also reported around that time, including xanthine, caffeine, atropine, colchicines, coniine, nicotine, sparteine, and many others. By 2008 more than 12,000 alkaloids had been identified due to emergence of chromatographic and spectroscopic methods in the twentieth century that had led the development of the chemistry of alkaloids and their functional role in plants' defense against herbivores and pathogens (Caporale 1995; Wink 1999).

With reference to alkaloid biosynthesis, three major technical advancements have led to veritable development in phytochemistry. The first includes the radio labeled precursor's introduction that permitted the chemical explication of the biosynthesis of alkaloids in 1950s. Second, the better use of plant cell cultures as an ample source of biosynthetic enzymes during the 1970s. These enzymes could further be isolated, purified, and characterized. Finally, in the 1990s, the extensive application of molecular methods led to the identification of many genes involved in alkaloid biosynthesis such as indole, tropane, and benzylisoquinoline. The initial events of signal transduction pathways, signal perception and gene promoters function have since been scrutinized in connection with the alkaloid metabolism regulation.

Many biologically active alkaloids have been reported for decades and used as an important source of medicines. These alkaloids are produced by diverse group of organisms such as plants, animals, bacteria, and, fungi (Goyal 2013). Plant extracts containing the naturally occurring alkaloids have been used across the globe to cure several diseases (Sofowora et al. 2013; Atanasov et al. 2015, 2021).

Alkaloids have a large number of biological activities including antiasthma, antimalarial, anticancer, vasodilatory, antiarrhythmic, antihyperglycemic, analgesic, an antibacterial activities (Marella et al. 2013; Russo et al. 2013; Cushnie et al. 2014; Kittakoop et al. 2014; Qiu et al. 2014; Thawabteh et al. 2019). They are also used in traditional or modern medicine, or as precursors for the discovery of a new drug. Several other alkaloids have various pharmacological activities such as stimulants (e.g., nicotine, cocaine, caffeine, theobromine) and psychotropic activities (e.g. psilocin) (Hussain et al. 2018; Kurek 2019). Only a small number of those naturally occurring alkaloids have been scrutinized through appropriate laboratory tests. Moreover, alkaloids production by natural means is quite vulnerable because of the dependency on climatic condition, hence the production of alkaloids is limited. However, the recent involvement of commercial interest in the field has triggered the exploration of techniques for altering the production of alkaloids. A detailed understanding of alkaloid biosynthetic pathway genes, transcription factors and their mode of action is essential for improved production of alkaloids. Considering the above facts, different techniques such as plant tissue culture, gene editing by CRISPR/Cas9, miRNA-mediated manipulation of these secondary metabolites and nanoparticles (NPs)-based enhancement are being used extensively with the aim to elevate and stabilize the SM production for a large-scale use at industrial level. This chapter focuses on a set of biotechnological tools that have the potential to enhance the production of different alkaloids which can further be used for drug discovery.

2 Alkaloids Biosynthetic Pathway

The initial steps in the alkaloid biosynthetic pathway are the most critical, as they direct the flux away from primary and towards the particular metabolism. They are chemically and enzymatically important because they are engaged in the production of new molecular structures (Kishimoto et al. 2016). Moreover, they play an introductory role in the pathways' evolution.

Four major steps involved in the first phase of biosynthesis of complex alkaloids: (1) amine precursor accumulation, (2) aldehyde precursor accumulation, (3) iminium cation formation and (4) a Mannich-like reaction, which involves the "scaffold-forming" (Lichman 2021). The most important difference in these general steps is whether they are intermolecular or intramolecular. For example, alkaloids derived from polyamine usually form a cyclic iminium through intramolecular condensation, followed immediately by an intermolecular Mannich-like reaction. On the contrary, the Pictet–Spengler reaction produces major families of alkaloids, such as the benzylisoquinolines that involve the production of an iminium intermediate through intermolecular condensation, subsequently by an intramolecular Mannich-like reaction (Lichman 2021).

3 Transcriptional Factors-Mediated Regulation of Alkaloids

Transcription factors are the master proteins inside the living cells that show a sequence-specific DNA binding. They are known to activate or repress transcription of a wide range of target genes. They tend to bind with *cis*-regulatory elements such as enhancer or DNA promoter region lying adjacent to the genes. Many biological processes are being controlled by the regulation of gene expression. Many TFs showed specific interaction with one another that ultimately control many biological processes such as response to the environment, cell cycle progression, metabolism, and development (Dehghan Nayeri 2014). Thus, TF-mediated regulation occurs in a cell-specific manner as well as at the cellular level. The TF activity is regulated at the transcriptional level. Also, the transcriptional regulation establishes several vital agronomical traits (Li et al. 2019). Therefore, studies on the location and relative expression of TFs would prove to be highly valuable in elucidating the role of proteins interaction and their biological functions.

Numerous TFs have been identified and characterized that are involved in the alkaloid biosynthesis in many plant species, including those of steroidal glycoalkaloids such as tomatine in *Solanum lycopersicum*, solanine in *Solanum tuberosum*; nicotine alkaloids in *Nicotiana* species; monoterpenoid indole alkaloids (MIAs) such as vincristine and vinblastine in *Catharanthus roseus*; morphine in *Papaver somniferum* and benzylisoquinoline alkaloids such as berberine in *Coptis japonica* (Yamada and Sato 2013). A number of TFs have been reported to regulate the alkaloid biosynthetic pathway (Table 1).

3.1 AP2/ERF Transcription Factors

ORCA1 and ORCA2, belonging to the family AP2/ERF, were the first transcription factors (TFs) isolated that regulate the alkaloid biosynthesis in *Catharanthus roseus* (Liu et al. 2016). The JA-responsive regions in *Strictosidine synthase* (*STR*) promoter were manipulated for isolating these TFs by using the yeast one-hybrid method (Menke et al. 1999; Pré et al. 2000; Siberil et al. 2001). Later, using the activation tagging approach, another AP2/ERF-domain protein, ORCA3, was isolated from *C. roseus* (van der Fits and Memelink 2000). Belonging to different subfamilies, these TFs ORCA1 and ORCA2 are involved in the drought-responsive expression of the gene, ORCA1 homologous to DREB2A and DREB2B (Sakuma et al. 2002), whereas in defense-responsive expression of a gene, ORCA2 is highly homologous to tomato Pti4 and tobacco EREBP (Ohme-Takagi and Shinshi 1995; Gu et al. 2000). Moreover, ORCA1 had little transcriptional activity in vivo but it shows in vitro binding with the JA-responsive element of *STR* promoter, and does not induce the *ORCA1* gene expression when treated with methyl-JA (MeJA). On the contrary, ORCA2 not only shows transcriptional activity in vivo but also binds to the JA-induced response, thus suggesting that ORCA2 promotes the transcriptional activity of *STR* promoter (Menke et al. 1999). Several MIA biosynthetic genes were induced by the *ORCA3* gene overexpression e.g., *STR*, *desacetoxyvindoline 4-hydroxylase* (*D4H*)), *tryptophan decarboxylase* (*TDC*), and *cytochrome P450 reductase* (*CPR*). Overexpression of *ORCA3* helps in the accumulation of tryptamine and tryptophan, which ultimately enhance the overall production of MIA, thus suggesting that ORCA3 is a positive regulator of MIA biosynthesis. Recently, ORCA4, ORCA5, and ORCA6 have been reported to be present in the same genomic region, which the ORCA2 and ORCA3 belong to (Paul et al. 2017, 2020; Singh et al. 2020).

Also, in tobacco AP2/ERF TFs have been isolated and characterized. These TFs produce a broad range of secondary metabolites such as anatabine, nicotine, and

Table 1 List of Transcription factors (TFs) regulating the alkaloid biosynthesis in medicinally important plants

TFs family	TFs name	Plant species	References
AP2/ ERF	NtERF32	*Nicotiana tabacum*	Sears et al. (2014)
	NtERF189	*Nicotiana tabacum*	Shoji et al. (2010)
	NtERF221	*Nicotiana tabacum*	De Boer et al. (2011)
	GAME9/ JRE4	*Solanum lycopersicum*	Cárdenas et al. (2016) and Thagun et al. (2016)
	OpERF2	*Ophiorrhiza pumila*	Udomsom et al. (2016)
	CrERF5	*Catharanthus roseus*	Pan et al. (2019)
	ORCA3	*Catharanthus roseus*	van der Fits and Memelink (2000)
	ORCA4	*Catharanthus roseus*	Paul et al. (2017)
	ORCA5	*Catharanthus roseus*	Paul et al. (2020)
	ORCA6	*Catharanthus roseus*	Singh et al. (2020)
WRKY	OpWRKY1	*Ophiorrhiza pumila*	Xu et al. (2020)
	OpWRKY2	*Ophiorrhiza pumila*	Hao et al. (2021)
	OpWRKY3	*Ophiorrhiza pumila*	Wang et al. (2019)
	PsWRKY	*Papaver somniferum*	Mishra et al. (2013)
	CrWRKY1	*Catharanthus roseus*	Suttipanta et al. (2011)
	CjWRKY1	*Coptis japonica*	Kato et al. (2007)
bHLH	CrMYC1	*Catharanthus roseus*	Chatel et al. (2003)
	CrMYC2	*Catharanthus roseus*	Zhang et al. (2011)
	SlMYC2	*Solanum lycopersicum*	Cárdenas et al. (2016)
	RMT1	*Catharanthus roseus*	Patra et al. (2018)
	NtMYC2a	*Nicotiana tabacum*	Zhang et al. (2012) and Sui et al. (2021)
	NtMYC2b	*Nicotiana tabacum*	Zhang et al. (2012) and Shoji and Hashimoto (2011a, b)
	NbbHLH1	*Nicotiana benthamiana*	Todd et al. (2010)
	NbbHLH2	*Nicotiana benthamiana*	Todd et al. (2010)
MYB	BPF-1	*Catharanthus roseus*	Van der Fits et al. (2000) and Li et al. (2015a, b)
	OpMYB1	*Ophiorrhiza pumila*	Rohani et al. (2016)
bZIP	GBF1	*Catharanthus roseus*	Siberil et al. (2001)
	GBF2	*Catharanthus roseus*	Siberil et al. (2001)

anabasine (Imanishi et al. 1998). Using the luciferase-reporter assay, the transactivation activities of NtORC1/ERF221 and NtJAP1/ERF10 were established by the measuring expression of *PMT* (De Sutter et al. 2005; Rushton et al. 2008). Overexpression of *NtORC1/ERF221, ORCA3* homologue showed enhanced productivity of nicotine and other alkaloids containing pyridine ring (De Boer et al. 2011). Moreover, the *NtJAP1/ERF10* overexpression showed no such significant findings, thus suggesting that NtORC1/ERF221 plays a vital role in nicotine biosynthesis. Later, co-expression analysis in *Nicotiana benthamiana* revealed that *NtORC1/ERF221* and *NbbHLH1* TFs have synergistic effect on *PMT* and *QPT* expression (De Boer et al. 2011). Shoji et al. (2010) independently isolated the AP2/ERF domain transcription factor genes using the microarray technology in *nic* mutant of tobacco. This *nic* mutant has a reduced level of nicotine, which lacks *NIC1* and *NIC2* regulatory loci (Hibi et al. 1994; Katoh et al. 2007; Shoji et al. 2009). Furthermore, the expression level of nicotine biosynthesis genes such as *PMT*, *QPT, A622*, *AOX*, *QS* and *ODC* was found to be on the lower side along with *NtERF189* TF. Recently, Sui et al. (2019) have reported the regulatory role of *NtERF91* during alkaloid biosynthesis and its accumulation in tobacco. It has also been observed that nicotine biosynthesis and its biosynthetic enzyme genes were induced by MeJA in tobacco hairy roots. Silencing of *NtERF189*-through *RNAi* suppressed MeJA-induced biosynthesis of nicotine. However, there is a significant increase in the expression of *PMT* and *QPT* genes roots that overexpressed *NtERF189* with no MeJA-treatment. Alternatively, *NtERF179* overexpression showed a marginal increase in the transcript level of *PMT* and *QPT* and thus increased the overall content of alkaloid in both conditions i.e., MeJA-treated and untreated ones (Shoji et al. 2010). In conclusion, AP2/ERF TFs are the transcriptional activators of nicotine biosynthesis that control the JA-signaling cascade, even though they might have diverse functions.

3.2 WRKY Transcription Factors

WRKY TFs are the largest TF families that regulate the genes responsible for many growth and developmental processes in plants and act as the major regulators in defense responses to biotic and abiotic stresses. Kato et al. (2007) first isolated the WRKY gene from *Coptis japonica* cultured cells, which generally produce berberine-type alkaloids. Silencing of CjWRKY1 through RNAi showed a reduced expression of berberine biosynthetic genes. However, RNAi of CjWRKY1 did not alter the expression of other genes that control the berberine biosynthesis such as *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*), *dehydroquinate shikimate dehydrogenase* (*DQSDH*), and *chorismate mutase* (*CM*), and *3-deoxy-D-arabino heputulosonate 7-phosphate synthase* (*DAH7PS*). Additionally, overexpression of *CjWRKY1* showed increased expression levels of the berberine biosynthetic genes, while primary metabolism remained unaffected. These investigations suggested that

CjWRKY1 plays a crucial role in regulating the biosynthesis of berberine in *C. japonica* (Kato et al. 2007).

The role of the WRKY transcription factor in MIA biosynthesis has also been investigated. A large number of *WRKY* genes were isolated and characterized from *Catharanthus roseus* in MeJA-induced tissues (Suttipanta et al. 2011). It was observed that *CrWRKY1* was highly expressed in roots that showed a response to ethylene, gibberellic acid, and MeJA. However, in hairy roots, the overexpression *CrWRKY1* gene resulted in enhanced expression of *TDC*, while there is repression of both the *ORCA2* and *ORCA3* genes, whereas the *STR*, *CPR, and G10H* genes showed no alteration in expression. CrWRKY1 bound to the W-box in the *TDC* promoter directly, suggests that CrWRKY1 might activate the *TDC* gene and suppress the expression *ORCA* genes. Further, the overexpression of *CrWRKY1* increased the accumulation of serpentine while the catharanthine accumulation was reduced significantly (Suttipanta et al. 2011). Therefore, CrWRKY1 positively controls the serpentine biosynthesis in roots by promoting the *TDC* or by suppressing the *ORCA* genes.

3.3 bHLH Transcription Factors

The first basic Helix-Loop-Helix (bHLH) TF elucidated in alkaloid biosynthesis is *CrMYC1* which belongs to group B and was isolated and characterized using the yeast one-hybrid method (Chatel et al. 2003). Upon MeJa treatment, the expression of *CrMYC1* gene was found to be upregulated; however, this up-regulation was slower than that of *STR* (Chatel et al. 2003). MYC2, belonging to the bHLH TF family, was reported to be involved in regulating the biosynthesis of nicotine alkaloid (Todd et al. 2010; Zhang et al. 2012; Shoji and Hashimoto 2011a). Todd et al. (2010) isolated new regulatory genes involved in the nicotine alkaloid biosynthesis by performing functional screening in two steps. They first prepared the cDNA library to isolate new TF genes from roots of *Nicotiana benthamiana* treated with MeJA, and later performed virus-induced gene silencing where they showed suppression of these genes.

This investigation revealed that repression of *NbbHLH1* and *NbbHLH2* TFs (bHLH transcription factor genes) significantly decreases nicotine content in the leaf tissues upon MeJA-treatment (Wang et al. 2015). Alternatively, silencing of *NbbHLH1* and *NbbHLH2* through RNAi considerably reduces the transcript levels of *PMT*, *QPT*, *QS, MPO, AOX* and *ODC* genes, while overexpression of these TFs marginally increases the nicotine level. Similarly, *NtMYC2* genes were isolated from *Nicotiana tabacum*, which are homologue to *NbbHLH1* and *NbbHLH2*. RNA-silencing of *NtMYC2a* and *NtMYC2b* showed lower expression of genes associated with nicotine and berberine biosynthesis (Shoji and Hashimoto 2011a; Zhang et al. 2012). Furthermore, the inhibition of *NtMYC2b* led to decreases in the transcript level of AP2/ERF (Shoji and Hashimoto 2011a), suggesting that the expression of nicotine biosynthetic genes is directly controlled by NtMYC2.

Another bHLH TF, i.e. CrMYC2, has been isolated from *C. roseus* in MIA biosynthesis (Zhang et al. 2011). The expression of *CrMYC2* gene was found to be highly upregulated in the MeJA-treated tissues.

RNA silencing of CrMYC2 showed a significant reduction of both MIA accumulation in MeJA-treated plants and *ORCA3* transcript level of *C. roseus* cultured cells, while an insignificant reduction of expression in the *STR* and *TDC* genes was observed. *CrMYC2* overexpression induces the transcript level of the *ORCA2* and *ORCA3* gene, which suggests that *CrMYC2* activates the expression of *ORCA3* upon MeJA treatment. Zhang et al. (2011) reported that the expression of STR promoter is not directly upregulated by *CrMYC2*, while NtMYC2 is. Additionally, with the *CjbHLH1* (non-AtMYC2-type bHLH transcription factor) sequence, a BLAST search of ESTs of BIA-producing plants revealed that only extremely homologous genes were retrieved (Yamada et al. 2011). Silencing of *CjbHLH1* expression through RNAi results in a decreased expression of the berberine biosynthetic genes, while the expression of genes associated with primary metabolism remains unaltered. However, the upregulation of *CjbHLH1* genes moderately increases the expression of berberine biosynthetic genes. These investigations suggested that *CjbHLH1* acts as a positive regulator of berberine biosynthetic genes and other transcription factors associated with the regulation of berberine biosynthesis (Yamada et al. 2011; Yamada and Sato 2021).

3.4 Other Transcription Factors

Another elicitor-responsive element was identified located in the upstream region of the *STR* promoter. Using the yeast one-hybrid method, *CrBPF-1* gene was isolated with the help of this cis-element. CrBPF1 is a MYB-like protein that regulates the expression of the TIA biosynthetic gene *STR* (Li et al. 2015a, b). Overexpression of the *CrBPF1* gene resulted in increased expression of both the indole and terpenoid biosynthetic pathways genes. Additionally, *CrBPF1* overexpression showed enhanced expression of 11 out of 13 genes that are assumed to act as transcriptional regulators of TIA and other associated pathway genes. Interestingly, an enhanced expression for both TIA transcriptional activators and repressors was observed for *CrBPF1* overexpressed lines (Li et al. 2015a, b; Liu et al. 2016). CrBPF-1 promotes the expression of *STR* promoter, although its activity was found to be weak in comparison to ORCA2. Additionally, Zhang et al. (2011) reported that the combination of CrBPF-1 and CrMYC2 did not direct the *STR* promoter expression, therefore, indicating that CrBPF-1 could not interact with CrMYC2.

Plant MYB TFs can be divided into four groups such as R2R3-MYB, R1R2R3-MYB, 4R-MYB, and MYB-related proteins. Many R2R3-MYB TFs have been found involved in controlling the specified metabolites, such as camalexin, glucosinolate, and flavonoid biosynthesis (Frerigmann et al. 2015; Zhou and Memelink 2016), but only a few MYB TFs have been reported so far that are engaged in the regulation of alkaloid biosynthesis (Rohani et al. 2016). Also, two bZIP TFs have been identified in C. roseus. Similarly, C2H2-type zinc finger proteins, GATA TF, and AT-hook motif nuclear localized TFs have also been reported. Moreover, three TFs genes that encode TFIIIA-type zinc finger proteins such as *ZCT1*, *ZCT2*, and *ZCT3* have also been reported to negatively control the expression of the *TDC* and *STR* promoters. ZCT1 silencing showed little variation in the transcript levels of MIA biosynthetic genes and MIA accumulation, thus suggesting that the three ZCT repressors might function redundantly (Rizvi et al. 2016). Since CrMYC2 and ORCA TFs stimulate the expression of ZCT genes (Sui et al. 2018; Singh et al. 2020), ZCT TF might fine-tune the spatiotemporal expression of MIA biosynthesis genes (Pauw et al. 2004).

4 Improvements to Enhance In Vitro Alkaloids Production

The alkaloid production depends highly on the developmental and physiological stages of the plant or its cells. Various reports have established that endophytic fungi and plants produce secondary metabolites via mutualistic symbiosis. Fungal endophytic, *Choanephora infundibulifera* and *Curvularia* spp. found in *C. roseus* stimulate vindoline amount by 229–403% (Pandey et al. 2016). The *Taxus chinensis* suspension cells co-cultured with *Fusarium mairei*, produce 38 times higher paclitaxel within 15 days than that by uncoupled culture. Along with genetic engineering, many metabolic manipulations have been applied to attain the desirable production of alkaloids at industrial scale. Two of these metabolic manipulations were: (1) screening and selection of highly produced cell lines and stimulation of biosynthetic activities via numerous methods and (2) optimization of growth and production medium.

4.1 Metabolic Manipulations

4.1.1 Cells Screening

To attain highly industrious cell clones, germplasm screening method for selection is implied. The callus culture of *Rauvolfia serpentina* was categorized into yellow-green and blue-white fluorescent cell strains under 365 nm UV light, resulting in the production of more reserpine by the yellow-green strain than by the blue-white strain (Yamamoto and Yamada 1987). In five transformed hairy root clones of *C. roseus* grown in liquid culture, the levels of ajmalicine, catharanthine and serpentine alkaloids in these clones were higher in comparison to other cell suspensions. The research also suggested the presence of 3 times higher vindoline content in two clones than in other reported cell cultures (Bhadra et al. 1993). Reports suggest that from eleven cell lines of *C. roseus* originated from protoplast the catharanthine and ajmalicine production was substantially higher when the cell aspect ratio i.e. cell length: width was greater than 2.8 (Kim et al. 1994).

4.1.2 Elicitors

In nature, production of various secondary metabolites is immediately increased when the plant confronts the abiotic or biotic environmental stresses. It is believed that certain secondary metabolites work as defensive agents against the stresses. This led researchers to use elicitors or artificial stresses such as drought, growth retardants, low or high temperature, yeast extract, a saline medium, fungal carbohydrates, MeJA, microbial toxin and chitosan, usually in in vitro cultures, to amplify the production of the desired compound. The vincristine or vinblastine production was not affected by incorporation of a drought-producing agent, PEG (Polyethylene glycol), in the *C. roseus* callus culture media (Iskandar and Iriawati 2016). Approximately, four times higher ajmalicine was yielded than the control, when *C. roseus* callus culture was treated with growth stressing agents, mannitol and KCl (Zheng and Wu 2004; Zhao et al. 2001). Further, a twofold rise in the galanthamine concentration was observed on addition of MeJA initially during the cultivation of *Leucojum aestivum* cell culture (Ivanov et al. 2013). The enhanced production of atropine and biomass accumulation has been observed in hairy root cultures of *Datura metel*, when abiotic, nanosilver and $AgNO_3$; and biotic, *Bacillus cereus* and *Staphylococcus aureus*, elicitors were incorporated in *D. metel* (Shakeran et al. 2015). The atropine amount in hairy roots was greatly enhanced by nanosilver compared to other elicitors. The rise in the concentration of hyoscyamine and scopolamine was observed in altered roots of *Brugmansia candida* on chitosan incorporation (Pitta-Alvarez and Giulietti 2019).

4.1.3 Culture Conditions Optimization

To achieve the maximum and harmonious production of the desirable substance, it is essential to optimize the culture conditions and culture media, along with the basic conditions necessary for cell culture to attain an adequate cell-growth rate containing a particular level of alkaloids. The equivalent combination of NAA and BA produces the maximum biomass accumulation and hyoscyamine alkaloid production. The growth rate of culture was higher when grown in light and lower when grown in dark; in contrast the alkaloid amount was somewhat higher under dark conditions. Moreover, cell suspension cultures with low inoculum density gave the shortest doubling time, i.e., 2.93 days, while cultures with a high inoculum attained 13.47 days. When the liquid MS medium was accompanied with kinetin in the cell culture of *Aconitum napellus*, a high growth rate and a greater aconitine production were observed. A system has been established for cell suspension cultures of *C. roseus* to increase both the accumulation and catharanthine growth in a single-stage culture of 14–21 days, by manipulation of a standard MS culture medium, with optimized inclusion of lactose (as carbohydrate source), kinetin and NAA (as growth regulators) (Smith et al. 1987). Thus, the production of a certain amount of alkaloids by culturing cell suspension in a bioreactor requires a balanced combination of internal culture conditions and technical design. Optimization of pH medium, gas exchange flow rate, agitation speed, dissolved oxygen, supply of nutrients, cell inoculum density, and temperature are amongst the dynamics of custodian importance (Ruffoni et al. 2010; Georgiev and Weber 2014).

4.1.4 Genetic Engineering

Numerous achievements have been established for manipulating several metabolic pathways by using particular genes which encode regulatory proteins or biosynthetic enzymes. The level of expression of the *CrRR2* gene from *Catharanthus roseus* is very low; even the treatment with cytokinins does not trigger its transcription. In contrast, the expression level of the *CrRR3* gene from *C. roseus* is root specific and the transcripts are rapidly upregulated and remain unaltered even when treated with abscisic acid, NaCl or jasmonic acid; however, it was raised with trans-zeatin (Papon et al. 2003). Based on the information about biosynthetic pathways, a logical strategy must be established to provoke the transcript levels upregulation of pathway genes. In MeJA-elicited cultures of *C. roseus*, the expression levels of *tdc, str*, and *sgd* was three-nine times higher after 24 h. The increase in gene expression levels was upregulated in ajmalicine, strictosidine, and tabersonine after 5 days (Glokany et al. 2009).

5 miRNA-Mediated Regulation of Alkaloid Biosynthesis

Functional proteins which are encoded by genes depend on various regulatory factors such as transcription factors (TFs), microRNAs (miRNAs), etc. Among these, miRNAs are small endogenous, non-coding RNAs of about 20–24 nucleotides length, which mainly regulate the gene expression at the post-transcriptional level by the degradation of their target mRNAs or by translational inhibition. They play a crucial role in the biosynthesis of plant secondary metabolites, particularly for medicinal plants. Various studies have shown that plant miRNAs tend to target TFs involved in various growth and development processes (Liu et al. 2016). Thus, any change in the expression level of miRNAs leads to significant alteration in the growth and developmental processes, which ultimately affect the production of metabolites in a plant cell.

Plant secondary metabolites are mainly active components, which are the basis of medicinal effects of a plant. It is important to study the biosynthetic pathways and their complex regulatory networks in medicinal plants. In the recent years, miRNAs have emerged as a potent candidate for enhancing the plant secondary metabolites including alkaloids. The genetic engineering technique for targeting the coding regions of transcripts by a conventional method to enhance the bioactive compounds is limited. However, miRNAs act as negative regulators of the gene by targeting several genes in a complex and are known to be the key players in plant regulatory network. For this reason, miRNAs act as a novel target for directly increasing the metabolite production (Sabzehzari and Naghavi 2019; Waheed and Zeng 2020). Several studies have shown that the alteration of a single miRNA gene could drastically improve both the metabolite production and the crop yield under different circumstances like stress or fluctuation in temperatures (Sabzehzari and Naghavi 2019).

Many researchers have reviewed the biogenesis, structure and functions of miRNAs in medicinal plants (Bartel 2004, 2009; Sabzehzari and Naghavi 2019). There is a separate database for miRNA, viz. miRbase, which stores information (such as sequence, structure, function and source) associated with miRNAs. A total of 38589 miRNAs have been reported from different species (miRBase v22.1). To obtain enhanced production of important secondary metabolites through miRNAs is an interesting strategy. Numerous studies have been conducted on unraveling the regulatory role of miRNAs in the production of alkaloids from important medicinal plants.

5.1 miRNAs-Mediated Regulation of Alkaloids in Medicinal Plants

5.1.1 miRNAs Regulating Alkaloids Biosynthesis in Opium Poppy

Opium poppy (*Papaver somniferum*) is known to produce various alkaloids such as morphinan and has been widely studied from diverse medicinal perspectives (Ziegler and Facchini 2008). In the capsule latex, poppy synthesizes important secondary metabolites, i.e., benzylisoquinoline alkaloids (BIAs). However, the content of BIAs is limited in the capsule latex. In poppy, a total of 20 miRNAs had been identified and validated (Unver et al. 2010). Boke et al. (2015) have shown that miRNAs (pso-miR13, psomiR408, and pso-miR2161) are involved in the biosynthesis of BIAs. They predicted that pso-miR13 targets *7-OMT*, and psomiR408 targets a reticuline oxidase-like protein, while pso-miR2161 targets *4-OMT* genes. The *7-OMT* and *4-OMT* genes are involved in the conversion S-reticuline to slaudanosin and (S)-3′-hydroxy-N-methylcoclaurine to S- reticuline, while reticuline oxidase converts S-reticuline to (S)-scoulerine in BIA biosynthesis. Therefore, these miRNAs can be used as a potential tool for manipulation of morphine content in this important medicinal plant.

5.1.2 miRNAs Involved in the Nicotine Biosynthesis

Tobacco *(Nicotiana tabacum)* is commonly used as a model organism for investigating the biosynthesis of alkaloids. It is known for the production of many secondary metabolites including alkaloids such as nicotine, used in pharmaceuticals, chemicals and pesticides industries. Nicotine biosynthetic pathway has been comprehensively studied; however, the miRNA-mediated regulation of nicotine biosynthesis is poorly understood. Li et al. (2015a, b) elucidated the possible role of miRNA-mediated regulation of nicotine biosynthetic pathway. They identified miRX17, miRX19, miRX20 miRX27 that target *QPT1*, *PMT2, CYP82E4* and *QPT2* genes, respectively. These genes (like *QPT1*, *QPT2*, *CYP82E4*, and *PMT2*) control the nicotine biosynthesis and its accumulation in tobacco. QPT convert quinolinic acid to nicotinic acid mononucleotide, PMT2 converts putrescine to N-methylputrescine, while as CYP82E4 coverts nicotine to nornicotine, respectively. Fu et al. (2013) showed that the downregulation of miR164 after topping increases the nicotine content by upregulating the *NtNAC-R1* gene in tobacco roots. Recently, identified a new target that exposes the miRNA's function in nicotine biosynthesis, using small RNA and degradome sequencing. Combined analysis of transcriptome datasets and degradome analysis showed 51 novel miRNA-mRNA interactions that may regulate nicotine biosynthesis in tobacco.

5.1.3 miRNAs Involved in the Regulation of Taxol Biosynthesis

Taxol is commonly used as an anticancer drug with a market value above $2 billion per year (Malik et al. 2011; Talbot 2015). Taxol was isolated from *Taxus baccata* and has been employed in the treatment of various types of cancer such as breast, pancreatic, ovarian, and a number of other carcinomas (Zhu and Chen 2019; El-Sayed et al. 2020). It is present in lower concentrations in the Yew bark; making the price of taxol so high. Efforts are, therefore, on to find out efficient methods for enhancing the taxol content in Yew.

It was observed that taxane 13α hydroxylase and taxane 2α-O-benzoyltransferase (taxol biosynthetic enzymes) are the potential targets of miR164 and miR171, respectively. Hao et al. (2012) showed that miR164 and miR171 are the negative regulators of taxol biosynthesis. Therefore, silencing of miR164 and miR171 can be a promising tool to enhance taxol content in Yew. Similarly, miRNA-4995 regulates the terpenoids biosynthesis by targeting 3-deoxy-7-phosphoheptulonate synthase, which ultimately alters the picroside-I production in *Pirorhiza kurroa* (Vashisht et al. 2015). The list of miRNAs, their target gene, and plant source are depicted in Table 2.

Table 2 List of miRNAs involved in the regulation of different plant secondary metabolites

Plant	miRNA	Target gene	Effected PSM	References
Papaver somniferum	pso-miR13	*7-OMT*	Morphinan	Boke et al. (2015)
	pso-miR2161	*4-OMT*		
	psomiR408	*Reticuline oxidase-like protein*		
Nicotiana tabacum	miRX17	*QPT1*	Nicotine	Li et al. (2015a, b)
	miRX27	*QPT2*		
	miRX20	*CYP82E4*		
	miRX19	*NAC-148*		
Taxus baccata	miR164	13α hydroxylase	Taxol	Hao et al. (2012)
	miR171	taxane 2α-O-benzoyltransferase		
Rauvolfia serpentina	miR396b	kaempferol 3-O-beta-D-galactosyltransferase	Flavonol glycosides	Prakash et al. (2015)
Mentha spp	miR156	bHLH	Flavone/flavonol biosynthesis	Singh et al. (2016)
Panax ginseng	miR854e, miR854b and miR854c	*Farnesyl diphosphate synthase* *Squalene epoxidase (SE)*	Ginsenosides	Mathiyalagan et al. (2013)
Panax ginseng	miR1439b, and miR1439h	*Beta amyrin synthase*		
Picrorhiza kurroa	miRNA-4995	One enzyme regulating the terpenoids biosynthesis	Picroside-I	Vashisht et al. (2015)
Catharanthus roseus	miR-5021	Two enzymes of TIAs biosynthesis	Terpenoid indole alkaloids (TIAs)	Pani and Mahapatra (2013)
Helianthus annuus	miR2911	*γ-tocopherol methyl transferase*	α-tocopherol	Barozai et al. (2012)
Salvia sclarea	miR828a and miR948a	*MYB12 Lipoxygenase*	Flavonoids	Legrand et al. (2010)
Ferula gummosa	miR1533	*SPL7*	Terpenes	Sobhani Najafabadi and Naghavi (2018)
	miR5021	*SPL11*		
	miR5658	*ATHB13*		

6 Gene Editing by CRISPR/Cas9

Plants produce many metabolites and nutrients that are either used as raw material or directly assimilated by humans. Recently, new plant genome editing techniques have been exercised in order to attain a variety of important agronomical traits like increased nutrient and metabolite production and also resistance to different stresses (Chen and Gao 2015). Zinc-finger nucleases (ZFNs) and transcription activator-like endonucleases (TALENs) were protein-based powerful gene-editing tools, which have been used in the recent past for manipulating the genomes (Urnov et al. 2005; Malzahn et al. 2017; Dasgupta et al. 2020). These technologies utilize Fok-I endonuclease to knock out the target gene of interest by initiating double-strand breaks (DSBs) in the target DNA. As targeting precise sequences in the genome can be problematic, these systems need to develop two different proteins (Kim et al. 1996; Li et al. 2011). The presence of these DNA sequences along with both the proteins challenged the efficiency of these systems to align and dimerize correctly with the essential spacer length (Händel et al. 2009; Urnov et al. 2010). This can conquer with the breakthrough of CRISPR/Cas9 system, which does not involve dimerization of protein (Mali et al. 2013). Gene editing by CRISPR/Cas9 system involves artificially designed guide RNAs that identify the PAM sequence in the target site (Cho et al. 2013). Later, it has been employed by many researchers in various plant species including *Nicotiana benthamiana*, *Nicotiana tabacum*, *Arabidopsis thaliana*, *Brassica oleracea*, *Solanum tuberosum*, *Glycine max*, *Zea mays*, *Marchantia polymorpha*, *Oryza sativa*, *Populus tomentosa*, *Triticum aestivum* etc. (Nekrasov et al. 2013; Baltes et al. 2014; Woo et al. 2015; Alagoz et al. 2016; Jiang et al. 2021).

Opium poppy (*Papaver somniferum*), from the family of Papaveraceae is known to biosynthesize morphine (La Valva et al. 1985). It also yields many BIAs. The biosynthesis of BIA begins with the dopamine and the 4-hydroxyphenylacetaldehyde (4-HPAA) condensation to produce (S)-norcoclaurine (Ilari et al. 2009). Following a methylation and hydroxylation steps, (S)-norcoclaurine gets converted into S-reticuline, which is catalyzed by 3′-hydroxy-N-methylcoclaurine 4′-O-methyltransferase (4′ OMT) (Morishige et al. 2000; Choi et al. 2002; Ziegler et al. 2005). S-reticuline resulted into various end products like papaverine, morphine and noscapine via different BIA pathways. A recent report has suggested that in opium poppy, BIA production can be manipulated by altering some specific genes expression in the BIA pathway. Recently, studies in opium poppy about over-expression and TRV-mediated gene silencing exposed that the alkaloid biosynthetic amount could be manipulated in a tissue-specific manner (Frick et al. 2004; Apuya et al. 2008; Hosseini et al. 2011; Desgagné-Penix and Facchini 2012). Both the over-expression and the silencing of *3′-hydroxyl-N-methylcoclaurine 4′-O-methyltransferase* (*4′ OMT2*) and (*R, S*)-*reticuline 7-O-methyltransferase* (*7OMT*) genes indicated their regulatory roles in different tissues in BIA production (Gurkok et al. 2006). The prior approaches impacted gene expression particularly at the post-transcriptional level resulting in a substantial decrease in gene expression but did not eliminate the gene function. This is imperative because the metabolites production continues and the biosynthetic pathways do not cease, masking the overall phenotypes regulated by the targeted genes. Thus, the gene knock-out strategies application, like CRISPR/Cas9, can assist in addressing these challenges and expanding our understanding of the genes of interest. CRISPR system components are generally expressed by synthetic vectors like Cas9 endonuclease and sgRNAs in animal and plant systems (Baltes et al. 2014). The efficacy of the CRISPR/Cas9 system can be raised by using the backbones of viral vector for the guide RNAs expression in plant systems. CRISPR/Cas9 system was reported to knock out *4′ OMT* gene involved in the biosynthesis of some biologically active compounds in opium poppy, using both the viral-based and the synthetic delivery systems.

7 Nanoparticles Based Enhancement

It is well-known that nanoparticles (NPs) are the particles ranging from 1 to 100 nm in size. Due to their large surface-area to volume ratio, NPs are unique in biological activities, catalytic reactivity, thermal conductivity, and chemical steadiness. Owing to such effects, NPs are exclusively used in health, cosmetic and chemical industries as well as in space and agriculture sectors (Prasad et al. 2017; Iqbal et al. 2019; Nasrollahzadeh et al. 2019; Kumar et al. 2021a, b, 2022; Sharma et al. 2021; Siddiqi and Husen 2021; Husen 2022). The commonly used nano-materials include silver (Ag), gold (Au), copper CuO/Cu_2O, iron (Fe_3O_4/Fe_2O_3), titanium (TiO_2), zinc (ZnO), magnesium (MgO) and silicon (SiO_2) (Husen and Iqbal 2019; Husen et al. 2019). Interestingly, plants exhibit constant interaction with NPs present in the environment including soil, water and air.

7.1 NPs as Elicitors of Secondary Metabolism

Recent studies have established that plants can act as a major source for the formation of NPs either in their active or inactive form (Kralova and Jampilek 2021). Various plants are known to accumulate metals, which further undergo reduction to form NPs intra-cellularly. This is evident from studies where gold NPs were synthesized from alfa alfa plants. Similarly, the accumulation of AU NPs has been reported in the epidermis, and the vascular and corttical tissues of *Brassica juncea* and *Medicago sativa* (Husen et al. 2019). Interestingly, most of the studies have concentrated upon usage of inactive plant parts as a reduction sink for synthesizing NPs. The secondary metabolites in plants viz., flavones, alkaloids, terpenoids, aldehydes, ketones, phenolics, saponins, tannins, phenolics and many others play an imperative role in the reduction of metals. Several studies have reported the relevance of NPs to positive attenuation of the content/composition of secondary metabolites in plants (Fig. 1).

Confirming the above, it was observed that silver nanoparticles (AgNPs) synthesized from *Acalypha indica* L. leaf extract resulted in enhanced total phenolic content (TPC) in the treated *Bacopa monnieri* (L.) Similarly, under the influence of Cu NPs in vivo grown pennell plants showed increased contents of secondary metabolites including the alkaloids which in turn enhanced the antioxidant capacity of the plant (Mishra et al. 2019; Singla et al. 2019). Treatment of shoot tips of *Atropa belladonna* with manganese trioxide NPs increased the production of alkaloids,

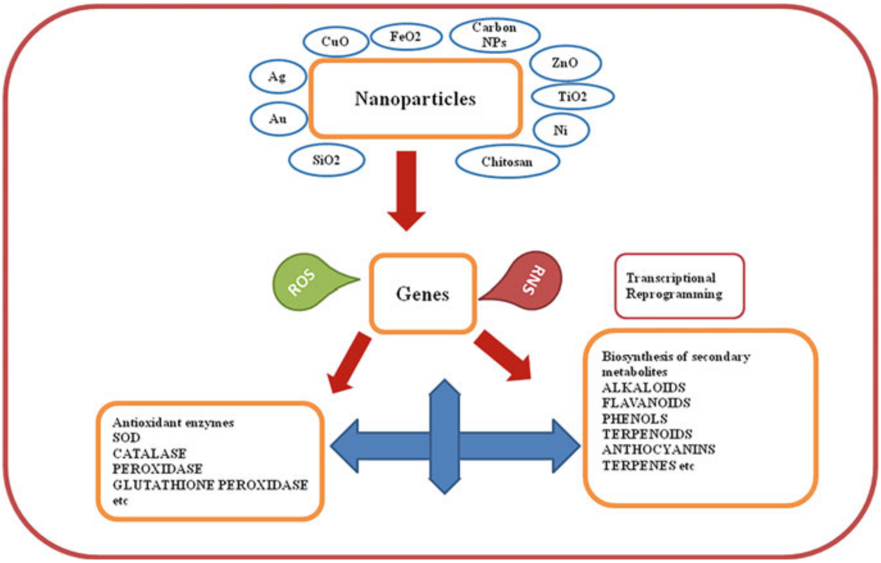

Fig. 1 Schematic representation of NPs as elicitors of secondary metabolites

phenolics as well as flavonoids in a dose-dependent manner, which in turn activated the anti-oxidant defence enzymes (Taghizadeh et al. 2019).

A study performed using *Capsicum annuum* L. cultures which were supplemented with chitosan NPs, showed enhanced amounts of alkaloids, phenols and proline respectively. This resulted in amplification of organogenesis along with augmentation of peroxidase and catalase (Asgari-Targhi et al. 2018). Elicitation of the seedlings of *Catharanthus roseus* with Multi-walled carbon nanotubes, grown in MS medium, enhanced the level of phenol and alkaloids by 1.7-fold. This was in synchronization with increased catalase, peroxidase, PAL and deacetylvindoline-4-O-acetyltransferase (DAT) genes. In *Hyoscyamus reticulatus* hairy root culture introduced with iron (Fe) oxide (NPs), tropane alkaloids were found to be raised by a 900 mg for 24 h treatment (Moharrami et al. 2017).

7.2 Potential Applications of NPs

NPs are known to possess numerous applications in industries due to the presence of a broad range of plant secondary metabolites. Some important applications are associated with enzymes that are used as biocatalysts in modern biology. It was observed that the stability of α-amylase was enhanced by using naringin functionalized magnetite nanoparticles (Defaei et al. 2018). Ag NPs obtained from *Chenopodium aristatum* and *O. sanctum* showed a high-quality catalytic activity for degrading 4-nitrophenol (Singh et al. 2018; Yuan et al. 2017). Likewise, Ag NPs from *Thuja occidentalis* extract can be used as a plant growth regulator and also as a soil conditioner (Das et al. 2018). *Lawsonia inermis*-based Cu NPs have been used efficiently to prepare electrical-conducting nano biocomposites (Cheirmadurai et al. 2014). Also, CuO NPs synthesized from the extract of *Ocimum tenuiflorum* have been used as non-enzymatic glucose biosensor (Dayakar et al. 2017).

The tendency to chelate metal ions by PSMs for the formation of steady complexes and their ability to further conjugate with NPs can be marked as a new milestone in their use for harvesting the natural products. The NP-secondary metabolite complex upon green synthesis strongly establishes a link between the type of NP and the compound class. During the nano-harvesting, the metabolites are adsorbed onto NPs and released into the medium from plant cells (Khan et al. 2017). Nano-harvesting can be used for the identification of bioactive compounds by minimizing the use of organic solvents. Therefore, it provides a novel methodology for the use of nonmaterial for isolation and analyzing the bioactive properties of plant- based compounds.

Interestingly, green synthesized NPs can be explored to develop highly efficient tools for the extraction of specific compounds from crude extracts. The application of NPs-secondary metabolite conjugation can further be used in biotechnology industries, as the NP-based delivery of important biomolecules will enhance their therapeutic potential. This is evident from the study where hesperitin-conjugated gold nanoparticles showed good results to cure hepatocellular carcinoma (Krishnan et al. 2017). Besides, NPs have shown antibacterial, anti-fungicidal, antiplasmodial, anti-inflammatory, antidiabetic, antiviral and antioxidant properties.

8 Conclusion

In conclusion, the advanced biotechnological and metabolomics approaches could be used as a viable alternative for enhancing the pharmacologically and commercially important bioactive compounds, such as alkaloids from distinct medicinal plants, and ensure their availability. More focused efforts in this area can lead to the discovery of many unknown medicinally important secondary metabolites. There are still many underexplored areas which can be utilized for altering the biosynthesis of natural products in plants. Therefore, the manipulations or engineering of biosynthetic pathways of natural products in medicinal plants must be taken as a challenge so that these bioactive compounds can be further utilized for drug development.

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Genome-Editing Strategies for Enhanced Stress Tolerance in Medicinal Plants

Vincent Pamugas Reyes, Abriel S. Bulasag, Sourav Ranjan Mohapatra, Kajal Samantara, and Shabir Hussain Wani

Abstract Plants are considered an essential source of numerous therapeutic means for humans. These plant-derived medicines are utilized for various pharmaceutical products including different drug formulations. However, the large-scale production of these plants has become a challenge due to various factors such as susceptibility to diseases, pests, and poor tolerance to abiotic stresses. Even though advances have been made to improve breeding pipelines for medicinal plants, certain bottlenecks have remained. To broaden the horizons of conventional breeding strategies, current genome editing tools are assisting precise gene editing in terms of stress resilience, adaptation, and marketability, in a shorter time frame. This chapter summarizes the utilization of engineered nucleases to transmute the genomic targeted site with an artificially designed and precise controlled genetic circuit in a plant system. In addition, the latest progress in the gene-editing approach including the remarkable instances of the CRISPR system application for stress tolerance in medicinal plants was also highlighted. Lastly, potential threats and future outlooks in using genome-editing tools for medicinal plants are also addressed.

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A. Husen, M. Iqbal (eds.), *Medicinal Plants*,
https://doi.org/10.1007/978-981-19-5611-9_16

Keywords CRISPR/Cas9 · Genome editing · Genetic engineering · TALEN · ZFN · Medicinal plant

1 Introduction

Plants sustain a wide range of human needs such as food, biomaterials, and medicine. According to the World Health Organization (WHO), ~80% of the world's population relies on the use of allopathic and traditional medicines that are derived from medicinal plants, also known as herbal medicines (World Health Organization 2013). In addition, 50% of the newly developed medicines are plant-derived or may contain the active constituent from the plant (Teng and Shen 2015). Generally, herbal medicines are known to be a safe and effective alternative to synthetic medicines as they are less toxic (Parveen et al. 2020a, b; Abate et al. 2021; Husen 2021, 2022). In the United States of America, about 8% of hospital admissions are due to the adverse or side effects of synthetic drugs (George 2011). Therefore, people are shifting to herbal medicines hoping that it is less harmful, compared to synthetic medicines. The use of medicinal plants has existed for thousands of years, and even today, people living in far flung areas treat their illness by using plants growing in the wild (Atique et al. 1985a, b; Anis et al. 2000; Husen et al. 2021). For example, Ayurveda was established in the Himalayas during 4500–1600 BC (Kunwar et al. 2006; Padma 2005), whereas Unani system of medicine originated in Greece by the efforts of Hippocrates and his disciples around 400 years BC, developed in Arabia and Persia during the sixth to thirteenth century AD, and later prospered in the Indian sub-continent (Iqbal et al. 2018; Parveen et al. 2020a, 2022). In China, the traditional Chinese medicine (TCM) originated during 3000 BC, while Kampo in Japan existed during 500 AD (Yu et al. 2006). Over the recent years, utilization of herbal medicine has made a significant leap. According to Muriuki et al. (2012), the demand for herbal medicine in Kenya has increased enormously and led to the formalization of the market. In addition, herbal medicines are now prescribed for therapeutic and dietary supplements, and by the end of 2030, the global market for herbal medicines is expected to reach $178.4 billion (Global Industry Analysts Inc 2021).

The high demand for medicinal plants and their derivatives has led to increased harvesting, primarily in their wild habitats. According to a study, the demand for wild resources in these medicinal plants has increased by 8–15% in Europe, North America, and Asia (Ross 2005). The preference for medicinal plants varies across cultures and customs. For example, countries in Asia have different ethnic groups which entail their respective cultures and customs. In most cases, the information on the utilization of medicinal plants in these groups is just passed on across generations (Anis and Iqbal 1986; Anis et al. 2000; Beigh et al. 2003a, b). There are numerous unique folk claims in the ethnomedicine literature about the efficacy of several individual plants (Atique and Iqbal 1992; Atique et al. 1985a, b, 1986, 2009; Husen et al. 2021; Husen and Iqbal 2021; Husen 2021, 2022; Asfaw et al. 2022), which do require pharmacological and clinical verification. Several studies conducted on animal models have demonstrated that these medicinal plants have the potential to become an effective remedy against a variety of illnesses. For example, a study conducted by Abdel-Mageid et al. (2018) has shown the potential effect of garlic (*Allium sativum* L.) and curcumin nanoparticles against complications in diabetic induced mice. Chen et al. (2019) demonstrated the effect of oral administration of green tea (*Camellia sinensis*) reduces oxidative stress and enhances the restoration of cardiac function in diabetic rats. Thomson et al. (2002) used ginger (*Zingiber officinale* Rosc.) as a potential anti-inflammatory and antithrombotic agent using mice as the animal models. In their study, high oral doses (500 mg/kg) of ginger were more effective in reducing prostaglandin-E_2 (PGE_2), a bioactive lipid associated with inflammation.

Turmeric (*Curcuma longa*) has been shown as a potential remedy for neurological diseases, skin diseases, and dyspepsia (Hewlings and Kalman 2017). Another well-known traditional medicine is the Gingko (*Gingko biloba* L.). Punkt et al. (1997) demonstrated the benefit of ginkgo extract through changes in enzyme activity in the myocardium of diabetic and hypoxic diabetic mice. Saini et al. (2014) studied the protective effect of *Ginkgo bilob*a extract on STZ- induced diabetic cardiomyopathy in mice. The treatment showed that the *Ginkgo biloba* extract can attenuate cardiac dysfunction in mice. This was evident with decreased LV collagen content, protein content, LVW/BW ratio, and serum LDH level. The root and rhizome of *Panax ginseng* are used as traditional medicine by Chinese people for thousands of years to treat various disorders such as anorexia and hemorrhage (Goldstein 1975; Liu and Xiao 1992; Xiang et al. 2008). Recently, Hwang et al. (2021) used Rg3-enriched red ginseng extract in an in vitro study on human lung cancer cells to elucidate the role of mitophagy in cell death induced by Rg3-RGE. Interestingly, the Rg3-RGE was found to induce apoptotic cell death by inhibiting mitophagy and cause a significant decrease in cell viability and increase apoptosis via mitochondrial dysfunction. Ju et al. (2021) conducted a study on the capability of hydrolyzed red ginseng extract (HRGE) for learning and memory improvement of mice treated with scopolamine. The oral administration of HRGE demonstrated that it can give neuroprotective effects and reduce scopolamine-induced learning and memory deficits in vivo through upregulation of nuclear-factor-E2-related factor 2 (Nrf2) and antioxidant enzymes. The success in various in vivo and in vitro studies of these medicinal plants has great promise for future medicinal research and application.

For thousands of years, people have started domesticating various plant species that are beneficial as food and alternative medicine (Parveen et al. 2020b). The farming system of plants often takes place in multiple cropping systems, which include agroforestry systems (Rao et al. 2004). But over the years, climate change has resulted in extreme weather disturbances such as typhoons, drought, floods, colder winter, and hotter summer. In addition, pests, disease-causing viruses, and bacteria have evolved making plants more susceptible to their attacks (Fig. 1). In major crops such as rice, maize, wheat, barley, and sorghum, the marker-assisted breeding (MAB) approach is effective for improving disease resistance, abiotic stress tolerance, and yield productivity (Angeles-Shim et al. 2020; da Silva et al.

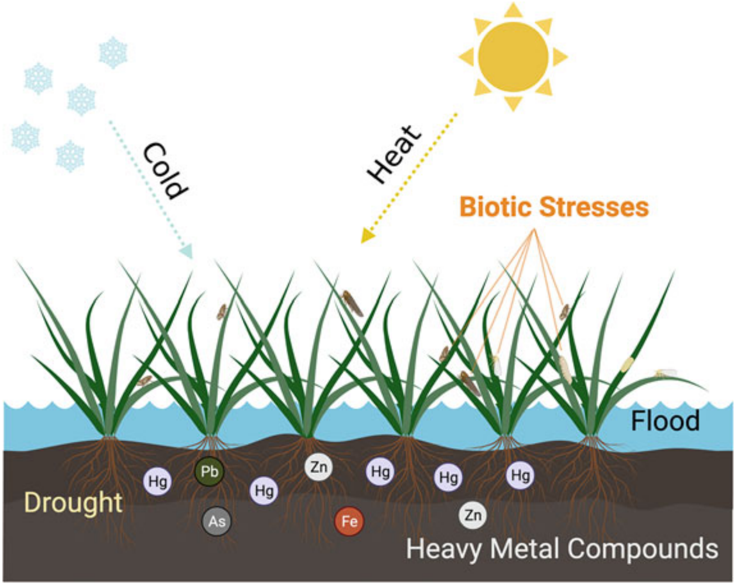

Fig. 1 Types of stresses that affect the performance of plant

2020; Hernandez et al. 2020; Reyes et al. 2021; Yohannes et al. 2015; Zhao et al. 2012). MAB can also be applied in the case of medicinal plants to improve their tolerance to various biotic and abiotic stresses to meet the growing demand of our population.

The advances in the field of genetics and molecular biology have led to the development of innovative tools to know the genomic composition of an organism through DNA sequencing (Margulies et al. 2005; Sanger et al. 1977; Sanger and Coulson 1975). Recently, some techniques of genome editing have allowed researchers to add, remove, and alter, genomic regions in a more efficient manner (Chevalier et al. 2002; Jinek et al. 2012; Pavletich and Pabo 1991; Rothstein 1983). This technique has been applied for enhancing heat and cold tolerance, bacterial and fungal resistance, and yield improvement in rice (*Oryza sativa* L.), wheat (*Triticum aestivum*), maize (*Zea mays*), tomato (*Solanum lycopersicum*), cotton (*Gossypium* spp.) and soybean (*Glycine max*) (Gao et al. 2020; Jacobs et al. 2015; Li et al. 2017, 2018a, b; Shan et al. 2014; Zhang et al. 2021). Although most medicinal plants grow in the wild, the success of genome editing tools could also be utilized to enhance their biotic and abiotic stress resistance and tolerance (Fig. 2). We intend to highlight here the major genome editing tools that can precisely modify the plant genome and its application for enhancing stress tolerance and resistance in medicinal plants. The potential threats and future perspectives in using genome-editing tools for medicinal plants would also be discussed.

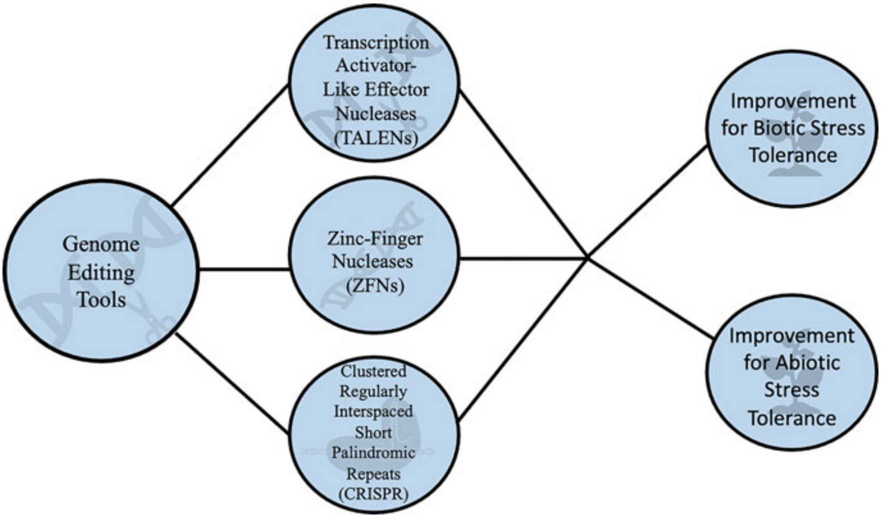

Fig. 2 Types of genome-editing tools for enhancing abiotic and biotic stress tolerance

2 Improving Stress Tolerance in Medicinal Plants

Various stresses such as drought, cold, salinity, heavy metal toxicity, and disease susceptibility are the major constraints in plant production. Efforts have been made to improve the stress-resistance and tolerance capacity of plants, as also their phytoremediation ability, by using different means and techniques (Ansari et al. 2013; Iqbal et al. 2015). Most of the plants carry therapeutic significance due to possessing a huge diversity of secondary metabolites. Thus, medicinal plants encompass a large number of species with wide array of biological specificities and characteristics. Unlike the cultivated cash crops, most medicinal plants grow in the wild. However, with increasing demand for their products, their cultivation and breeding are now necessary to ensure a high quality, profitable and sustainable production. The availability of "omics" technologies has led to the identification and utilization of beneficial genes and QTL in breeding programs for major crops (Cavanagh et al. 2013; Kitony et al. 2021; Reyes et al. 2021)**.** To date, some research efforts have been devoted to understanding the genomic composition of some medicinal plants. The draft genomes of medicinal plants such as ginseng (*Panax ginseng*), plume poppies (*Maclaeya cordata*), Chrysanthemum (*Chrysanthemum nankingense*), Bitter melon (*Momordica charantia*), Rhodiola (*Rhodiola crenulate*), tomato (*Solanum lycopersicum*), garlic (*Allium sativum*), *Aloe vera*, ginger (*Zingiber officinale*), guava (*Psidium guajava*) have been reported and are available for further genetics and breeding studies (Fu et al. 2017; Kim et al. 2018b; Liu et al. 2017; Song et al. 2018; Sun et al. 2020; The Tomato Genome Consortium 2012; Urasaki et al. 2016). However, these technologies are still underutilized, especially for plants that only have medicinal value.

Table 1 General molecular networks involved in stress adaptation in plants

Type of molecular networks	Examples	Mode (s) of action	Stress	References
Reactive oxygen scavengers	Enzymatic (Ascorbate peroxidase; glutathione cycle enzymes; alternative oxidase); non-enzymatic (ascorbate, flavones, anthocyanins)	Detoxification of ROS	Soil salinity; heavy metals; drought; biotic stress	Akram et al. (2017) and Das and Roychoudhury (2014)
Stress proteins	Late embryogenesis abundant (LEA) proteins	Chaperones; protein stabilization; membrane stabilization; ion sequestration	Drought; cold stress	Chourey et al. (2003), Ding et al. (2019), and Mouillon et al. (2006)
Transcriptional control	Transcription Factors: ethylene-responsive element binding protein (EREBP); zinc finger TF (*Alfin 1*)	Regulation of transcriptional activities	Wide array of biotic and abiotic stresses	Banerjee and Roychoudhury (2020), Gangola and Ramadoss et al. (2020), Ramadoss et al. (2020), Sharma et al. (2020), and Surabhi and Badajena (2020)
Growth regulators	Cytokinin and brassinosteroids	Hormone homeostasis	Drought; cold; salinity stress	Ahammed et al. (2015), Bielach et al. (2017), and Verma et al. (2016)

Generally, genes related to stress adaptation are classified into (a) functional genes, and (b) regulatory genes (Shinozaki et al. 2003). The functional genes are composed of enzymes and metabolic proteins such as detoxification enzyme, water channel, heat shock protein (HSP), and late embryogenesis abundant (LEA) protein. On the other hand, regulatory genes encode regulatory proteins such as transcription factors (TF), protein kinases, and protein phosphatases, which are crucial for signal transduction and gene expression for stress response (Wang et al. 2016a, b, c). For the past decades, scientists are trying to understand the genetic networks that are involved in plant adaptation to stress tolerance. As a result, several products of major and minor genes, which affect the adaptive strategy of plants toward certain stresses, have come to light (Table 1).

2.1 Genome Editing Tools

Nowadays, genome editing is highly preferred over traditional breeding and marker-assisted selection program due to faster generation advancement (Driedonks et al. 2016). Zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR) are the well-known genome editing tools. These tools provide a venue to introduce targeted mutations, insertion/deletion (InDel), and precise modification in the genome using specific nucleases (Jain 2015). Over the years, these have been applied for the improvement of model crops. For example, Arazoe et al. (2015) demonstrated that the tailor-made TALENs system that they developed was able to improve resistance in rice against blast caused by *Pyricularia oryzae*. Shan et al. (2015) demonstrated the success of using TALENs to increase 2-acetyl-1-pyrroline (2AP), a major fragrance compound, in rice. The ZFNs were used to stack genes conferring two herbicide resistances in embryonic maize suspension cultures, allowing for co-segregation of genes to progeny (Ainley et al. 2013). Using CRISPR/Cas, Shi et al. (2017) generated maize lines that carry genome-edited *AGROS8* with improved grain yield under drought stress conditions. Zhou et al. (2015) used CRISPR/Cas9 system to modify the *EBEPthXo2* region of the rice sucrose transporter encoding gene *OsSWEET13*. This resulted in disease resistance lines due to the inhibition of *X. oryzae* pv. *oryzae* TALE-PthXo2-mediated activation of *OsSWEET13*. Although these tools are well established in model crops, studies on their utilization for improving resistance to abiotic and biotic stresses in medicinal plants remain limited.

2.1.1 Zinc Finger Nucleases (ZFNs)

ZFNs are primarily composed of zinc finger protein domains, which are capable of sequence-specific binding, connected to a nuclease domain, and cause DNA cleavage (Fig. 3). Of a tethered array of zinc finger protein domains each recognizes approximately 3 bp of DNA (Moore et al. 2001). Target sequences are bound by zinc finger nucleases (ZFN) and then dimerized by *Fok*I (*Flavobacterium okeanokoites*) nucleases, a type II restriction enzyme (Kim et al. 1996; Miller et al. 2007). The ZFNs sequence specificity is determined by zinc finger proteins (ZFPs) that are composed of tandem arrays of C2H2 zinc-fingers (ZFs). Each of these ZFs recognizes a 3-bp DNA sequence, and 3–6 ZFs are used to generate ZFN subunit that binds with a 9–18 bp DNA sequence. DNA repair processes are then induced by the DSBs generated by ZFN cleavage. An improvement in the error-prone NHEJ came through homology-directed repair (HDR) that resulted in either gene editing or site-specific integration.

Although promising, this tool also imposes some setbacks. One of the caveats of ZFN is the poor targeting density (Kim and Kim 2014). It was previously reported that although each ZF recognizes a 3-bp DNA sequence, no open-source collection

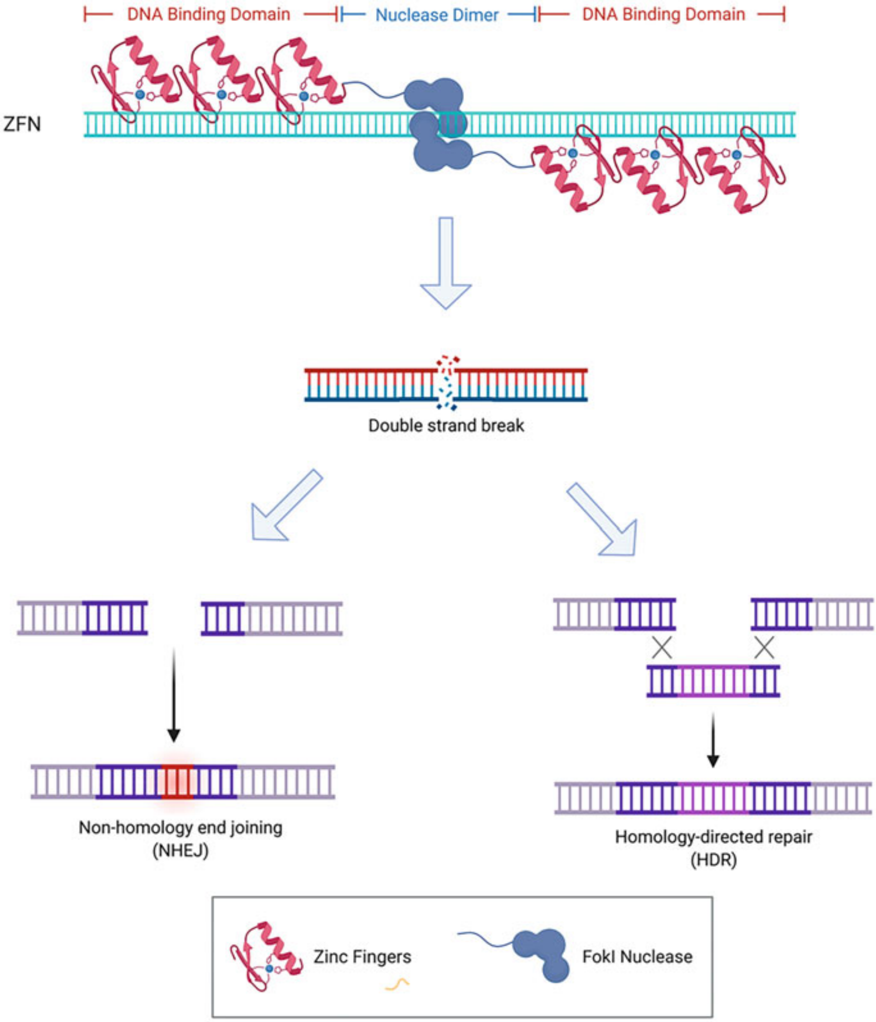

Fig. 3 Basic working principle of zinc finger nuclease (ZFN) genome editing

of 64 zinc-fingers covers all possible combinations of the triplet sites (Bae et al. 2003; Segal et al. 1999).

2.1.2 Transcription Activator-Like Endonucleases (TALENs)

Over the years, TALENs have emerged as an alternative to ZFNs for inducing targeted double-stranded breaks and genome editing. Like ZFNs, TALENs are comprised of nonspecific *Fok*I nucleases together with a customizable

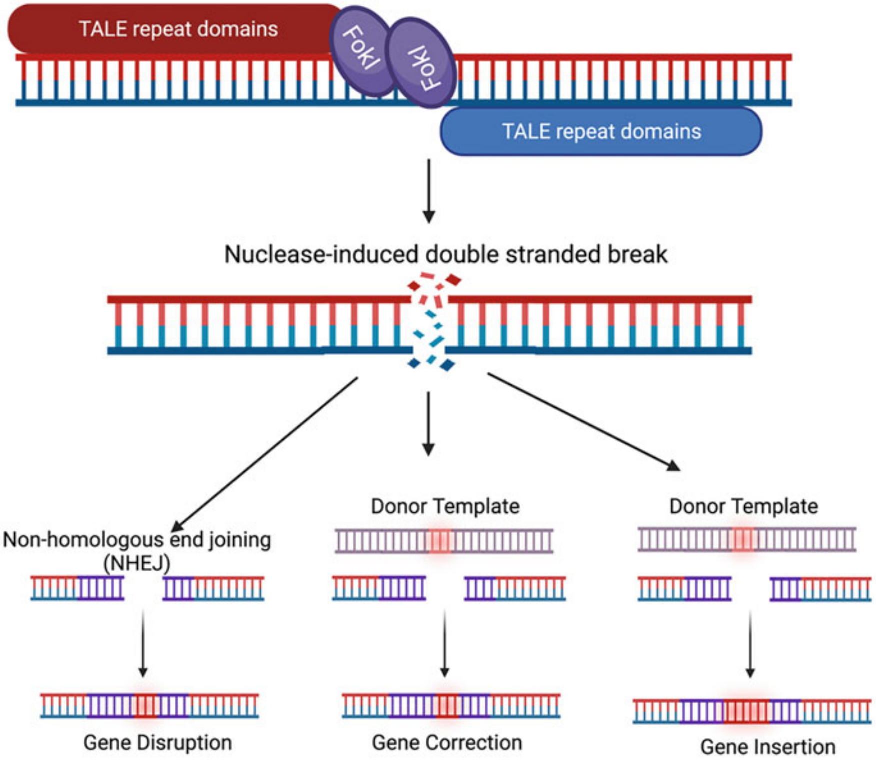

Fig. 4 Basic working principle of a Transcription Activator-like endonuclease (TALEN) genome editing

DNA-binding domain (Fig. 4). The DNA-binding domain in this tool is derived from transcription activator-like effectors (TALENs), proteins secreted by *Xanthomonas* spp. bacteria (Boch and Bonas 2010). Many researchers prefer TALENs as it could be easily and rapidly modified through the use of simple protein-DNA code that facilitates the modular DNA-binding TALE repeat domains to individual bases in the target site (Joung and Sander 2013).

The development of DNA encoding TALE repeat array is considered to be challenging due to the required assembly of multiple nearly identical repeat sequences (Joung and Sander 2013). Over the years, several platforms have been developed to facilitate the assembly of plasmids that encodes TALE repeat arrays. Generally, these methods are grouped as (1) standard restriction enzyme and ligation-based cloning (Huang et al. 2011; Sander et al. 2011); (2) golden gate cloning (Cermak et al. 2011; Weber et al. 2011); and (3) solid-phase assembly (Briggs et al. 2012). The architectures of TALENs such as the length and sequence composition of the N and C terminal TALE-derived sequences are crucial factors while deciding on the type of assembly method.

Taken altogether, TALENs can be applied to introduce knockout mutations and to confer resistance in plants by disrupting the target sites of naturally occurring TALEs associated with pathogenicity.

2.1.3 Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9

To resolve limitations in the use of ZFNs and TALENs, the CRISPR/Cas (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein) system has revolutionized genome editing in terms of protein synthesis, design, and validation. CRISPRs were initially identified in *E. coli* in 1987 by Ishino and his team; this historical journey is reviewed in Ishino et al. (2018) while analyzing a gene associated with the conversion of alkaline phosphatase. In 2012, Jinek et al. discovered that the bacterium *Streptococcus pyogenes* could be utilized for genome editing and genetic engineering. As compared to ZFNs and TALENs, CRISPR/Cas has a DNA-binding moiety of RNA instead of protein. In addition, it uses RNA sequence as the specificity determining element for double-strand breaks (DSBs) and requires only the single guide RNA (sgRNA) which is relatively cheaper and more efficient (Kozovska et al. 2021; Lino et al. 2018).

A single guide RNA (sgRNA) is assembled in vitro by combining a spacer, a CRISPR RNA (crRNA) and a transactivating CRISPR RNA (tracrRNA). Different CRISPR-associated proteins (Cas), originally discovered as Cas9, are then paired with the sgRNA depending on the intended purpose and cloned into a vector, either individually or in tandem, before transformation into plant cells (Arora and Narula 2017).

The most widely used Cas, Cas9, is a multifunctional protein that is made up of two nuclease domains (HNH and RuvC). It also needs to be adjacent to a $5'$-NGG-$3'$ protospacer adjacent motif (PAM) (Jinek et al. 2012). Transformation is then enabled either through particle bombardment or introduction via protoplasts. Double-strand breaks (DSBs) are then introduced following the expression of the sgRNA and nickase protein in plant cells. This triggers a DNA repair machinery that leads to the modification of DNA sequence and ultimately to protein coded by the sequences, eventually being manifested in the desired phenotype. Specifically, DSBs may either be repaired by non-homologous end-joining (NHEJ) or homology-directed repair (HDR) leading to either gene knockout or gene knock-in modification, respectively. Likewise, different inducible expression systems using the CRISPR/Cas system have been developed for the expression/overexpression of the guide RNAs (gRNAs) against the target genes of interest (e. g. CRISPRa/CRISPRi Synergistic Activation Mediator, SAM). Mutations are then screened by PCR, molecular markers, selection media, sequencing, and/or western blot, among other applicable analytical procedures (Vats et al. 2019). Figure 5 describes a generalized process for CRISPR/Cas9-mediated genome editing in plants.

Pioneering studies utilizing CRISPR-mediated genome editing in plants were conducted in *A. thaliana*, *Nicotiana benthamiana* (Li et al. 2013) and *O. sativa*

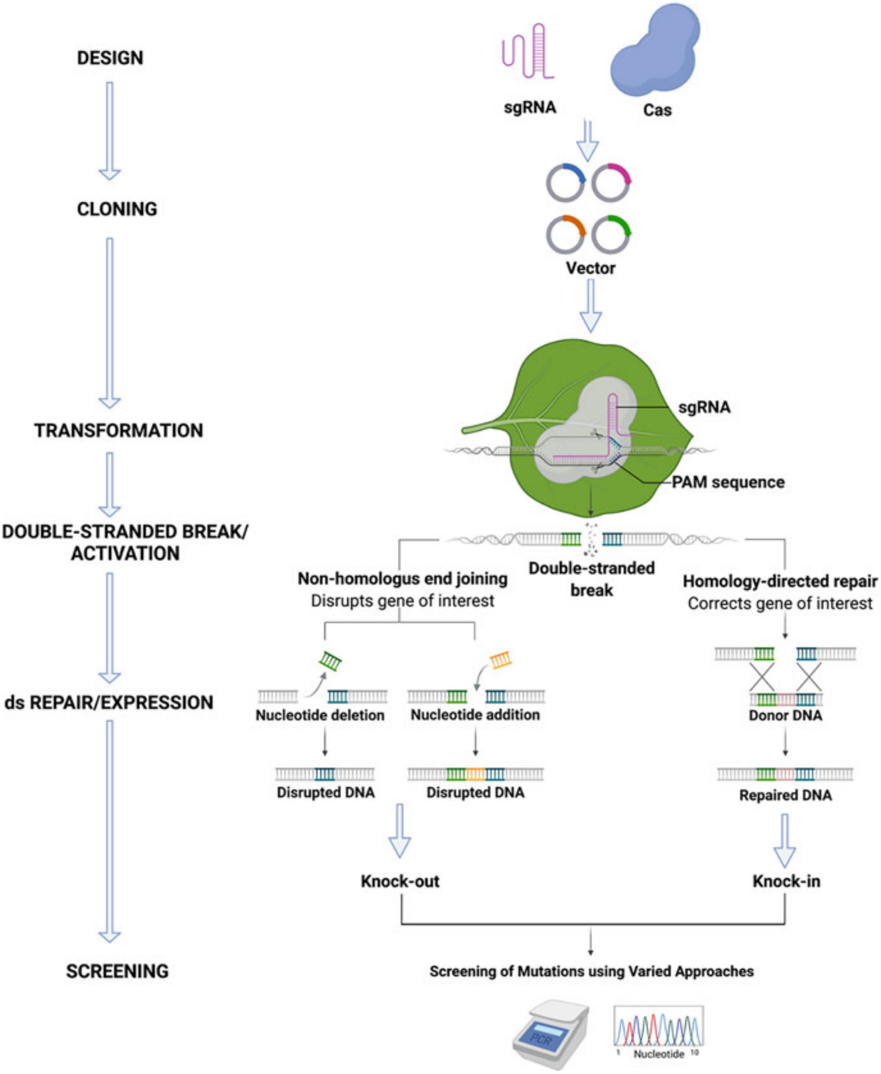

Fig. 5 Generalized process for clustered regularly interspaced short palindromic repeats (CRISPR/ Cas9)-mediated genome editing in plants

(Shan et al. 2013). While, the simplicity and high-throughput capability of CRISPR/ Cas9 have democratized its use and hence found certain applications in medicinal plants such as *Linum usitatissimum* (Sauer et al. 2016), *Ocimum basilicum* (Navet and Tian 2020), and *Citrullus lanatus* (Tian et al. 2018), much more extensive studies were conducted on commercial cash crops such as rice, corn, orange, pepper, tomato, potato, barley and wheat.

3 Applications of Genome-Editing Tools on Select Medicinal and Model Plants

3.1 Abiotic Stress

Knockout mutants for a highly conserved C-repeat binding factor (CBFs), *SlCBF1*, were generated to investigate its role in tolerance to cold stress in tomato (Li et al. 2018a, b). Slcbf1 mutants showed greater chilling injuries, with downregulation of CBF-related genes. Likewise, membrane damage, as evidenced by higher electrolyte leakage and malondialdehyde (MDA) accumulation, was also exhibited by *slcbf1* mutants. Higher peroxide content and antioxidant activities were also associated with the chilling stress.

On the other hand, genes implicated in heat tolerance were extensively studied in some major crops and model plants. Mitogen-activated protein kinases (MAPKs) were posited to perform critical roles in responding to several biotic and abiotic stresses. *SlMAPK3* gene-edited mutants exhibited higher tolerance to heat in tomato as compared to the wild-type (Yu et al. 2019). Likewise, mutants were observed to have increased antioxidant enzyme activities as well as lesser ROS content and upregulated expressions of HSFs and HSPs. In contrast, mutants exhibited reduced membrane damage and less severe wilting symptoms. *GmHsp90A2* is a positive regulator under heat stress in soybean (Huang et al. 2019). Overexpression mutants led to reduced chlorophyll degradation and lipid peroxidation under heat stress. Moreover, *GmHsp90A2* was seen to interact with *GmHsp90A1* by facilitating its entry to the nucleus.

Being sessile organisms, plants need to be efficient in regulating their most important resource, the water. Hence, genome editing technologies were also employed to engineer tolerance under severely limited water conditions. Several tomato genes were also implicated in drought tolerance. Knockout of *SlMAPK3*, a tomato MAPK gene, led to up- or down-regulated expressions of several drought stress-responsive genes such as *SlLOX, SlGST*, and *SlDREB*. Proponents gleaned that *SlMAPK3* confers drought tolerance by protecting cell membranes from oxidative damage and modulating the transcription of stress-related genes (Wang et al. 2017). On the other hand, *SlNPR1* knock-out mutants exhibited reduced drought tolerance with phenotypic traits of plants under drought stress: increased stomatal aperture, higher electrolyte leakage, and reduced antioxidant activity, as compared to the wild type (Li et al. 2019a, b). This also led to the down-regulation of several key drought-tolerance genes (*SlGST, SlDHN*, and *SlDREB*). Knockout of the tomato gene *SlLBD40* led to enhanced drought tolerance as compared to overexpression and wild-type tomato plants (Liu et al. 2012).

In tomato, CRISPR/Cas9-mediated genome editing of the gene *SlHyPR1*, a negative regulator of the salinity stress response, led to increased tolerance to salinity across all developmental stages (Tran et al. 2021). The summary of studies that utilized genome editing for improvement of abiotic stress tolerance is presented on Table 2.

Table 2 Genome editing of pharmacologically important crop species inducing abiotic stress tolerance

Plant	Technique	Phenotype (type of stress tolerance)	Target gene/gene family/promoter	References
Solanum lycopersicum	CRISPR/Cas (knockout)	Cold stress	*SlCBF1*	Li et al. (2018a, b)
		Heat tolerance	*SlMAPK3*	Yu et al. (2019)
		Drought stress	*SlLOX, SlGST and SlDREB*	Wang et al. (2017)
		Salt stress	*SlNPR1*	Li et al. (2019a, b)
			SlLBD40	Li et al. (2020)
			SlHypR1	Tran et al. (2021)
Glycine max	CRISPRa	Heat tolerance	*GmHsp90A2*	Huang et al. (2019)

A *Zingiber officinale* stress gene, *ZoCPDK1*, exhibited a rare coupling between a nuclear localization sequence of the junction domain and the consensus sequence in the EF-hand loops of the calmodulin domain (Vivek et al. 2013). Interestingly, upregulation of *ZoCDPK1* is rapidly induced in rhizomes by high salinity, drought, and JA treatment but not by abscisic acid treatment and low temperature. Further heterologous overexpression of *ZoCDPK1* in Nicotiana tabacum conferred tolerance to both high salt and drought stress, as suggested by results in various stress-dependent physiological parameters (e.g., seed germination, photosynthetic efficiency). In addition, transgenic tobacco exhibited 50% more growth, compared to wild type under various stress conditions. Likewise, *ZoCPDK1* has upregulated stress-related genes (*RD21A* and *ERD1*) in tobacco plants. These results outline the critical role of *ZoCPDK1* in regulating salinity and drought stress. Transient fluctuations in cytosolic calcium concentration in plants have been associated with abiotic stress conditions such as high salinity, extreme temperature and mechanical perturbations. As such, calcium-dependent kinases (CDPKs), which perform a critical role in modulating calcium levels, can be valuable targets for genome editing tolerance to multiple stresses. It also suggests that genome editing through heterologous expression of medicinal plants' stress genes in well-established plant models could be a promising system to be explored in the future research.

Several genes/genome targets in *Medicago sativa* have been reported as promoting tolerance to various abiotic stresses, i.e., drought stress tolerance genes such as *miR156* and *SPL9* (Arshad et al. 2017; Hanly et al. 2020); tolerance to freezing stress such as *SOD* (McKersie et al. 1993); and salinity tolerance genes such as *SOD, CAT*, and *CKX* (Wang et al. 2016a, b, c; Li et al. 2019a, b). It is notable however that while the abovementioned studies employed genetic engineering approaches such as *A. tumefaciens*-mediated transformation and RNAi silencing, proponents have yet to use the CRISPR/Cas-mediated gene editing approaches.

Genome-editing of promising gene targets associated with the production of metabolites with pharmacological value is described below. Targeted mutagenesis was performed to engineer the secondary metabolite production in *Dioscorea zingiberensis* (Feng et al. 2018). Transformation after knock-out was facilitated by *Agrobacterium tumefaciens*. The sgRNA was designed to target the first exon of the farnesyl pyrophosphate synthase gene (*Dzfps*) through the *OsU3* promoter. Squalene, a pharmacologically sought-after metabolite, was significantly reduced in *Dzfps* mutants as compared to wild-type, suggesting a successful genome modification. It underscores a promising strategy to genome engineered *D. zingiberensis* for the desired metabolite. Likewise, a similar approach was used to target a gene, *4′-OMT2* regulating the synthesis of benzylisoquinoline alkaloids (BIAs) in opium poppy (*Papaver somniferum* L.) (Alagoz et al. 2016). Viral-based TRV and synthetic plasmids were used as vectors before *A. tumefaciens* transformation. Indel knockouts, generated through a non-homologous end-joining genome repair mechanism, showed significantly reduced BIAs such as morphine and thebaine. Findings suggest the applicability of *4′-OMT2* gene in opium poppy as a promising target for genome-engineered BIAs. Lastly, sgRNA targeting the gene for rosmarinic acid production (SmRAS), under the U6 promoter, was explored for the traditional Chinese medical herb *Salvia miltiorrhiza* (Zhou et al. 2018). The RAS expression level and phenolic metabolites such as rosmarinic acid were significantly decreased among homozygous mutants. The abovementioned studies provide empirical proof for CRISPR/Cas9-mediated bioengineering of promising metabolites from pharmacologically important plants. Similarly, CRISPR/Cas9-mediated targeted mutagenesis was employed to knockout phytoene desaturase (PDS) in *Medicago truncatula* (Meng et al. 2017). PDS is a critical gene for the biosynthesis of lycopene, a potent antioxidant. Monoallelic and biallelic homozygous mutants were considerably produced using the approach, which suggests the applicability of the pipeline employed for the genome editing of PDS.

Various genes/transcription factors have also employed CRISPR/Cas9-mediated approaches toward managing the abiotic stress tolerance in model plants and key crop species: *SbWRKY30* (Yang et al. 2020); *GmDREB* (Zhou et al. 2020); *ARGOS8* (Shi et al. 2019); *TaDREB2* and *TaERF3* (Kim et al. 2018a, b); *GhRDL1* and *GhPIN1* (Dass et al. 2017; Haque et al. 2018) and *AREB1* (Paixão et al. 2019). These are also putative targets that may be explored for medicinal plants.

Genome-editing using the CRISPR/Cas9 platform has also led to the development of tolerance to multiple abiotic stresses in different model plants and crop species targeting genes belonging to the ERF (ethylene-responsive factors) family: Arabidopsis (*OST2, UGT79B2/UGT79B2*); rice (*OsER1, OsER2, OsERRF109, OsBIERF1, OsBIERF2, OsBIERF3*), cassava (*MeKUP*), and banana (*MaAPS1* and *MaAP2*) (Debbarma et al. 2019). All these developments in recent years show the utility of genome-editing in engineering the climate-resilient crops.

3.2 Biotic Stress

Targeting resistance genes to major pathogenic groups also holds a lot of promise as evidenced by the various studies conducted on this theme. Gene targets like SlDMR6 in tomato provided resistance to a host of bacterial pathogens such as *P. syringae*, *P. apsica* and *Xanthomonas* sp. (de Toledo Thomazella et al. 2016; Langner et al. 2018). On the other hand, its homolog SlMLO1 provided resistance to powdery mildew caused by *Oidium neolycopersici* (Nekrasov et al. 2017). Gene editing of another DMR6 homolog in wheat *TaMLO-A1* conferred similar resistance to *Blumeria graminis* (Wang et al. 2014). Similarly, CRISPR/Cas-mediated knockout of the grape gene VvMLO7 reduced susceptibility to Erysiphe necator (Pessina et al. 2016). Another grape gene target, *VvWRKY52*, which codes for a transcription factor, involved four gRNAs in the CRISPR/Cas9 construct to confer partial resistance to *B. cinerea* (Wang et al. 2018). Pioneering efforts in transiently introducing gene-edited NPR3 (Non-Expressor of Pathogenesis-Related 3), also conferred resistance in *Theobroma cacao* to *Phytophthora tropicalis* (Fister et al. 2018). Several studies employed CRISPR/Cas9 systems on virus-related gene targeting. Gene-targeted mutagenesis of the *eukaryotic translation initiation factor 4E* (*eIF4E*), a cap-binding protein conferred resistance to several plant viruses (zucchini yellow mosaic virus, papaya ringspot mosaic virus, and the cucumber vein yellowing virus) belonging to Family Potyviridae (Chandrasekaran et al. 2016). Some other notable viruses targeted by CRISPR/Cas9 gene-mediated resistance include beet severe curly top virus (BSCTV) (Ji et al. 2015); tomato yellow leaf curl virus (Tashkandi et al. 2018); and banana streak virus (Tripathi et al. 2019); among many other. Thus, gene editing through the CRISPR/Cas9 system is a robust and promising tool conferring tolerance to the plethora of biotic stressors (Table 3).

4 Difficulties in Improving Stress Tolerance in Medicinal Plants

The rapidly expanding scope of applying genome editing in the crop improvement domain is vast and has offered various prospects in molecular biology research. However, despite having several improvements made to medicinal plants, various challenges have to be faced in materializing an efficient plant genome alteration.

4.1 Off Target Mutation

One important areas of concern are the off-target mutation since CRISPR/Cas9 can target unintended sequences, mainly if gRNA is not designed carefully and validated in silico before wet-lab experiments. Hence, it may cause deleterious effects on the

Table 3 Genome editing of pharmacologically important crop species inducing resistance to a specific pathogen

Plant	Technique	Phenotype (resistance to specific pathogen)	Target gene	References
Solanum lycopersicum	CRISPR/Cas (knockout)	*Pseudomonas syringae*, *P. capsici*, *Xanthomonas* sp.	*SlDRM6*	de Toledo Thomazella et al. (2016) and Langner et al. (2018)
		Oidium neolycopersici	*SlMLO1*	Nekrasov et al. (2017)
		Tomato yellow leaf curl virus	*TYCLV1*	Tashkandi et al. (2018)
Theobroma cacao	CRISPR/Cas (knockout)	*Phytophthora tropicalis*	*NPR3*	Fister et al. (2018)
Vitis vinifera	CRISPR/Cas (knockout)	*Erysiphe necator*	*VvMLO7*	Pessina et al. (2016)
Musa sp.	CRISPR/Cas (knockout)	*Banana streak virus*	*BSV*	Tripathi et al. (2019)

plant genome and may pose a potential menace to the environmental integrity. Hence, the risks and benefits, both should be evaluated for precise modification through genome alteration (Rodriguez 2016; Yang et al. 2013). Another important problem linked with genome editing, especially with CRISPR/Cas9 is the cleaving of identical or highly-homologous sequences, which can lead to cell death or unintentional transformation (Fu et al. 2013; Zhang et al. 2015).

4.2 Declining Species and Genotype-Dependent Transformation

Despite many technological advancements, transformation and regeneration remain strenuous for most medicinal plants (Altpeter et al. 2016). Currently, two development regulator (DR) genes named *Baby Boom* and *Wuschel2* along with ideal phytohormones have improved the efficiency of transformation in crops. Hence, future research endeavours require special focus on different forms of genome editing, in addition to targeted mutagenesis, gene insertion, and base replacement to improvise the transformation ability in medicinal crops.

4.3 Broadening of Targets

Gene or genetic refinement of medicinal crops during any trait modification must be assured by genome editing (Kwon et al. 2019; Oliva et al. 2019). However, limited knowledge of biological processes involving the genes, their routes, networking, as well as their interactions with various environmental factors create challenges to integrating the multidisciplinary approaches suitable for genome editing (Araus et al. 2018).

4.4 Regulatory Norms of Genome Editing in Medicinal Plants

The chances of things moving in the reverse direction may arise in stress resistance genome editing programs. For instance, the consequence of erroneous edits can lead to the conversion of some desirable crop varieties into invasive weeds. Therefore, it is essential to emphasize more the regulatory norms of CRISPR applications and other antecedent gene-editing technologies viz., TALENS and ZFN. Another issue is the regulation of patenting of genome-edited organisms where there are many economic interests involved giving rise to frequent controversies and frictions among biotechnological companies. Therefore, emphasis must be given to regulating the patent system efficiently to commercialize or release safe and healthy plants for humankind.

4.5 Need for a Promising Delivery System

Although genome editing possesses many beneficial applications yet it lacks enough in substantial plant genome manipulation and an improved delivery system. Problematic delivery systems create difficulties for implementing cost-effective gene editing in crop plants. However, the delivery mechanism via pollen-mediated transformation can prevent the species specificity limitations and reproduction by pollination or artificial hybridization. The delivery systems that are based on nanotechnology and virus particle-like structures can also be considered promising (Husen and Iqbal 2019); for instance, the carbon nanotubes, which have been used to transmit DNA to mature plant leaves, contributing to efficient protein expression (Demirer et al. 2018). Other nanomaterials, like layered double hydroxides (Mitter et al. 2017), mesoporous silica nanoparticles and poly ethylenimine (Cunningham et al. 2018), also possess tremendous potential to increase the supply of delivery vehicles as they cause little cellular disruption, have low toxicity, and gain high efficiencies in transformation.

5 Future Perspectives for Efficient Outcomes in Medicinal Plants

Gene editing may accelerate the medicinal plant domestication. Modern crops have been selectively bred for several years, leading to the introduction of important features, which allow high-quality, nutrient-rich food to be harvested mechanically (Osterberg et al. 2017). Genome editing may also be considered for manipulating the mitochondrial and chloroplast genomes. Editing could be used in identifying cell lineages for understanding the patterns underlying plant growth, creating genetic circuits to combine and transduce signals, and establishing plant biosensors to detect internal and external signals. It would be imperative to establish more efficient editing technologies capable of combining or "stacking" mutated alleles. Besides, it is recommended to achieve greater precision with RNA base editing ("RESCUE" and "REPAIR") technologies in the future.

6 Conclusion

The utilization of genome editing tools is widely established for model crops such as rice, wheat, barley, and maize. However, only a few studies have been implemented in medicinal plants, although a significant increase has been observed over the years in the utilization of these plants as supplementary medicine and for aesthetic purposes.

In the future, the application of these tools would be necessary due to the increasing demand for crops and to develop climate-smart medicinal plants. The ability of genome editing tools to generate specific and genome-wide editing in the diversity of plants has produced remarkable advancements in genetics and breeding research. These tools have emerged as robust tools for the improvement of varieties of traits such as yield, disease resistance, and stress tolerance in plants.

Application of these tools in medicinal plants is still in its infancy owing to the lack of genome references. These genome references are necessary to identify key genes that are involved in biotic and abiotic stress tolerance in medicinal plants. The recently and widely adapted tool, CRISPR/Cas9, promises great potential in medicinal plant research. The rapid pace and development of this tool can immensely contribute to the understanding of gene regulatory networks which are associated with stress response and adaption of medicinal plants.

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Phytoremediation Potential of Medicinal Plants

Brian Robert Shmaefsky and Azamal Husen

Abstract The demand for traditional herbal medicines has grown exponentially worldwide in the twenty-first century. Much of this growth is in economically disadvantaged nations seeking sustainable and inexpensive options for treating endemic and emerging epidemic diseases. In wealthy nations, much of increased desire for medicinal plants is for the formulation of cosmetics and nutraceuticals. Research studies for identifying and confirming the effectiveness of medical plants is globally competitive. However, having an arsenal of effective medicinal plants is impractical without research that investigates the profitable cultivation of indigenous and non-native medicinal plants. Much of the medicinal plant cultivation research investigates the impacts of environmental stress factors on plant development and growth, leading to the discovery of physiological mechanisms that protect plants from unfavorable environmental conditions. Besides benefiting the plant, these mechanisms make the plant a candidate for phytoremediation, a viable option for removing pollutants from contaminated soil and water. The physiological mechanisms that contribute to phytoremediation are involved in extracting, immobilizing, or removing salts, metals, organic compounds and radionuclides from soil and water. In medicinal plants phytoremediation properties have the potential of precluding the safe use of the plant. In contrast, certain properties can allow the safe use of the plants for human consumption while removing contaminants from soil or water.

Keywords Environmental stress · Phytoremediation · Medicinal plants · Medicinal plant safety · Plant physiology

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A. Husen, M. Iqbal (eds.), *Medicinal Plants*,
https://doi.org/10.1007/978-981-19-5611-9_17

1 Introduction

1.1 What Is Phytoremediation?

Phytoremediation is a biotechnology strategy of using plants for removing a variety of pollutants from contaminated sites (Jabeen et al. 2009; Shmaefsky 2020). It uses a plant's physiological stress responses and rhizosphere organisms as inexpensive and reliable tools to remove some of the most hazardous or persistent pollutants in regions with few financial resources available for pollution remediation in soils and waterways (Schwitzguébel 2015). The physiological processes, symbiotic relationships, and abiotic environmental factors that make plants suitable for environmental cleanup by phytoremediation often counteract the utility of these plants for agricultural and medicinal uses (McCutcheon and Jørgensen. 2008). Phytoremediation is becoming a popular pollution remediation strategy in economically developing countries because it is inexpensive and, in many circumstances, more reliable than technological approaches for removing the hazardous or persistent pollutants (Schwitzguébel et al. 2011).

The idea of using plants for bioremediation is attributed to Robert Richard Brooks' research studies on hyperaccumulating plants in 1960s CE (Brooks 1998). Hyperaccumulating plants are naturally capable of growing in soils or water with high concentrations of metals that would normally harm other plants. They can tolerate large concentrations of metals in their tissues while exhibiting no signs of cytotoxicity. Some of these plants have specialized metal transporter proteins that facilitate the uptake of metals that are typically not transported into cells (Rascio and Navari-Izzo 2011). Brooks directly and indirectly contributed to the discovery of hundreds of hyperaccumulating plants selectively capable of up-taking and accumulating various metals such as aluminum, arsenic, cadmium, cobalt, copper, chromium, lead, manganese, mercury, molybdenum, nickel, selenium, thallium, and zinc (Brooks 1998). Later it was discovered in a host of studies that certain hyperaccumulating plants could uptake radioactive materials (Fulekar and Singh 2010). In many studies, uptake parameters such as bio-concentration factor, translocation index, Cr distribution within the plant, and tolerance index of plants, have been considered to determine the remediation potential of plant species and genotypes (Diwan et al. 2010a, 2012; Ansari et al. 2015, 2018, 2021). Currently, there are at least 450 plant species from at least 45 angiosperm families confirmed to be the metal hyperaccumulators (Yan et al. 2020).

Studies conducted in the 1990s by academic researchers and the US Environmental Protection Agency (Kling 1997) paved the way for using plants for the bioremediation of organic contaminants in soil and water. These plants were not the bioaccumulation plants used for remediating metals; rather, these plants were capable degrading or detoxifying a variety of organic chemical pollutants in soil and water. The organic chemicals these plants could remediate included crude oil, explosives, herbicides, landfill leachates, pesticides, petrochemicals, and wastewater components. Currently, many types of plants are being used for phytoremediation,

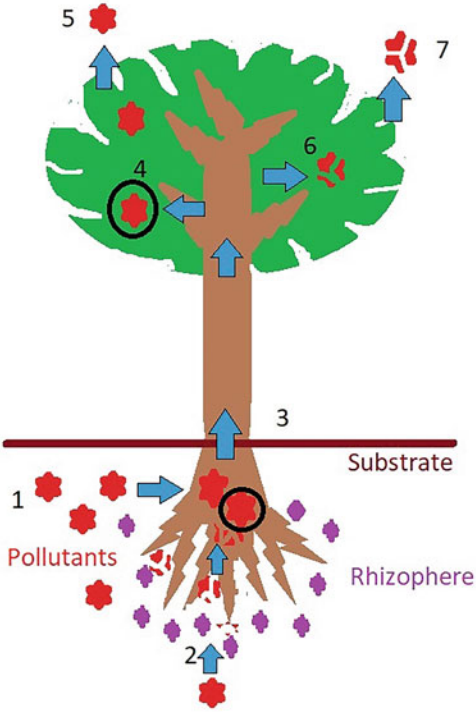

Fig. 1 Phytoremediation is the adsorption of contaminants and compartmentalization of the contaminants into root cell vacuoles. In some plants phytoremediation is induced by rhizosphere metabolism. In certain plants contaminants then accumulation in roots or in aerial organs upon root-to-shoot xylem transport. Particular contaminants are degraded by rhizosphere, root, or leaf metabolism which allows volatilization of the toxic compound. Phytoremediation typically occurs in the following sequence. Metals and organic compounds (red stars) absorbed on (1) soil particles or cell walls or (2) induced by rhizosphere metabolism enter the roots. Contaminants are sequestered in the roots (black circle around contaminant) or are (3) transported to the aerial parts. Contaminants are (4) sequestered or (5) or removed by transpiration. Contaminants can be (6) degraded by leaf metabolism be removed by (7) volatilization of the toxic compound

and many others are being explored, including the plants known to have medicinal properties (Gandotra et al. 1999; Gawronski and Gawronska 2007; Iqbal et al. 2015; Ansari et al. 2021). Some external applications also affect the phytoremediation capacity of plants. Saifullah et al. (2016) reported that sulfur fertilization improved the photosynthetic and transpiration rates and consequently increased the straw and grain yields of wheat grown on Pb-contaminated soil. It enhanced not only the S and Zn contents of different plant parts but also Pb deposition in roots, its translocation from roots to shoot, and accumulation in grain. Thus, besides mitigating the toxic effect of Pb and improving the plant growth, sulfur enhanced the phytoextraction capacity of wheat plants. In certain cases, plants used for phytoremediation are later harvested for use as herbal remedies (Mafakheri and Kordrostami 2020) (Fig. 1).

1.2 Safety Implications of Phytoremediation

During the phytoremediation process, it is possible for plants to accumulate the hazardous materials in their reproductive and vegetative structures (Ansari et al. 2013a, b; Chen et al. 2019). This can contaminate plant extracts, poultices, and dried tissues used for herbal medical preparations. Not all phytoremediation activities result in the accumulation of harmful materials in plant tissues. However, it is crucial to understand the several types of phytoremediation processes that can interfere with the preparation of medicinal plants for therapeutic and veterinary applications (Abubakar and Haque 2020). Many studies are now indicating that certain types of phytoremediation activities are known sources of medicinal plants contaminants (Kosalec et al. 2009; Dökmeci and Adiloğlu 2020).

An understanding of the factors that influence phytoremediation is necessary for predicting the chance of encountering harmful materials that can accumulate in medicinal plants collected in the wild (Fischer et al. 2017; Lajayer et al. 2017), cultivated in fields or greenhouses (Gupta 2017), and grown in vitro (Ansari et al. 2013a; Cardoso et al. 2019). It is essential to realize that phytoremediation mechanisms are natural features of plants that evolved and coevolved in response to abiotic stressors and assist with plant survival. The major abiotic factors that induce phytoremediation responses are drought, salinization, high metal and metalloid concentrations, exposure to harmful and biocidal organic compounds, extreme soil or water pH fluctuations (Zhu 2016). Biotic factors such as plant pathogens and rhizosphere microbiome interactions (Thijs et al. 2016) can diminish or enhance phytoremediation. Manipulating these stress factors as an attempt to discourage phytoremediation could negatively affect the growth of plants and the ability of plants to synthesize the medicinal compounds (Rai et al. 2020).

The phytoremediation potential of medicinal plants creates concerns about the possible toxic contamination of a plant's biomass (Song and Park 2017), and may impact the governmental policies and regulations pertaining to the use of medicinal plants (Djordjevic 2017). Public health concerns regarding medicinal plant safety are a growing interest all over the globe (Husen 2022). Aside from evaluating safety issues associated with the efficacy and side effects of medicinal plant preparations, any potential harmful contaminants must be assessed to protect the welfare of users (Ang-Lee et al. 2001; Ekor 2014). Contaminant-free medicinal plant preparations are becoming an emerging issue as physicians embark on testing medicinal plants in complementary, alternative, and integrative health applications with conventional drugs and therapies (Fasinu et al. 2012). There are numerous evidence-based studies on plant contaminates that complicate the use of alternative and integrative medicine (Ernst et al. 2001). In addition, contaminants could alter the additive, antagonistic, and synergistic interactions of the complementary treatments (Clark et al. 2021) as well as exacerbate any side effects (Niggemann and Gruber 2003).

1.3 Implications of Phytoremediation on Medicinal Plant Policies and Regulations

There are many constraints on the use of plants or plant-based products used in the agricultural, cosmetics, medical, and textile industries. One important concern about plant-basted materials is their safe use as a component or an ingredient in consumer products and medicines (Parveen et al. 2020). Medicines are particularly scrutinized for safety concerns as well as for their effectiveness. Safety guidelines and policies for medicinal compounds vary based on whether they are over-the-counter, pharmacy controlled, prescription, or government regulated controlled substances. Guidelines and oversight also differ from one country to another, which creates inconsistencies in the quality of medicinal plant products (WHO 2022; Rägo and Santoso 2008).

The global market for herbal medicine is growing significantly. It is estimated that annual sales of medicinal plant products will reach US$550 billion by 2030. Much of the growth in demand is in the geriatric population. Asian countries are the fastest growing market for medicinal plants. However, there is a growing demand for medicinal plants in Africa, Europe, Latin America, and North America also (Fact. MR 2022). This increased desire for herbal medicine is stimulating the expansion of medicinal-plant farming in regions that may favor phytoremediation activity in the plants (Davis 2012).

New developments in genetically modified medicinal plants are being done to improve the pharmacomics and nutriomics of the plants (Yan et al. 2006). A plant's pharmacomic properties take into account how the genetic attributes of a drug recipient affect the likely responses to therapeutic drugs. Some genetic modification procedures used on medicinal plants could introduce phytoremediation properties into these plants while attempting to improve growth in stressed environments (Shmaefsky 2010).

The growing demand for medicinal plants is building a large agrotechnology sector specific for medicinal plants. This must encourage some type of consistency and quality control in the production of these plants. The recognition of medicinal plant farming as a sustainable practice consistent with the United Nations Sustainable Development Goals is encouraging countries to further develop and conserve medicinal plant resources (Sharrock and Jackson 2017). However, this endorsement is also putting pressure on the production of medicinal plants that are free of hazardous contaminants that can be introduced by phytroremediation processes (Silori and Badola 2000).

There are initial attempts at self-regulating the medicinal plant sector to set growing and processing standards. Proponents of medicinal plant standards identified key limitations about the utility of medicinal plants, such as (1) inadequate standardization, (2) a lack of quality specifications, (3) potentially serious drug interactions, and (4) a lack of scientific data related to drug safety (Kataria et al. 2011). These limitations are the basis for governmental polices about medicinal plant standards in the European Union (Knöss and Chinou 2012) and the United States

(Wu et al. 2020). Other countries are considering adhering to any global standards that ensure medicinal plant safety as a way of protecting their populations as well as making the medical plant products globally competitive. Regulations related to the safety of medical plant contaminants will likely be evaluated using standard current pharmaceutical testing procedures (Liu et al. 2019) and emerging technologies such as genotoxicology assay investigations (Sponchiado et al. 2016). The United States Food and Drug Administration regulations (FDA 2007) and World Health Organization recommendations for medicinal plant safety will likely be suggested as global models for standards and regulations (WHO 2005).

The by-products and waste products of medicinal plant production could have a profitable secondary use in the expanding plant-based products market, particularly for the use in animal-based protein substitutes, food packing and textiles (Shogren et al. 2019; PBPC 2020). The regulation of contaminants will be based on the product life cycle of the materials made from the medicinal plant by-products and waste products (Varela-Ortega et al. 2021). To be globally competitive, tt is essential to assume that any medicinal plant products used for consumer products follow United States Food and Drug Admintration (FDA 2021), United States Department of Agricutlure (USDA, Herbal Informaton 2022a) and the World Health Organization (WHO 2007). It is also prudent for medical plant products sold in local markets as herbal nutritional crops to satisfy guidelilnes proposed by the Food and Agriculture Organization of the United Nations (FAO 2022).

Many of the contaminants introduced by phytroemediation into medical plants are quality control and safety concerns proponents of entities promoting medicinal plant standardization (Leung and Cheng 2021; Beressa et al. 2021). Testing methods targeting the safety of medicinal plants are being developed and used more frequently on laboratory animal models (Alelign et al. 2020) and on animal cell tissue cultures (Saad et al. 2006; Akhtar et al. 2016; Kahaliw et al. 2018). Past studies show that tissues and extracts of medical plants undergoing phytoremediation in contaminated environments can become acutely cytotoxic and unfit for any medical or consumer use (Ramborger et al. 2021; Oladoye et al. 2022). This situation obviously works against generally accepted safety recommendations, regulations, or standards and would necessitate methods for removing the cytotoxic compounds in order to market the plants or plant products (EU 2022).

2 Phytoremediation Principles

2.1 Phytoremediation Types

Brooks' initial studies on plant bioremediation led to numerous studies on the mechanisms of phytoremediation plants (Brooks 1998). It is now known that phytoremediation involves a complex set of normal metabolic processes carried out in plants and in some instances the bioremediation processes of microbes in the rhizosphere (Agarwal et al. 2020) and in root endosymbionts (He et al. 2019).

These pathways evolved in response to abiotic stress factors related to drought (Des Marais and Juenger 2010) and biotic factors associated with natural organic contaminants, such as waste products and decomposition intermediate molecules, in soil and water (Embrandiri et al. 2016). Plant responses to drought typically allow plants to implement metabolic processes that facilitate tolerance to high levels of metallic and mineral salts. Soil or water pH fluctuations outside of a plant's normal range can also induce plants to carry out drought responses. This is done as a means of protecting the plant from high levels of macronutrients and micronutrients created by pH oscillations that effect ion solubility in soil and water (Fujii 2014). A host of studies discovered that temperature stress also induces tolerance to high levels of anions and cations as a means of reducing oxidative damage associated with extreme temperature damage (Zhang et al. 2022). Plants vary greatly in their stress responses, and this in turn makes some plants better candidates for being a high-function phytoremediation plant (Jabeen et al. 2009). Studies also show that certain rhizosphere microbiome compositions facilitate or hinder phytoremediation depending on the microbiome in the soil (Palmroth et al. 2007).

Researchers who work with phytoremediation often classify the plants' remediation mechanisms into two categories: stabilization or containment mechanisms and removal or degradation mechanisms. Stabilization or containment prevent pollution from migrating into nearby soil or water. Containment binds pollutants to soil particles or to the rhizosphere. The pollutant particles can be immobilized in their native form or in a chemically converted form. Stabilization of the chemically converted form is typically achieved with redox reactions that are often facilitated with enzymes released by plant roots or rhizosphere microbiome organisms (Shackira and Puthur 2019). Removal or degradation mechanisms typically involve biodegradation pathways using enzymes within the plant tissues or in the rhizosphere. In plant biodegradation, the pollutants are initially broken down by enzymes secreted by the roots. The breakdown products are either absorbed by the roots or further degraded and metabolized by soil microorganisms. Any breakdown products taken up by the plant tissues and degraded further (Jee 2016). In general, metal salts cannot be degraded and are thus immobilized, but organic pollutants can be broken down and hopefully converted into less hazardous compounds.

2.2 Stabilization or Containment Mechanisms

The stabilization or containment mechanisms in phytoremediation exploit stress responses to drought, high salinity, and pH fluctuations. Plants with stabilization or containment properties typically secrete root exudates that bind ions and certain organic compounds to soil particles. The secretions are also capable of binding soil particles to encapsulate the pollutants. Some of these plants have a high transpiration rate and maximize the rate of pollution entrapment by moving large volumes of water to their roots. Plants capable of stabilization or containment are usually divided into two categories: phytostabilization plants and hydrolytic control plants (FRTR 2020).

Phytostabilization is the use of plants to immobilize contaminants in the rhizosphere or soil. Soil immobilization occurs when plants or the rhizosphere microbiome produce secretions that reduce contaminant movement. In certain plants, enzymes are used to bind contaminants by converting decaying mater into humus in a process called humification. The humus reduces the soil contaminants by absorbing and binding to minerals, metals, and many types of organic molecules (Jabeen et al. 2009). Lignin released by roots acts similarly to humus. Plants can also capture contaminants in their roots by deposing lignin in their root endodermal cells, forming the Casparian strips. This internal lignification traps and often accumulates pollutants inside the roots (Naseer et al. 2012).

Hydraulic control is a type of phytoremediation that slow the movement of contaminated groundwater or waterways. These plants are selected for their deep root system and high groundwater uptake rates, which is driven by a high transpiration rate. The rapid water uptake of these plants provides migration control for mobile pollution plumes and outflows. Many of these plants carry out other phytoremediation processes, including those that cause the uptake of pollutants into the plant tissues (Etim 2012).

2.3 Removal or Degradation Mechanisms

The removal and degradation in phytoremediation process typically degrade and uptake soil or water contaminants. Many phytoremediation plants may require a co-metabolism relationship with microorganisms in order to carry out remediation (Hooda 2007). Certain plants can accumulate particular minerals and metals in their living tissues to levels that may be up to thousands times greater than is normal for most plants. They are generally effective at taking up heavy metals and radioisotopes (Tonelli et al. 2020). Other types of plants can accumulate organic compounds (Fan et al. 2018). The removal and degradation plant mechanisms are categorized as phytoaccumulation, phytoextraction, phytotransformation, phytovolatilization, rhizodegradation, and rhizofiltration (Jabeen et al. 2009).

Phytoaccumulation takes advantage of specific plants to take up and accumulate large amount pollutants through stress-adapted organelles and transpiration mechanisms. These plants focus on the uptake of anions and cations. Mineral and metal uptake requires that they are dissolved in soil moisture or groundwater to be absorbed by the plant roots. Once absorbed, the materials are transported throughout the plant. Many phytoaccumulators store the compounds in roots and leaves, where the minerals and metals accumulate. Halophytes are a group of phytoaccumulation plants that evolved to survive in high saline environments. Many halophytes are specifically adapted to tolerate high levels of salts, and may not tolerate toxic metals (Jlassi et al. 2013). The biomass of these can be used for metals recovery but may not be suitable for consumer products and medicinal compounds.

Plants that carry out phytoextraction specialize in the uptake of organic compounds, and typically do not hyperaccumulate metals. These plants are unique from other plants in being able to break down and absorb complex organic chemicals, such as phthalic esters (PAEs), polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs), polychlorobiphenyls (PCBs), petroleum hydrocarbons (PHC), pesticides, and other volatile organic compound pollutants (VOCs) (Chen et al. 2013). The organic compounds are taken up in their native form into the plant roots. The molecules are absorbed as chemicals dissolved in soil moisture, groundwater, or surface water. Some plants can absorb the pollutants in soil vapor. The molecules are then transported from the roots to the leaves. These molecules are either accumulated or metabolized in the leaves and transpired into the atmosphere (Iqbal et al. 2015). Recent phytoremediation investigations are discovering plants that can phytoremediate organic air pollutants, such as VOCs and particulate matter, from the atmosphere by leave absorption through the stomata. The fate of the organic molecules in the plant tissues is still under investigation (Brilli et al. 2018). The impacts of organic compound phytoremediation activities on medicinal plants varies greatly based on the type of phytoremediation and is being studied in crop plants used for phytoremediation (Haller and Jonsson 2020).

Phytotransformation, also known as phytodegradation, refers to the use of plants to break down organic contaminants. The plants used in phytotransformation take up the organic materials through the roots and perform the bioremediation intracellularly. Biodegradation is typically achieved using hydroylases that attach hydroxyl functional groups to the contaminant molecules or oxidases that modify contaminant functional group. The contaminants are often modified with a second phase of metabolism using detoxification enzymes (Iqbal et al. 2015). Phytotransformation is relatively inexpensive and has been shown effective against atrazine, PCPs, pesticides, petrochemicals, and TNT (Newman and Reynolds 2004). Certain halophytic plants can perform phytotransformation on polyvalent metals such as chromium with the outcome of converting the harmful forms of chromium into micronutrients (Caçador and Duarte 2015).

Phytovolatilization uses plants to remove contaminants from the soil or water and then evaporate or volatilize the contaminants through the leaves. Phytovolatilization exploits transpiration and sometime phytotransformation to remove contaminants from soil and water. In this process, plants uptake the contaminants in the roots. The contaminants are then transported to leaves where these are removed by transpiration as a volatile substance. Many of the compounds are degraded or detoxified before being transpired. This process is most effective on organic pollutants. Phytovolatilization has also been used to remediate mercury, which is converted to its elemental form. Other studies used phytovolatilization to remove arsenic and selenium from soil and water (Arya et al. 2017).

Rhizodegradation, often called phytostimulation, is carried by plant root systems and microorganisms in the rhizosphere. The rhizosphere is a thin region of soil modified by a complex mixture of root exudates and a unique microbiome made up of bacteria, fungi, and protists. The nature of the rhizosphere, and any rhizodegradation activity, is determined by the plant-soil-microbiome

Table 1 The major types of phytoremediation properties that can be found in medicinal plants

Process	Mechanisms	Pollutants	Substrate
Phytoaccumulation	Hyperaccumulation Vacuolar storage Osmotic tolerance	Mineral salts Metals	Soil Water
Phytoextraction	Vacuolar storage Hyper-Transpiration	Organic compounds	Soil Water Atmosphere
Phytotransformation	Vacuolar storage Plant detoxification Transpiration	Organic compounds Metals	Soil Water
Phytovolatilization	Hyper-Transpiration Limited detoxification	Organic compounds Mercury, selenium, arsenic	Soil Water
Rhizodegradation	Vacuolar storage Microbial detoxification Transpiration	Organic compounds Mineral salts Metals Xenobiotics	Soil Water
Rhizofiltration	Microbial detoxification Plant detoxification Microbial detoxification Transpiration	Metals	Water

interrelationship and soil conditions. To maintain interrelationship, plant roots excrete acids, alcohols, and sugars as a carbon source for microorganisms. This enriches microbial activity in rhizosphere (Iqbal et al. 2015). Certain soil microorganisms are capable of bioremediation and contribute to microbial degradation of soil contaminants. Rhizosphere dynamics has been the subject of basic ecological research for many years. However, it is only recently that these findings are being applied to agriculture, land management, and phytoremediation. The plant-microbiome environment is proving effective at degrading metals, organic pollutants, radionuclides, and xenobiotic compounds (Dzantor 2007).

Rhizofiltration is a variation of rhizodegradation and is similar to phytoextraction in aquatic systems or water-saturated soil environments. In this application of bioremediation, the aquatic rhizosphere acts as a filter that uptakes and degrades water contaminants (Hanus-Fajerska and Koźmińska 2016). It can be used for remediating groundwater and surface waters. The aquatic rhizosphere is made up of a microbiome biofilm encapsulated in plant root exudates and secretions (Lee et al. 2013). Rhizofiltration is a stress response in aquatic or wetland plants when exposed to harmful metals such as Cu (2+), Cd (2+), Cr (6+), Ni (2+), Pb (2+) and Zn (2+). The rhizosphere microbiome enhances the ability of phytodegradation companion plants to completely mineralize or partially break down contaminant compounds through metabolic action within the plant tissues (Table 1).

3 Phytoremediation Physiology and Environmental Factors

3.1 Phytoremediation as a Stress Response

Phytoremediation is a type of plant stress adaption for an immobile life cycle. Unlike mobile organisms, plants cannot escape abiotic stressors such as drought, salinity, extreme temperatures, and water inundation. So, it is essential for plants to express traits that efficiently counteract environmental harm. Stress responses require receptors that can detect a stress factor and then communicate a signal to general or specific stress response mechanisms. Ancestral plants made use of SNF1-related protein kinase 2s (SnRK2s) cell signaling pathways to initiate environmental stress responses. SnRK2s is related to the SNF1/AMPK protein kinases that are highly conserved in eucaryotes (Craig et al. 2018). SNF1 (sucrose non-fermenting 1) adapts yeast cells to glucose limitations stress and shifts to metabolic pathways that permit yeast to use other cellular respiration carbon sources (Hedbacker and Carlso 2008). AMPK (AMP-activated protein kinase) is found in mammals and regulates cellular and somatic energy homeostasis (Bright et al. 2009). It is suggested that plants' ancestors initially used SnRK2s for low energy sensing, or low energy syndrome (LES), signaling pathways, in response to photosynthesis efficiency in aquatic environments (Kulik et al. 2011). The first land plants adapted LES signaling to deal with the osmotic stress associated with fluctuations in water salinity. It has been shown in algae that SnRK2s is activated when it is phosphorated by the phytohormone abscisic acid (ABA) (Al-Hijab et al. 2019). ABA accumulates in plants that are under osmotic stress. It initiates SnRK2s by first binding to a PYR/PYL/RCAR receptor complex. The ABA-PYR/PYL/RCAR complex then removes a PP2C Group A protein phosphatase type 2C (PP2C), which protects the SnRK2s phosphorylation site.

It is hypothesized that the phytromendiaiton stress response was one of many outcomes of the diversification of the LES signaling system in adapting to terrestrial life (Zeng et al. 2020). Phytoremedation in many plants likely diverged from the LES-derived pathways for remediating oxidative stress due to elevated concentraitons of ions in the soil and the high levels of oxygen in the Earth's atmosphere (Ahmad and Prasad 2012). Phytoremdiation properties, particulary for heavy metal tolerance, have also been associated with a mitogen-activated protein kinase (MAPK) pathway that is normally involved in biotic stress, such as fungal infections (Opdenakker et al. 2013). Further natural selection events led to the development of a variety of plant hormone receptors and environmental signal receptors that regulate phosphoration cascade systems for responding to other abiotic and biotic stressors, including predation and pathology (Lamers 2020). Evolutionary modifications to the low energy sensing pathways involve transcriptional, translational, and metabolic reprogramming that is necessary for stress adaptation (Tomé et al. 2014) and has the consequence of down-regulating plant secondary metabolite pathways. These adaptations to stress can unfortunately reduce the production of therapeutic compounds in medicinal plants and introduce unfavorable metabolites that can harm people (Isah 2019).

Many of strategies used to understand plant stress and phytoremediation were tested on *Arabidopsis*, a popular generalizable model for understanding the impacts of abiotic stress on phytoremediation plants (Clemens 2001). The genus demonstrates the properties of non-phytoremediation and phytoremediation plants, and information on its genome, mitochondrial genome, proteome, and metabolome is well-studied (Huang et al. 2011). Studies on *Arabidopsis* suggest that genes for phytoremediation stress responses promote stress tolerance by regulating gene expression through a variety of signal transduction pathways (Nakashima et al. 2009).

3.2 Biotic Factors Enhancing Phytoremediation

An understanding of the phytoremediation physiological processes helps explain the implications of phytoremediation on the effectiveness and safety of medicinal plants. Medicinal plants can be subjected to in-situ and ex-situ stresses before being marketed, which include biotic and abiotic factors, many of which are induced by environmental stress. Direct stress is not the only factor that induces phytoremediation processes in plants. Under certain environmental conditions, the microbiome of the rhizosphere may indirectly induce phytoremediation. Rhizosphere organisms can help stimulate root proliferation and plant growth, thereby increasing a plant's ability to uptake inorganic and organic chemicals from the soil (Imadi et al. 2016). Another indirect effect of rhizosphere microorganism is their ability to improve plant fitness with a subsequent increase in a plant's ability to carry out phytoremediation in response to stressors (Yan et al. 2020). Direct influences of rhizosphere on phytoremediation include assisting plants with phytoremediation potential under stressed environments (Jing et al. 2007). Rhizosphere organisms known to facilitate phytoremediation are arbuscular mycorrhizal fungi (Göhre and Paszkowski 2006), legume rhizobia (Fagorzi et al. 2018), and other plant-growth-promoting (PGP) bacteria such as *Azospirillum fluorescens*, *Azospirillum lipoferum*, *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, and *Pseudomonas putida* (Souza et al. 2015).

It is believed that the symbiotic relationship between terrestrial plants and the rhizosphere microbiome began as aquatic algae-bacteria symbiotic relationships before the evolution of terrestrial plants. In some situations, the symbiosis resulted in phytoremediation activities that are being applied to remediate wastewater (Ji et al. 2018). These symbiotic relationships led to the rhizosphere-induced phytoremediation activities in aquatic and terrestrial vascular plants. Plants produce compounds that attract, retain, and nurture the microbiome which the rhizosphere microorganisms mutually provide the plants with nutrients and help plants elicit responses to environmental stress. The relationship between the rhizosphere and plants creates a delicate balance of keeping the plants healthy, but inducing stress responses also diverts normal plant functions. Unfortunately, some symbiotic relationships induce immune response reactions such as the production of antimicrobial lectins, as a means for plants to keep endophytic relationships under control (Jiang et al. 2010). For medicinal plants, differences in rhizosphere communities could impact the quality, purity, and growth rate of the plants (Qi et al. 2012).

3.3 Abiotic Factors Enhancing Phytoremediation

As discussed earlier, abiotic stress factors enhance phytoremediation activities in any plant. Plants that are identified for their phytoaccumulation, phytoextraction, phytotransformation, and phytovolatilization properties respond more intensely to abiotic stress (Cobbett and Meagher 2002). This acute stress response improves the survivability of those plants but may also impart outcomes that affect their edibleness or medicinal utility. Some of the abiotic stress induction pathways overlap with the stress response pathways elicited by biotic factors. Abiotic stresses that produce or enhance phytoremediation properties are osmolarity, pH, temperature, and environmental contaminants. Osmolarity changes can by caused by drought, salinization, and environmental contaminants. These contaminants can also cause oxidative damage stress responses. Temperatures above and below the tolerance range for a plant will induce stress responses.

Plant responses to osmolarity stress are set in motion to protect the plant from possible damage. One representative osmolarity stress response is exhibited by the medicinal plant *Salvia sclarea* when it is exposed to excess toxic metals. Salvia is a zinc-accumulator and can tolerate high toxic levels of zinc in the leaves. It responds to zinc by sequestering calcium, iron, and manganese ions in the leaves to protect the photosynthesis by enhancing the photosystem I (PSI) and photosystem II (PSII) activities (Dobrikova et al. 2021). Another response to excess zinc, as a representative metal, is an increase in the synthesis of anthocyanins and phenolics in the leaves. This response is likely involved in protecting the plant from oxidative damage. Lipid peroxidation and electrolyte leakage were used as indicaters of oxidative damage (Anjum et al. 2015). This response in medicinal plants helps the plants survive osmotic stress, but the plants may accumulate harmful levels of metals in tissues causing an upset in the ratios of secondary metabolites (Masarovicova et al. 2010).

Drought and salinity stress typically share common stress responses that induce phytoremediation activities. The initial plant response to drought and salinity are a reduction or cessation of root and shoot growth, closure of stomata, and a decline of photosynthetic activity. To limit stress damage, plants make use of AtPR5K2 kinase to phosphorylate ABI (*Arabidopsis thaliana* abscisic acid insensitive) signal transduction adapting proteins that mediate abscisic acid (ABA) and sugar responses associated with plant growth (Baek et al. 2019). This signing pathway is independent form the SnRK2s low energy sensing signaling system. The ABI-1 activates mechanisms involved in desiccation tolerance, energy utilization, and tissue growth

(Brocard-Gifford et al. 2004). Some of the desiccation tolerance responses activate cell transport mechanisms that facilitate salt exclusion, salt extrusion, salt dilution, and compartmentation of ions (Wu 2018). Drought and salinity improve the tolerance of medicinal plants to those growing conditions (Koocheki et al. 2008), and there is evidence it may improve the yield of metabolites of medicinal value (Selmar and Kleinwächter 2013).

Phytoremediation stress mechanisms are enlisted in response to low levels hazardous environmental contaminants such as heavy metals and toxic organic substances. Heavy metals usually activate four individual mitogen-activated protein kinases (MAPKs): SIMK, MMK2, MMK3, and SAMK. MAPK pathways are normally activated in response to plant hormones and during cell division. Environmental challenge, such as heavy metal or radioisotope pollution, also induces the MARK system (Jonak et al. 2004). The dynamics and kinetics of the stress response are determined by the type and concentration of heavy metals, which are first expressed in roots. Stress responses to toxic metals are handed by enzyme pathway involved in the homeostasis of essential metal micronutrients, and is expressed the most in hyperaccumulator plants. In order to tolerate heavy metals, the stress response sets in motion three primary mechanisms: overexpression of transport systems that enhance sequestration of heavy metals in plant tissues, tissue-specific expression of proteins and metabolites involved in detoxification and antioxidation, and the deployment of metal chelators that immobilize the contaminants in the tissues (Viehweger 2014). Roots can also secrete antioxidation compounds and chelators in the rhizosphere (Iqbal et al. 2015; Montiel-Rozas et al. 2016). Evidence shows that heavy metal impacts on medicinal plant growth and metabolites make them hazardous for human health (Ditta et al. 2022).

Soil or water pH is another environmental stress that can set phytoremediation pathways in motion. Hydrogen ion concentration in soil is known to regulate the C2H2-type transcription factor STOP1 within the plant cell nucleus. STOP1 involves pathways that protect plants from aluminum and proton toxicity (Sawaki et al. 2009). C2H2 transcription factors are involved in host of plant responses including the root and flower development (Ye et al. 2021). They are also involved in regulation of tissue necrosis in response to pathogens. It has been shown that pH changes could also signal stress from light intensity changes, drought, and low level of oxygen. The pH stress response may affect plant metabolite synthesis in ways that impact the quality of medicinal plants, especially those with phytoremediation potential. The effect of pH stress adaptation mechanisms in medicinal plants is highly specific. Antioxidant activity in medicinal plant extracts is decreased at alkaline pHs, with a subsequent increase in prooxidant activity. In effect, this situation reduces the stability of plant extracts by reducing the concentration of antioxidants in the plant tissues (Bayliak et al. 2016).

Organic compound pollutants typically initiate oxidative stress and osmotic stress responses that also remediate metal concentration stress (Shiri et al. 2015). However, the stress pathways for organic compounds remediation express the genes for a variety of antioxidant enzymes including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX). Chelators are also expressed to immobilize or sequester the organic compounds (Rauser 1999). Without this stress response, plants exhibit a decrease in chlorophyll *a* and *b* accompanied by an increase in phenanthrene concentration. Phenanthrenes are opioid agonists found in medicinal plants used for the relief. Vulnerable plants also undergo apparent deformation of chloroplasts and the collapse of other cellular structures (Liu et al. 2009). Studies show that the rhizosphere microbiome can play a large role in promoting the phytoremediation of organic compounds even in plants that do not exhibit phytoremediation potential (Jambon et al. 2018). Many of the organic chemical pollutants degraded during phytoremediation do not accumulate in the tissues for long. However, pesticides, herbicides, and other xenobiotic compounds may be derivatized into water-soluble intermediates that render the plant tissues harmful for use in medicinal products. There is much variability on the concentration of harmful compounds that are retained in phytoremediation plant tissues (Saier and Trevors 2010).

Temperature stress is usually categorized into three types: freezing stress, chilling stress, and high temperature stress. Each type of temperature stress has different adaptation responses. Freezing stress is one of the most severe stressors on a plant and causes the production and release of damaging reactive oxygen species (ROS) that contribute to cell function disruption and cell injury. Plants respond to the ROS molecules by activating many of the antioxidant phytoremediation pathways expressed during the heavy metal or organic compound exposure (Baek and Skinner 2012). Chilling stress in vulnerable plants interferes with all physiological processes such as mineral uptake, photosynthesis, cellular respiration, water regulation, and other metabolic pathways. Chilling results in the overproduction of glutathione, which then accumulates and oxidizes in affected cells. The oxidized glutathione then becomes a signal to produce catalase and SOD (Gechev et al. 2003). Responses to high temperature stress in plants that reduce tissue damage and protect cellular functions, exhibit diversity. Induction of the heat stress response is signaled by the Arabidopsis CNGC2 gene. This gene expresses one part of the membrane cyclic nucleotide gated Ca^{2+} channel (Bita and Gerats 2013). Activation of the channel plays a role in the restructuring of lipid membranes (Horváth et al. 2012). Another mechanism for initiating heat stress responses is the expression of heat shock proteins (HSP) that protect the plant from oxidative damage (Firmansyah and Argosubekti 2020). This latter mechanism is likely to be related to other oxidation stress responses and would be more active in plants that carry out phytoremediation. It appears that temperature stress interferes with the protective features of phytoremediation and render the plants susceptible to environmental abiotic stressors (Sarma et al. 2021). Overall, temperature stress responses influence the expression of secondary metabolite biosynthesis genes in ways that decrease the quality of medicinal plants. These outcomes vary greatly from one plant to another based on taxonomic differences and phytoremediation potential of plants (Li et al. 2020).

4 Medicinal Plants with Phytoremediation Potential

4.1 *Taxonomy of Phytoremediation Medicinal Plants*

Medicinal plants can be found in almost all major phylogenetic group of plants. The same is true for plants with phytoremediation potential. Phytoremediation has been documented in the chlorophyta (green algae) (Liu et al. 2019), bryophyta (mosses) (Tesser et al. 2021), pteridophyta (ferns) (Praveen and Pandey 2020), coniferophyta (conifers) (Ouatiki et al. 2021), and magnoliophyta (flowering plants) (Shmaefsky 2020). The focus of most phytoremediation investigations has been on the flowering plants. Flowering plant families that possess many species with phytoremediation potential include the Amaranthaceae, Asteraceae, Brassicaceae, Cannabaceae, Cannaceae, Careophylaceae, Chenopodiaceae, Cyperaceae, Fabaceae, Poaceae, Pontederiaceae, Salicaceae, and Typhaceae (Gawronski and Gawronska 2007; Anjum et al. 2014). Phytoremediation has also been demonstrated using algae (Bwapwa et al. 2017), lichens (Cansaran-Duman and Aras 2015), and fungi (Khalid et al. 2021) that can be used for medicinal purposes. A major factor that determines the phytoremdiation potential of a taxonomic group is the evolutionary history of the plant in response to environmental stresses encountered during its speciation (Maestri and Marmiroli 2011).

Phytroemediaton databases are the most efficient resources for obtaining informaton plants with with phytoremediaton potentential. One database designed for people with interest in phytoremediation is available at http://www.steviefamulari.net/phytoremediation (Famulari and Witz 2015). Databases are also being compiled for plants that express targetted phytoremediation such as the PytoPet database of plants that can phytoremediate petroleum compounds (Farrell 2000). The U.S. Environmental Protection Agency Office of Superfund Remediation and Technology Innovation maintains a phytoremediation projects on a broad spectrum of phytoremediaiton applications (Green and Hoffnagle 2004). Unfortunately, there is currently no extensive database specifically identifying medicinal plants known to carry out phytoremediation.

Most of the literature on medicinal plant phytoremediation focuses on exploting the phytoremediaton potential of the plants and not on the medicinal utility of those plants. In many instances the plants are harvested and disposed either to reclaim the pollutants or dispose of the wastes in hazaroud wastes sites (Shmaefsky 2020). However, there are studies using these plants for phytoremediation purposes and then using the contaimnated biomass for energy production (Sharma et al. 2021). Another study investigates the feasibilty of harvesting phytoremediaiton medicinal plants for fibers and animal feed (Abubakar et al. 2021).

Some studies encourage using medicinal plants for their medicinal properties as well as environental remediation potentials. One representative article discusses how a green economy can be developed by selling medicinal plant products from plants used to remeditate polluted sites (Pirzadah et al. 2019). The authors do mention public health safety concerns and the need for new regualtions to address product safety when phytoremediation plants are used in cosmetics and health products. Another study in a medicinal and aromatic plants compendium also promotes harvesting medicinal plants from phytoremediation sites as a source of medicinal compounds (Kumar et al. 2021). Again, caution is advised while processing the plants to ensure they are free from hazardous contaminants.

4.2 Contaminants in Phytoremediation Medicinal Plants

Medicinal plants pharmacopoeias have been published for many human diseases and for almost all regions of the world (Fitzgerald et al. 2020). In an effort to promote the herb agriculture market, the Unites States Department of Agriculture provides resources on medicinal plants (USDA 2022b). Unfortunately, it is a tedious task finding a comprehensive collection of medicinal plants that are able to carry out phytoremediation alone or in cooperation with the rhizosphere. The need to know this is because of the "annoyance factor" of medical plants with phytoremediation potential. As mentioned earlier, phytoremediation can alter the effectiveness and the safety of plants and plant extracts.

Standard operating procedures on medicinal plants proposed by the World Health Organization voice concerns about potential sources of contamination in plants and plant products. Possible sources of contaminants have been identified as phytoremediation stress responses (Chan 2003) and rhizosphere symbionts (Kneifel et al. 2002). Studies on medicinal plants safety in China are raising concerns about heavy metal contaminants in medicinal plants causing human harm (Chen et al. 2021). However, the concentration of several HMs has increased several fold in some ecosystems as a result of anthropogenic activities (Kosalec et al. 2009). Besides the hazardous environmental contaminant metals that can build in during phytoremediation (Ar, Cd, Ni, Hg, Pb, Se, and radioisotiopes), medicinal plants undergoing phytoremediaiton can build up harmful levels of natually occuring metals such as Mn, Mo, Zn, Fe, Cu, and Cr (Khan et al. 2008; Diwan et al. 2008, 2010b) (Table 2).

Aside from phytoremediation contaminants, phytoremediation stress responses can change the metobolyte composistion of medical plants. The following results are possible: increases in medicinal metaoblites, decreases in medicinal metabolites, and the production of harmful non-medicinal metabolites. The metabolites affected by phytoremediaton include terpenoids, phenolics, flavonoids, alkaloids, glycosides, and anitoxidant compounds. Studies also show that significant amoutns of lectins can also build up in plants during phytoremediation responses to abiotic factors (Bezrukova et al. 2011). Sources of foreign organic molecules in medicinal plants can be a byproduct of hydrocarbon, complex organic compound, xenobiotic phytoremediation. Pollutant molecules stored during phytovolatilization (Limmer and Burken 2016), phytotransformation (Nzengung et al. 2003), and rhizoremediation (Gerhardt et al. 2009) can remain in plant organs containing the medicinal compounds.

Table 2 List of contaminants of concern that can be found in the tissues and rhizosphere of medical plants exacerbated by phytoremediation activities

WHO classification	Examples	Phytoremediation source
Arsenic and toxic metals	Arsenic, lead, cadmium, mercury, chromium	Soil, water
Radioactive contaminants	Cs-134, Cs-137	Atmosphere, soil, water
Persistent organic pollutants	Residues and degradation products of aromatics, chlorinated hydrocarbons, hexachlorocyclohexane, benzene hexachloride, bromopropylate, chloropicrin, ethylene dibromide, ethylene oxide, methyl bromide, sulfur dioxide, RDX and HMX	Soil, water
Microbial toxins	Mycotoxins, Endotoxins	Rhizosphere microorganisms
Microbiological contaminants	Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella species, Shigella species, Escherichia coli, azotobacter, Azospirillum, Arthrobacter, Bacillus, Burkholderia, Enterobacter, Klebsiella	Rhizosphere microorganisms

The "WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues" recommends testing these substances

5 Conclusion

Phytoremediation properties in medicinal plants are both a benefit and a bane. The benefits are that medical plants with phytoremediation potential have physiological properties that allow them to survive in extreme and toxic environments. The bane is that medicinal plants with phytoremediation potential might be ineffective or unsafe if grown in environments that induce phytoremediation stress responses. It is best to assume that medicinal plants belonging to certain plant families are likely to have phytoremediation potential. Hyperaccumulating plants are the most critical concern because they are more likely to store harmful minerals and metals in their tissues. The number of plants capable of organic pollutant phytoremediation has not been accurately estimated. Almost all plants show some degree of organic pollutant phytoremediation, especially those that have root endosymbionts or are in soils with rhizosphere microorganisms that have bioremediation properties. The impacts of organic compound phytoremediation stress response activities on medicinal plants varies greatly based on the type of phytoremediation carried out by the plant or the rhizosphere. Epigenetic studies depict that non-phytoremediation plants may be able to be epigenetically regulated to respond to abiotic stress as if the plant was a phytoremediator. Medicinal plants that are not known to have phytoremediation potential should be assayed in vitro and in situ to determine if they carry out phytoremediation. The literature is replete with generalizable examples of in vitro and in-situ assay protocols. Information on assaying of plants for contaminates in their tissues is well researched in the phytoremediation literature. However, the standardization of evaluating harmful contaminants and secondary metabolite dynamics in phytoremediation plants is needed for better medicinal plant quality control and safety.

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