

ENVIRONMENTAL STRESS PHYSIOLOGY OF PLANTS AND CROP PRODUCTIVITY



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Environmental Stress Physiology of Plants and Crop Productivity

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FOREWORD

I am delighted to jot down the foreword for this book that deals with plants' response to numerous abiotic stresses and various approaches and concepts to mitigate their adverse effects. While the demand for food is increasing continuously, agriculture productivity is threatened by various environmental factors, often related to temperature change and warming. The most typical abiotic stresses affecting the plants are high temperature and drought, resulting in unbearable crop losses. Global temperature change further increases the frequency of warmth stress, flood, and drought, which have a negative impact on crop yield and poses a heavy challenge to global food security. Presently, the best priority to satisfy the worldwide food demand is to sustain and improve crop yield.

This book “**Environmental Stress Physiology of Plants and Crop Productivity**” edited by **Prof. Saroj Arora and Dr. Tajinder Kaur** presents a broad picture of plant responses to major abiotic stress factors, plants adaptations during stress conditions like the role of antioxidative system and plant growth regulators, biotechnological approaches for abiotic stress tolerance in plants for sustainable agriculture, and the role of secondary plant compounds and signaling molecules to modulate the oxidative stress in plants under stressful conditions. These topics, written by experts in their respective fields, make this book highly useful to the scientific community. It covers a good range of subjects that provide the reader a comprehensive overview of stress tolerance to varied environmental factors affecting plant growth and development.

I congratulate the editors for compiling this publication that provides the reader the simplest way forward in abiotic stress management to enable more productive agriculture. I am sure that the readers within the field of abiotic stress management, agriculture, and biotechnology will find this book very useful.

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PREFACE

The human population is increasing worldwide at a much faster rate and is expected to increase from ~7 billion to ~ 10 billion by the end of the year 2050. On the other hand, agricultural productivity is not increasing at the desired rate to feed all the people due to the negative impact of various environmental stresses. Stresses in plants include heat, cold, drought, flooding, salinity, radiations, heavy metal toxicity, and nutrient loss, limiting agricultural productivity. In the present scenario of global environmental change, increasing crop productivity and minimizing the losses in crop yield is a major concern for all nations to cope with increasing food requirements.

The present book “**Environmental Stress Physiology of Plants and Crop Productivity**” integrates a broad cross section of scientific knowledge and expertise about the mechanisms underlying plant responses against different environmental stressors. It is a timely contribution to a subject that is of eminent importance. Chapter *one* gives an overview of major abiotic stress factors affecting growth and development in plants. Chapter *two* and *three* focus on two major abiotic stressors, *i.e.*, drought and salinity, that adversely affect crop productivity and quality. Chapter *four* covers factors responsible for temperature variations around the globe, plants’ response to temperature variations, and its impact on crop yield. Chapter *five* covers the effect of mineral deficiency on plant stress responses. Chapter *six* deals with the role of nitric oxide in providing tolerance to plants under salt stress. Chapter *seven* highlights the role of antioxidative defense system of plants in mitigating the harmful effects of excessive production of reactive oxygen species under various types of stress factors. Chapters *eight* and *nine* discuss the importance of melatonin and phenylpropanoid, respectively, in mitigating the adverse effects of abiotic stress in plants. Chapter *ten* comprehensively deals with the role of different plant growth regulators in abiotic stress tolerance. Chapter *eleven* present an overview of genomics, proteomics, and metabolic approaches, and *twelve* deals with the advent of new technologies like CRISPR gene technology to develop plant resistance against various environmental changes.

We express our thanks to all the contributors. We would like to thank Prof. (Dr) K. Muthuchelian for writing the foreword. Finally, it is a profound pleasure to thank Bentham Science for taking up the publication of this book. We hope that this book will provide current knowledge on abiotic stress in plants and will lead to new discussions and efforts to deal with various environmental stress factors.

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Summary

The knowledge of plant responses to various abiotic stresses is crucial to understand their underlying mechanisms as well as the methods to develop new varieties of crops, which are better adapted. *Environmental Stress Physiology of Plants and Crop Productivity* will provide a timely update on plants' response to a variety of stresses such as salinity, temperature, drought, oxidative stress, and mineral deficiencies. The book will focus on how plants adapt to abiotic stress and methods of improving plants' tolerance to abiotic stresses. The book will be beneficial to scholars and researchers working in the field of botany, agriculture botany, crop physiology, soil science, and environment sciences.

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CHAPTER 1

Abiotic Stress in Plants: An Overview

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Abstract: Environmental stress is one of the major limiting factors for agricultural productivity worldwide. Plants are closely associated with the environment where they grow and adapt to the varying conditions brought about by the huge number of environmental factors resulting in abiotic stress. Abiotic factors or stressors include high or low temperature, drought, flooding, salinity, mineral nutrient deficiency, radiation, gaseous pollutants, and heavy metals. High salinity, drought, cold, and heat are the major factors influencing crop productivity and yield. The negative impact of various abiotic stress factors is the alteration in the plant metabolism, growth, and development and, in severe cases, plant death. Abiotic stress has been becoming a specific concern in agriculture leading to unbearable economic loss to the breeders. Thus, understanding these stresses will help in achieving the long-term goal of crop improvement, therefore, minimizing the loss in crop yield to cope with increasing food requirements. With this chapter, an attempt has been made to present an overview of various environmental factors that are hostile to plant growth and development, thereby leading to great loss in crop yield.

Keywords: Abiotic stress, Crop yield, Drought, Heat, Salinity.

INTRODUCTION

Plants continuously face unfavorable environmental conditions that affect their growth and development by altering their metabolic activities, eventually leading to plant death. Abiotic stress is defined as environmental conditions that cause alterations in plant growth and development and limit yield below optimum levels [1 - 3] and can result in unacceptable economic losses. Abiotic stress factors include water stress (drought, flooding, waterlogging), extreme temperatures

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(heat, cold, and freezing), too high or too low irradiation, insufficient mineral nutrients in the soil, excessive soil salinity, gaseous pollutants, and heavy metals. However, drought and salt stress pose severe threats to agriculture resulting in loss of crop yield. These threats may become more intense by global climate change and population growth [4]. The ability of crops to achieve their yield potential (maximum possible yield) if all inputs are non-limiting can be affected by a number of stress events. In most agricultural systems, crops hardly reach their yield potential due to stress events, be they abiotic or biotic. Water is often the largest restraint to limiting crop yield. Water deficiency reduces the rate of transpiration and photosynthesis in addition to the absorption of nutrients and water by the root surface, resulting in lower crop yield as compared to yield potential [5]. The average yield of major crop plants is reduced worldwide by more than 50% due to abiotic stress [6, 7]. It is predicted to become even more severe as desertification increases. The present yearly loss of arable area may double due to global warming by the end of the century [8, 9]. Crop production may be reduced to about 70%, with the majority of the crops performing at only 30% of their genetic potential with regard to yield [10]. The problem will become more intense by a simultaneous increase in population growth, creating more pressure on existing cultivated land and other resources [11]. Given the increasing human interference by humans with nature, only 2.75% of the global land area is not affected by some environmental limitations, according to its yearly report by Food and Agriculture Organization (FAO), 2018. It has been projected that more than 90% of the land in rural areas is distressed by various environmental stress factors at some point during the growing season [1].

Abiotic stress exhibits a huge challenge in our goal for sustainable food generation. Drought and rising temperature are two of the main abiotic stressors around the world that reduce crop productivity and influence the ability to meet the food demands of the rising global population, particularly given the current and growing impacts of climate change. Several studies have reported that increased temperature and drought can decrease crop yields by 50% [12]. Salt stress is also one of the major severe limiting factors for crop growth and production, which has been elevated mostly by agricultural practices such as irrigation [13]. More than 6% of the world's land is affected by salinity. Of the present 230 million hectares of irrigated land, about 45 million hectares, *i.e.*, 19.5%, are affected by salinity. Of the 1,500 million hectares under dry land agriculture, about 32 million hectares (2.1%) are salt-affected to varying degrees [14].

Mineral stress is another constraint in plant growth. Intensive agriculture practices in developed nations have lessened the availability of nutrients in the soil resulting in its low fertility and poor availability of nutrients required for plant

growth. However, plants can initiate a number of cellular, molecular, and physiological changes in response to this stress. Plant reactions to abiotic stressors are complex and dynamic; they are both reversible and irreversible. Plants respond to stress in a number of ways depending on the tissue or organ affected by the stress, in addition to its intensity and duration. Some plants complete their life cycle during less stressful periods and hence escape the effects of stress. On the other hand, some plants have evolved stress tolerance, avoidance, or resistance mechanisms that isolate plant cells from stressful conditions [15]. In this chapter, we present an overview of various environmental factors that cause stress in plants and thereby affect their overall growth and yield.

ENVIRONMENTAL CONDITION THAT CAUSE STRESS

Plants are exposed to various types of abiotic stressors like drought, salinity, cold and heat, waterlogging, hypoxia, and anoxia, which have a negative influence on the survival, biomass production, and yields of staple food crops by up to 70%, hence, putting at risk food security worldwide [16 - 18]. Water deficiency stresses imparted by drought, salinity, and temperature severity are the most prevalent abiotic stresses that limit plant growth and productivity. The response of plants to these abiotic stressors is multigenic, with plants responding to these stressors at the molecular level by regulating the synthesis of various secondary metabolites, which act as physiological buffers to nullify the bad effects of abiotic stressors. To understand the functional dynamics of the stress signal perception and regulation of the associated molecular networks, the stress tolerance capacity and capability of plants are being unraveled through high throughput sequencing and functional genomics tools. Plant acclimatisation to abiotic stress is a complex and coordinated response involving hundreds of genes and various signal transduction pathways activated in response to the various environmental factors throughout the developmental period of the plant [19 - 21].

Hence, a thorough understanding of the molecular pathways functioning in response to the abiotic stress in plants is essential for targeting any improvement in plant biomass or yield. A better and thorough insight into the plant responsiveness to abiotic stress will help in both traditional and modern breeding applications towards improving stress tolerance in plants. Genetic regulatory mechanisms occurring at the level of transcriptional regulation, alternative splicing molecular mechanisms, and the rapid generation of signal transduction regulatory proteins *via* ubiquitination, sumoylation, phosphorylation, and chromatin remodeling tend to influence complex signal transduction networks that act in turn to regulate processes such as membrane transport, the ascent of sap to maintain cellular ion homeostasis and the synthesis of the secondary metabolites.

Drought and its Effects on Crop Physiology

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Abstract: During natural conditions, plants undergo a series of biogenic stressors that are devastating to plant growth. Among these, drought stress is the most common, which alters molecular and morphological parameters in plants and thus has detrimental effects due to environmental injury and physico-chemical disturbances. These have led to the development of technologies that increase the quality and sustainability of crops under deteriorating soil, declining natural resources, and environmental stress. Effective agronomic and genetic methods for crop protection provide best management practices to combat drought conditions. This chapter aims to contribute to the development of approaches for sustainable agricultural management practices suitable for crop production during drought stress.

Keywords: Crop production, Drought stress, Plant responses, Stress management.

INTRODUCTION

Climate change disruptions are the primary concern in many developing nations due to increased vulnerability and limited capacity to manage the adverse effects of climate change on efficient crop production [1]. For economic growth, countries are mainly focused on farming whilst the agricultural industry is entirely dependent on nature [2]. Drought stress is the most critical factor which restricts crop growth and quality [3, 4]. Demand for food, fiber, and various other commodities has strained our capacity to grow high-quality agricultural products. Drought stress further compounds this problem by adding detrimental effects on crop quality in addition to the broader impact of drought on crop production, pri-

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marily resting on soil moisture level, accessibility of nutrients [5], and crop varieties [6].

Various studies regarding drought as an extreme stress factor have been extensively reported. A previous study depicted that the biochemical and molecular approach to meet the environmental stress in plants is specific, which cannot be inferred directly [7]. Technologically, additional advanced solutions are still required to maintain current crop production and resolve the problems of food security, growing population pressure, and declining resource base in response to climate change. Being a rich source of various nutrients like minerals, vitamins and proteins, these crops can play a vital role in escaping the global nutrition production gap. Nonetheless, there must be an effective use of available germplasm and technologies to sustain production at an increased risk of drought stress. The present chapter deals with the impact of drought stress on crop growth, the assimilation of nutrients and water content. Crop enhancement and drought tolerance management solutions are also discussed.

PLANT RESPONSES TO DROUGHT STRESS

Drought stress causes negative consequences on crop development in different stages of crops, which rely heavily on the extent of stress and its duration, as well as the phase of plant growth. These effects are depicted by changes in plant morphology, physiology, biochemistry, and molecular processes.

Morphological and Yield Responses

During germination or initial seedling growth, some field plants are highly susceptible to drought. Water scarcity disrupts seedling growth by reducing the imbibition of water and seedling capacity [8]. Limited water supply leads to osmotic disruptions, excess reactive oxygen species (ROS) production, and impaired cellular metabolism, resulting in changes in the DNA and protein structure, membrane disruption, diminished production of ATPs, and respiration [9], which ultimately results in loss of seed germination. About a 25% decline in plant height during water-stressed conditions was confirmed in the study conducted on citrus seedlings by Wu *et al.* [10]. Adverse effects on *Abelmoschus esculentus* stem length have also been identified. Drought distress diminished leaf growth in a variety of species of plants [8, 11]. Multi-specific differences within two *Populus* species during drought stress have also been observed by Wullschleger *et al.* [12].

Drought stress also caused a decline in the plant height of various field crops [13]. Deficiency in soil water affects the root development but had less impact on aerial areas resulting in the incline of root to shoot ratio. Different effects of drought

stress have been reported on root growth, for instance, the length of the root of *Catharanthus roseus* and sunflower increased [14]; however, the increase in wheat and maize root growth did not change [15]. A rise in the root length was due to increased abscisic acid levels in roots under drought conditions [16]. Under dry conditions and high humidity, the developed roots are generally fibrous and strong [17]. The development of seedlings can be affected by water shortage, which can cause a reduction of about 32% in wheat production. However, in dry conditions, the root size and root/shoot ratio increased in wheat. The loss of root to coleoptile water supply during drought stress prevents the development of coleoptiles [18].

Yield and Related Attributes

Drought stress causes a reduction in development and productivity, which ultimately decreases agronomic and crop yield attributes. In vegetables, water deficiency considerably lowered seed efficiency by restricting the growth of pods, increasing abortion of pods, and reducing seed size [19]. The number of branches and the overall seed yield considerably declined in soybean under drought stress [20]. Drought, in turn, has a detrimental effect on grain yields and crop yields. The decline in different characteristics of crop plants might be the result of stress-induced changes in biochemical and physiological practices and equivalent adverse consequences for plant reproductive organs with reduced productivity. In general, drought damages the plants; however, the seed development stage is perhaps more vulnerable than pre and post-reproductive phases to such stress factors.

Physiological and Biochemical Responses

Nutrient Relations

Besides diminishing plant growth and efficiency, plant nutrient uptake activity is also hampered during drought stress [19]. It restricts nutrient absorption and translocation in plants [21]. The low availability of soil moisture under drought stress diminished root growth and nutrient inflow [22]. Drought stress also restricts the nutrient assimilation function of enzymes. For example, during drought stress, the nitrate reductase activities in the Dhainicha nodules and *Phaseolus vulgaris* L. were considerably reduced [19]. To sum up, there is a major relationship between the acquisition of nutrients, soil moisture content, and soil temperature. Stress from dryness reduces plant nutrient supply, translocation, uptake, and metabolism.

CHAPTER 3

Salinity and its Effect on Yield of Field/Horticultural Crops

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Abstract: Salinity is considered a crucial environmental factor that limits the production of the crop in many parts of the world with marginal agricultural soils. It causes a reduction in agricultural productivity globally and renders an estimated one-third of irrigated land of the world unsuitable for the production of crops. A high concentration of salt can kill all the crops and plants. Salinity can affect the yield and growth of most crops, as the higher rate of salinity can cause both hyperosmotic and hyper ionic effects in plants, leading to an increase in the production of activated oxygen species, membrane disorganization, and metabolic toxicity. Its effects on the growth and development of plants include osmotic stress, ion toxicity, mineral deficiencies, biochemical and physiological perturbations, and combinations of these stressors. Salinity reduces Ca²⁺ availability that in turn decreases the mobility and transport of Ca²⁺ to growing regions of the plant when dominated by Na⁺ salts and thereby affects the quality of both reproductive and vegetative organs. The horticultural crops are mostly glycophytes that evolved under the conditions of low salinity of the soil. Nutrient uptake is directly affected by salinity, such as Cl⁻ reducing NO³⁻ uptake or Na⁺ reducing K⁺ uptake. The performance of crops may be affected adversely by salinity-induced nutritional disorders. These disorders resulting from salinity may affect the availability of nutrients, transporter partitioning, and competitive uptake within the plant. This chapter will elucidate the deleterious effect of salinity on the growth and development of crop plants.

Keywords: Agricultural soils, Crop, Ion toxicity, Nutritional disorders, Salinity.

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INTRODUCTION

Salinity is considered a major abiotic factor that limits the productivity and growth of plants due to increased use of soil salinization and poor quality of water for irrigation in many regions of the world [1 - 3]. Salts are the natural and main components of water and soils. The ions responsible for causing salination include Na^+ , Ca^{2+} , K^+ , Cl^- , and Mg^{2+} . The absorption of important nutrients like Ca, K, N, and Mg is affected by the excessive concentrations of Na^+ and Cl^- and the soils can also become sodic. Sodic soils represent many challenges as they have a very poor quality of structure, which prevents or limits water drainage and infiltration. In the plant tissues, water stress like salt stress leads to osmotic imbalances [4]. In the case of salt stress, the excessive amount of salt, usually Na^+ and Cl^- , leads to direct toxic and nutritional effects [5]. The toxicity of a specific ion is caused by the accumulation of chloride, sodium, and boron in the cells and tissues of transpiring leaves, having a deleterious effect on the growth and yield of crops and plants. The accrual of harmful ions might inactivate enzymes, hinder protein synthesis and photosynthesis, and injure the chloroplasts and other related organelles [6].

Soil salinity is the amount of excessive salt content in the soil, and the process of enhancing the content of salt is known as salinization. The presence of excess salt not only disturbs the structure of the soil, but also has the ability to attract water and thereby block its absorption by the roots of the plant. As a result, the plants may show signs of drought even at the time of waterlogging or wetting of the soil. The salt damage may also cause water pooling on the surface of the soil without penetration. The weathering of minerals also increases the salinity concentration of soil [7]. Another yield-limiting factor is nutrient deficiency, and this issue is gradually aggravated due to intensive cultivation as well as the injudicious and imbalanced utilization of fertilizers. The deficiency of phosphorus and nitrogen is widespread, followed by the deficiency of Zn. Zinc is a crucial protein component and acts as a functional, structural, or regulatory cofactor of several enzymes and plays a key role in the metabolism of the plant [8].

Salinity adversely affects the growth, yield, and biomass of the plants as the leaves become thicker and smaller than those of normal plants. The excess amount of chloride (but not sulfate) enhances the palisade cells' elongation, resulting in increased succulence [9]. It is anticipated that in time to come, these ecological stressors will turn out to be more frequent and extreme. At the same time, in 2050, it is estimated that the population of the world will experience a grave food shortage as it is presumed to reach about 10 billion [10]. Overcoming the problem of salt stress would have a positive impact on the production of agricultural crops. Plants have developed certain biochemical and physiological

mechanisms to regulate the stability of the intracellular environment through the accumulation of numerous solutes under saline conditions [11]. Proline, as an essential osmoprotectant, might contribute to protecting enzymes from oxidative damage and also provide osmotic adjustment during the condition of salinity [12]. The adjustment of osmotic level in plants maintains the turgor pressure of cells and the uptake of water by allowing the regularity of physiological metabolism [13].

Several attempts have been made to improve the ability of crops to tolerate salt by genetic transformations and traditional breeding programs. However, due to the complexity of the trait, commercial success has been very limited [14]. Salinity is one of the severe environmental and agrarian issues in semi-arid and arid areas that detrimentally affect the growth of crops and the productivity of agriculture [15, 16], thereby affecting 2.1% of dryland agriculture and 19.5% of irrigated land at a global scale [17]. Thus, the development and discovery of schemes or programs to ameliorate the adverse effects of environmental stressors have been receiving considerable attention. This chapter will highlight the effect of salinity on the growth and productivity of crop plants.

CAUSES OF SALINITY

Salinity can be defined as the concentration of dissolved mineral salts present in the soil. The mineral salts present in saline soil are comprised of electrolytes of cations and anions. The major cations found in saline soil are sodium, potassium, magnesium, and calcium. The major anions present are chloride, sulphate, carbonate, bicarbonate, and nitrate. Mineral salts soluble in water accumulate in the upper layer formed by the A and B zones of the soil profile and the lower layer of the soil comprising unconsolidated rock material affecting the crop production and environmental well-being. The natural causes of soil salinity are rain, wind, and weathering of rocks. Rain and weathering are major sources of salt. Mineral salts are major components of rocks and rainwater containing a small amount of salt which accumulates in the soil over a long period of time. Winds act as a major distributor of salts from weathered rocks and oceans to land, contributing to soil salinity. Natural catastrophes like tsunami play a vital role in introducing seawater to nearby lands. Tsunami waves contain huge amounts of ocean salts, and when they hit the ground, a generous amount gets deposited in the soil. Besides natural causes, anthropogenic activities are also responsible for soil salinity. Humans introduce salts in soil by following improper agricultural practices. Water used for irrigation and its poor drainage plays an important role in increasing soil salinity. Even if the water used for irrigation is of good quality, it contains a small amount of salt, which accumulates with time if water drainage systems are not efficient [18].

Temperature Rising Patterns and Insights into the Impacts of Consequent Heat Stress on Edible Plants

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Abstract: Rapid urbanization and land-use transition contribute to the rise in the thermal scale of cities as well as small towns and villages worldwide. The equilibrium between the incoming solar energy and the outgoing terrestrial energy regulates the temperature. Nevertheless, the temperature, as we know, varies from place to place, and it also affects the natural processes as well as surrounding flora and fauna. On the other hand, temperature beyond the physiologically optimal limit is called high temperature that adversely affects the growth and development of plants as it has significant impacts on both the vegetative and reproductive phases of the plant life cycle. The extremely high temperature is referred to as heat stress which is reported as one of the devastating abiotic stressors. In plants, heat stress triggers various morpho-physiological changes in plants that affect their growth and economic outcomes *via* accelerating reactive oxygen species generation, reduced carbon assimilation, degradation and denaturation of proteins, lipid peroxidation of membranes, *etc.* Several conventional and modern strategies have been employed to resolve heat stress-induced damages in plants. Therefore, the present work is an outcome of extensive literature focused on the factors responsible for temperature variations' patterns, morpho-physiological responses of crops, and impacts on the economic yields of edible plants.

Keywords: Heat stress, Germination, Growth and development, Physiological responses, Sustainable crop production.

INTRODUCTION

Since 1750 BC, the concentration of various greenhouse gases like carbon dioxide, methane, and nitrous oxide have increased significantly due to various anthropogenic activities [1]. The concentrations of carbon dioxide, methane, and nitrous oxide were 280 ppm, 715 ppb, and 270 ppm, respectively in 1750,

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which were recorded to increase up to 407.4 ppm, 1874.7 ppb, and 329.9 ppm, respectively. An increase in greenhouse gases has resulted in an increase of 0.75 °C in global temperature from 1906 to 2005 [1]. The highest rate of warming has been recorded in the last decade. The Intergovernmental Panel on Climate Change (IPCC) has predicted that the temperature will increase from 1.8-4.0 °C by the end of this century [1]. For the Indian region, an increase of 1.56-5.44 °C is predicted by 2080 [2].

Agriculture is the backbone of the economy of many developing countries including India, but the rising temperature will affect the economic yield of edible plants *via* direct or indirect effects. Higher temperatures may affect the yield both quantitatively and qualitatively, alter growth rates, evapotranspiration and photosynthesis rate, moisture availability through changes in irrigation pattern, *etc* [3 - 14]. This can lead farmers to invest more in loss dipping inputs like pesticides rather than yield-enhancing inputs like fertilizers. The negative effect of high temperature on agriculture includes a decline in food production that can threaten food security, thus entail specific agricultural measures to combat this [14]. Developing nations will be severely hit by temperature variations as 50% of the population rely on agriculture, and 75% of the population lives in rural areas [15]. In emerging economies like India and China, farmers and plant breeders will face extreme challenges as there will be an upsurge in food requirements with increasing population and a decline in calorie accessibility, which will increase malnutrition in children by 20% [16, 17].

Earlier, it was believed that the rising temperature would bring a net benefit to agriculture. However, recent studies have shown a portentous effect of climate change on agriculture as crops can tolerate temperature variations only at threshold level. After that, there can be a sharp decline in productivity [18 - 21]. Studies have shown a non-linear pattern between temperature and crop productivity [22 - 24]. Within a range of 5.5-32°C, the temperature has a positive effect on crop production, whereas temperature higher or lower than that range will negatively affect crop production [20, 25]. Recent studies have shown that with an increase of 1°C temperature, India will lose 4-5 million tonnes in wheat production and cause a decline in production of soybean, potato, groundnut, and mustard by 3-7% [7, 14]. Evocative evidence shows that an increase in temperature increases the risk of pests' attack, more crop diseases, and weed [26].

Moreover, an increase in temperature may alter the precipitation pattern [27]. Higher precipitation rates can cause disease invasion in crops, whereas a low precipitation rate can have a detrimental effect on crops, especially during the growing stages [28]. Lower precipitation rates may cause mild to severe droughts in arid and semi-arid areas, which can reduce the quality and quantity of livestock

and crop yield [14]. In India, there are two main cropping seasons, *i.e.*, Rabi and Kharif. Rabi season is mostly influenced by the south-west monsoon, whereas the Kharif season is influenced by the north-east monsoon. Reports have shown that the overall temperature rise is expected to be more during the Rabi season (winter) rather than the Kharif season (monsoon) [8]. Khan *et al.* (2009) reported that the average temperature in India would increase by 1.1-4.5°C in the Rabi season and 0.4-2.0 °C in the Kharif season [29]. The decline in crop yield can increase food prices in the state as well as the national level [30]. Thus, the temperature can play an important factor in affecting the agriculture and economy of India.

Kumar and Parikh (2001b) used the Ricardian approach and revealed that an increase of 2°C in temperature would result in 8.4% damage in farm-level net revenue [31]. Due to an increase in temperature, heat-induced biotic stress increases the attack of pests as well as diseases that prompt farmers to use pesticides in early growing seasons [32]. Jagnani *et al.* (2018) also postulated that a 10% increase in growing days also increases 10% usage of pesticides while reducing the usage of fertilizer by about 5% [32]. Rising temperature will further affect soil erosion, surface runoff, soil water content, biodiversity, organic nitrogen, and carbon content as well as cause an increase in salinization [33]. Therefore, adequate strategies are required to reduce the effect of temperature on soil fertility and improve crop growth and production. Elevated temperature induces physiological and morphological stress as well as molecular and biochemical alterations that affect the growth and yield of plants [15]. Heat stress is more protuberant during reproductive development as compared to the vegetative growth period in most crop species [34]. Elevated temperature induces various physiological changes in plants such as scorching of leaves and stem, root and shoot growth inhibition, leaf senescence, and abscission, or fruit damage that lead to dropping in food productivity [35]. In many cases, high temperature affects plant growth by disturbing the shoot net assimilation rate [36]. High temperature may also cause ion channel, water, and organic solute movement across the plant membrane, which can disrupt processes like photosynthesis and respiration. Moreover, a higher temperature may also cause electrolyte leakage from leaves [37].

TEMPERATURE RISING TRENDS IN INDIA AND WORLDWIDE

Monitoring and analysis of temperature on a regional as well as global scale have gained a lot of importance due to clear signs of global warming. According to the report of IPCC, the mean surface air temperature of the earth has been increased by 0.6°C [38]. Circadian irregularity in temperature trends has been reported from India, and it was found that warming over India has been exclusively contributed

CHAPTER 5

Physiological Role of Mineral Nutrients and their Uptake during Abiotic Stress

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Abstract: Soil acts as a source of nutrient elements, and the availability of nutrients is determined by soil properties. The different elements are grouped according to their biochemical behavior and physiological functions. The nutrients that are needed in moderately large amounts are called macronutrients. The plant macronutrients comprise nitrogen, potassium, phosphorus, calcium, sulfur, and magnesium. The micronutrients are required for plant growth in much smaller quantities than macronutrients. These micronutrients contain boron, copper, iron, manganese, molybdenum, and zinc. All of these nutrients are absorbed through the roots. Water will transfer the nutrients from soil to the roots of the plant. In this chapter, we will discuss the physiological role of essential and non-essential elements and the effects of some general environmental stressors, such as salinity, drought, and metals, on nutrient uptake by plants.

Keywords: Deficiency symptoms, Drought, Essential elements, Heavy Metals, Macronutrients, Micronutrients, Salinity.

INTRODUCTION

Mineral nutrients are classified as macronutrients and micronutrients. Plants require these nutrients to complete their life cycle. Macronutrients are required in large quantities by plants such as carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), and potassium (K). Of these, C, H, and O are non-mineral nutrients as they are found in air and water. Micronutrients are required in smaller

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amounts than macronutrients. The micronutrients include boron (B), copper (Cu), chloride (Cl), iron (Fe), manganese (Mn), molybdenum (Mo), and zinc (Zn). Macronutrients are primarily chief structural components of plants, and micronutrients are essential for plant growth. Fig. (1) shows the classification of essential elements and their general function in plant growth. Mineral nutrients may also increase or decrease the tolerance of plants to various biotic stress factors, such as pathogens and pests, and abiotic environmental stress factors, such as drought, salinity, flooding, and chilling [1]. The physiological role of macro and micronutrients are listed in Table 1.

The deficiency of mineral nutrients produces drastic effects on plant growth and development. The minimum supply or complete absence of any of the essential elements can exhibit typical symptoms that are specific to the particular elements in the plant functioning. This condition is known as nutrient deficiency, and its symptoms are called deficiency symptoms [2]. Although micronutrients are required in small traces, their deficiency in plants causes restricted growth, chlorosis, interveinal necrosis, and defoliation of leaves [3]. Table 2 highlights physiological and nutritional deficiency symptoms in plants. On the other hand, acute toxicity of micronutrients inhibits seed germination, photosynthesis, biosynthesis of chlorophyll, and plant growth, and under severe abiotic stress, the condition causes the death of the plant [4 - 8].

The non-essential elements, *e.g.*, cadmium (Cd) and chromium (Cr), are harmful to plant health [9]. Plants are also sensitive to the deficiency and excess concentrations of some metals and essential micronutrients like all living organisms. The metals such as Cd, Hg, As can be poisonous to plants at higher concentrations [10]. Various studies have been conducted to verify the effects of toxic metals on plants. Table 3 highlights the various studies conducted on the toxicity of essential and non-essential elements on plants. Cadmium and lead are reported to have an inhibitory effect on transpiration, photosynthesis, carbohydrate metabolism, and other metabolic activities [11]. Cadmium inhibits the chlorophyll biosynthesis, reduces total chlorophyll content and chlorophyll a/b ratio in *Brassica juncea*, while Pb toxicity causes toxicity of nucleoli, disturbance of mitosis, inhibition of root elongation, the appearance of chlorosis, inhibition of enzymatic activities, and decrease in photosynthesis [12]. Heavy metal contamination of agricultural soil has become a serious environmental concern due to its possible adverse ecological effects. The toxic metals are considered soil pollutants due to their extensive occurrence and acute and chronic toxic effect on plants grown in such soils [10].

Table 1. Physiological role of essential mineral nutrients in plants.

Mineral Nutrients			Primary Form of Uptake	Function	Deficiency Symptoms
Category	Nutrient	Symbol			
Macronutrients	Nitrogen	N	Nitrate, NO_3^- , ammonium, NH_4^+	Constituent of amino acids, proteins, nucleic acids, and some co-enzymes. Acts as donor atom in many enzymatically catalyzed reactions	Stunted growth, leaves turn yellow (chlorosis)
	Phosphorus	P	Phosphate, HPO_4^{2-} , H_2PO_4	Involved in photosynthesis, essential constituent of high energy intermediates like ATP, nucleic acids, and phospholipids in membranes	Purple or red anthocyanin pigmentation on leaves, premature leaf fall development of dead necrotic areas on the leaves, petioles, and fruits
	Potassium	K	Potassium ion, K^+	Regulation of stomatal opening and closing, essential for photosynthesis, maintains cytoplasmic pH	Development of dead necrotic areas at the tip and margin of leaves (scorching), greenback symptom in tomato is due to potassium deficiency
	Calcium	Ca	Calcium ion, Ca^{2+}	Important component of cell walls, control cell permeability, involved in cell signaling	Meristematic tissue of stem, leaves, and roots die, roots become short, brown, and stubby, younger leaves become distorted
	Magnesium	Mg	Magnesium ion, Mg^{2+}	Essential for chlorophyll (Magnesium porphyrin)	Interveinal chlorosis of the leaves, the appearance of anthocyanin pigment in leaves
	Sulphur	S	Sulfate, SO_4^{2-}	Essential for sulphur containing amino acids, e.g., cysteine, cystine, and methionine	Chlorosis of young leaves

CHAPTER 6

Nitric Oxide Mediated Modulation of Antioxidative Responses under Salinity Stress in Different Plant Species: A Review**Jaskaran Kaur¹, Varinder Kaur² and Jatinder Kaur Katnoria^{1,*}**¹ Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, Punjab 143005, India² Centre for Advanced Studies-Department of Chemistry, Guru Nanak Dev University, Amritsar, Punjab 143005, India

Abstract: Nitric oxide (NO) is a small-sized, short-lived, highly diffusible, gaseous, and bioactive molecule that regulates various physiological and pathological processes in plants. It also plays a role in development, germination, flowering, senescence as well as response to abiotic stress in plants. In recent years, the role of NO in tolerance of abiotic stress posed by salinity, drought, heat, cold, UV-B, and heavy metals in plants has been identified and gained importance in the field of plant research. Salinity stress triggers the production of reactive oxygen species (ROS) that leads to oxidative stress in plants, resulting in cellular destruction *via* lipid peroxidation, enzyme inactivation, and DNA damage. To combat this stress and to minimize its harmful effects, certain plants activate various ROS-scavenging enzyme activities. The role of exogenous NO, which acts as an indicator in triggering ROS scavenging enzyme activities and regulates antioxidant activities in plants to alleviate the toxic effects of salt stress, has been well established. NO has been considered to play a key role in reducing the excessive production of ROS in cells, improving secondary defense mechanisms, alleviating osmotic damage, and maintaining cell permeability. Thus, understanding the mechanisms of action of NO that help in effectively utilizing the crop cultivation under saline conditions and support better growth of the plants is the need of an hour. Considering this, the present review focuses upon the recent knowledge of the crucial role of NO in providing tolerance to plants under salt stress.

Keywords: Antioxidant enzymes, Abiotic stress, Hydrogen peroxide, Nitric oxide, Oxidative stress, Reactive oxygen species, Salinity, Sodium nitroprusside.

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INTRODUCTION

Salinity is the accretion of excessive salt contents in the soil and is one of the major stressors, especially in arid and semi-arid regions, that progresses the inhibition of crop growth, ultimately leading to crop death [1, 2]. Stress induced by salt is considered an alarming condition for the agricultural productivity of soil because it can severely limit food crop production and result in decreased crop yields [3]. In nations where irrigation is a necessary utility for crop production, salinity is considered to be an ever-present threat [4]. Soil salinity has been found to be raised all over the world in past decades due to various anthropogenic reasons, including agricultural practices as well as changes in climatic conditions [5]. High salinity in agricultural soil in some regions has been documented to cause losses to 20% of the crop production annually [6]. Although abiotic stress like the one induced by salinity causes harmful effects in the plant system, yet certain plants have the capability to survive under such stress by producing their defense mechanisms. As roots are the prime organs to sense the high salinity in regions under crop cultivation, modulation of the root system architecture in response to salt stress has been reported by Wang *et al.* [7]. Similarly, in order to relieve the oxidative damage, certain mangrove plants have been reported to develop an effective antioxidant system with low-molecular weight antioxidants and antioxidant enzymes [8, 9]. The antioxidative enzyme activity has been correlated to redox state under salt stress by certain authors [10, 11]. Although plants have the potential to combat oxidative stress to a certain extent, the phenomenon of free radical scavenging can further be improved by the application of exogenous ROS scavengers like nitric oxide (NO) donors [12, 13]. Various antioxidative compounds like putrecine [11], sodium nitroprusside (SNP) [14, 15], 2-phenyl-4,4,5,5-tetramethylimidazolinone-1-oxyl 3-oxide [16], diethylenetriamine (DETA) [17] and sodium ferrocyanide ($\text{Na}_4\text{Fe}(\text{CN})_6$) [18] have been used for production of nitric oxide. The proceeding part of the review focuses on the effects of salinity stress, the role of the antioxidative defense system in combating salinity stress, and the use of exogenous nitric oxide donors in combating the oxidative stress induced by salinity in different plants species.

EFFECTS OF SALINITY

Salinity, like other abiotic stressors, is shown to disturb intracellular ion homeostasis of plants [19] and results in membrane dysfunction [20]. Egbichi *et al.* [17] documented decreased dry weight of root and shoot as well as nodule weight and the number of nodules in *Glycine max* L. under salinity-induced stress. Salinity induced premature leaf senescence and loss of photosynthetic efficiency, leading to reduced carbon assimilation and ultimately reduced crop yield, which has also been reported [21]. Effects of treatment of sodium chloride (NaCl) on

plant height, stem thickness, fresh weight, dry weight, and accumulation of polyamines in leaves of *Cucumis sativus* L. (Cucumber) was reported [22]. Hyper accumulation of Na⁺ (sodium ion) in the cytoplasmic matrix has shown to pose direct harmful effects on plasma membrane (PM), causing electrolytes leakage and effects on various metabolic processes in the cytosol [23].

High salinity in the soil was shown to elicit two primary effects, such as osmotic stress and ionic stress in plants, which further reduced the ability of plants to take up water and minerals like potassium and calcium [24, 25]. Salinity has been reported to cause attenuation of metabolic activity [8, 26, 27], alteration in the functioning of tonoplast vesicles [4, 28], reduction in plant water potential [20], and alteration in nutrient uptake [29] in many plant species. Apart from all these, it has been shown to cause oxidative stress *via* enhanced reactive oxygen species (ROS) like superoxide radicals, hydrogen peroxide, and hydroxyl radicals [11, 30]. As the physiological changes have been associated with various harmful effects in plants, the antioxidant defense mechanism plays an important role in suppressing abiotic stress and increasing plant growth. The role of antioxidative enzymes in the alleviation of salinity stress has been discussed in the following section.

ROLE OF ANTIOXIDANT ENZYMES IN ALLEVIATION OF SALT STRESS

The reactive oxygen species can cause cellular injury through oxidation of lipids, proteins as well as nucleic acids, ultimately leading to cell death [23, 31]. However, to combat this oxidative stress, many plant systems have evolved antioxidative enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), peroxidase (POX), glutathione reductase (GR), and dehydro ascorbate reductase (DHAR) [11]. Meneguzzo *et al.* [10] co-related the sodium chloride (NaCl) salt stress-induced alterations in antioxidative enzyme activities with tolerance to salinity in wheat crops. Alleviation of salt stress by decreasing ion leakage and malondialdehyde (MDA), carbonyl and hydrogen peroxide (H₂O₂) content in *Hordeum vulgare* L. (Barley) was observed by Li *et al.* [32].

High contents of certain antioxidants such as ascorbic acid, carotenoids, and polyphenols have been reported to contribute to resisting salt-induced oxidative damage by Rajaravindran and Natarajan [33] and Zhu *et al.* [34]. Superoxide dismutase (SOD), guaiacol peroxidase (GPX), and catalase (CAT) could also effectively remove free radicals in a study conducted by Chen *et al.* [35]. As the equilibrium between free radical generation and its sequestration is the determining factor for the survival of plant systems under the stress environment,

CHAPTER 7

Reactive Oxygen Species Metabolism and Antioxidant Defense in Plants under Stress

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Abstract: Plants are exposed to different types of environmental stressors throughout the different developmental stages. Reactive oxygen species (ROS) are found to play key roles in the maintenance of normal plant growth and improving their ability of stress tolerance. ROS as a secondary messenger performs crucial cellular functions, including the proliferation of cells, apoptosis, and necrosis. Both the external environmental factors and intrinsic genetic programs regulate the morphogenesis of plants. ROS are also considered as by-products of the aerobic metabolism of the plant and are formed in certain cellular compartments like mitochondria, chloroplasts, and peroxisomes. Plants form a huge number of ROS species under unfavorable circumstances that are involved in the regulation of different processes, including programmed cell death, pathogen defense, and stomatal behavior. These reactions often exert irreversible or profound effects on the development of organs and tissues, leading to abnormal death or plant growth. Several molecular approaches to understand the signaling and metabolism of ROS have opened novel avenues in comprehending its key role in abiotic stress. Plants possess their own enzymatic and non-enzymatic antioxidant defense system to encounter ROS. The interconnecting activities of these defensive antioxidants reduce oxidative load and regulate the detoxification of ROS in plants. This book chapter will highlight the importance of ROS metabolism and the role of the antioxidant defense mechanism of plants in combating the deleterious effect of oxidative stress under stressful conditions.

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Keywords: Antioxidant defense system, Detoxification, Pathogen, Plant reactive oxygen species.

INTRODUCTION

In aerobic life, the production of reactive oxygen species (ROS) is regarded as an unavoidable chemical entity [1, 2]. The aerobic metabolic processes, including photosynthesis and respiration, unavoidably result in the formation of ROS in peroxisomes, chloroplasts, and mitochondria. The main characteristic feature of the various types of ROS is their capability to induce oxidative damage to DNA, proteins, and lipids [3]. ROS are the major aerobic metabolism byproducts, and their generation involves strong electron flow, which is confined to cellular compartments. About 2.7 billion years ago, in the earth's early reducing atmosphere, the molecular oxygen was first introduced by O_2^- evolving photosynthetic organisms that led to the advent of the unwanted byproducts as ROS [2]. Under favorable conditions, at basal levels, the ROS is being constantly generated. In plants, ROS exhibit a dual role, both acting as key regulators of development, growth, and defense pathways and as aerobic metabolism by-products which are toxic in nature. The disturbance between production and elimination of ROS leads to severe consequences within the cells [4]. A cell undergoes oxidative stress when the ROS levels surpass the range of the scavenging systems, resulting in oxidative change that causes cell damage and ultimately death. When the ROS production rate is small, the cells are in a reduced state, and ROS acts as second messengers in cell division, maintenance, organogenesis, differentiation, and abiotic and biotic maintenance [5]. Therefore, ROS levels must be kept within the correct range for plant safety. However, the different antioxidant mechanisms have the capability to scavenge the free radicals that protect from their damaging effects. Plants also produce ROS by the activation of several peroxidases and oxidases in response to various environmental changes [6, 7]. ROS includes superoxide radical ($O_2^{\cdot-}$), singlet oxygen (1O_2), hydroxyl radical (OH^{\cdot}) and hydrogen peroxide (H_2O_2), *etc.* They exert numerous changes in the cellular structure of plants along with physiological responses and degrade proteins, enzymes, and nucleic acid [1]. The metabolism of redox and its related signaling act as crucial machinery at the time of abiotic stress [8].

In the evolutionary process, plants developed a high degree of control over the production of ROS and used them successfully as a signaling molecule [9]. The ROS family play a role of a double-edged sword by acting as secondary messengers in some physiological phenomena and under certain environmental stress conditions such as salinity, drought, UV irradiation, cold, heavy metals, *etc.*, they are able to induce oxidative damages when there is a disturbance in the

delicate balance between the elimination and production of ROS, crucial for normal cellular homeostasis [10]. Plants have efficiently developed antioxidant machinery to ensure survival by involving two arms (i) non-enzymatic antioxidants like carotenoids, ascorbic acid (AA), reduced glutathione (GSH), α -tocopherol, flavonoids, and the osmolyte proline (ii) enzymatic components like monodehydroascorbate reductase (MDHAR), catalase (CAT), glutathione reductase (GR), guaiacol peroxidase (GPX), superoxide dismutase (SOD), ascorbate peroxidase (APX) and dehydroascorbate reductase (DHAR) [11].

To enhance the plants' tolerance against the harsh environmental condition, it is important to reinforce the comprehensive antioxidant systems and oxidative stress. The cells of the plant will remain in a state of "oxidative stress" if the level of ROS is raised than the inside antioxidant defense mechanisms. This shows retardation of growth under oxidative stress, such as root gravitropism, leaf, and flower abscission, seed germination, lignin biosynthesis in the cell wall, polar cell growth, and cell senescence [1, 8, 10]. In this chapter, the vital role of plants in controlling and regulating the excessive generation of ROS is elaborated.

REACTIVE OXYGEN SPECIES METABOLISM

The different types of ROS are produced within the plant cells in response to various abiotic, biotic, and other environmental cues. The levels of ROS are usually determined by a balance between the breakdown and production which is tightly controlled and achieved *via* highly complex and sophisticated antioxidant systems. It was found that both biotic and abiotic stresses cause the over-accumulation of ROS. Between the two types of stress, it is observed that the source of enhanced formation of ROS seems to be different. The accumulation of ROS is normally triggered primarily by the electron transport pathway damage in the mitochondria and the chloroplasts in case of abiotic stresses [12]. In the case of biotic stress, it was found that the infections of pathogens induce specific ROS-producing enzymes, including the cell wall peroxidases or plasma membrane NADPH oxidase complex [13]. In plants, ROS are present in molecular and/or ionic states. Ionic states comprise superoxide anions ($O^{\cdot-2}$) and hydroxyl radicals ($\cdot OH$), while molecular states chiefly include singlet oxygen (1O_2) and hydrogen peroxide (H_2O_2) [3, 9, 14]. ROS generate various oxidative stressors that affect diverse biochemical and physiological reactions controlled by different genes in plants. Singlet oxygen (1O_2) is generally produced by photosystem II (PSII) in chloroplast with high oxidizability potential. Though 1O_2 persists for a short time and is unstable in cells, it has an effect on photosynthesis. Superoxide anion ($O^{\cdot-2}$) is one of the antecedents of various ROS generation as randomness with high reducibility. $O^{\cdot-2}$ might uphold the constancy of plant stem cells [15]. Excessive $O^{\cdot-2}$ is also produced due to increased ROS levels that ultimately lead to cell death

Role of Melatonin - A Signaling Molecule in Modulation of Antioxidant Defense System in Plants: Amelioration of Drought and Salinity Stress

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Abstract: Melatonin (N-acetyl-5-methoxytryptamine) is a small (232 daltons), non-toxic, indole molecule first isolated from the pineal gland of cows and later on found in different tissues of plants and bacteria. Melatonin acts as a signaling molecule/messenger, which plays an important role in coping with various biotic and abiotic stress conditions. Biosynthesis of melatonin involves two key cellular organelles *viz.* mitochondria and chloroplast. The endogenously produced melatonin serves as an antioxidant signaling molecule during the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). The exogenous melatonin also functions in the same way during critical conditions by repairing mitochondria and deal with various stresses. The plants regulate the production of melatonin depending upon the conditions and regulate salt, drought, cold, heat, oxidative, and heavy metals-related stresses. Besides that, melatonin acts as a plant hormone and regulates numerous functions in plants, including growth, development, photoperiod, clearing oxygen species, rhizogenesis, photosynthesis, and enhances antioxidant activity. It acts as a multi regulatory molecule by regulating gene expressions as well as by cross-talks with phytohormones (auxin, cytokinin, salicylic acid, and abscisic acid) involved in plant growth and development. Therefore, understanding the mechanism of action of melatonin as a signaling molecule may serve as a novel strategy to combat various stresses in plants and animals. An attempt has been made in this chapter to discuss the important role of melatonin in modulating oxidative stress in plants during stressful conditions.

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INTRODUCTION

Melatonin is a small, indole biological signaling molecule present in bacteria, unicellular eukaryotes, plants, and animals. First, it was isolated from the pineal glands of cows in 1958, where it plays an important role in reversing the darkening effect of melatonin by stimulating hormone (MSH) [1, 2]. In animals, it modulates numerous functions by regulating the activity of antioxidant enzymes, circadian rhythms, reproduction, coronary heart disease, immune system, mental status, and response to anticancer agents [3]. It also enhances the nighttime rise of serum levels [4]. After that, in 1995, melatonin was discovered in plants where it acts as a secondary messenger and regulates various functions by involving two key cellular organelles, mitochondria and chloroplast. It is distributed in different parts of the plants depending upon the type of plant species *i.e.*, *Lycopersicon esculentum* Mill. (Fruits), *Beta vulgaris* L. (leaf), *Fragaria magna* (root), *Brassica rapa* (corm), *Oryzasativum* (seeds) [4]. It is a multi regulatory molecule that modulates senescence, antioxidant activity, growth and development, circadian rhythms, root growth, and other biotic and abiotic stresses (salt, drought, cold, heat, oxidative, and heavy metals) [5, 6] in plants. Plants face more environmental stress due to their sessile nature. The concentration of melatonin in plants varies from species to species and also depends upon the growth rate, location, organ, and harvest time [7]. Its content in plant specimen can be analyzed by various methods such as RIA (radioimmunoassay), ELISA (enzyme-linked immunosorbent assay), GC-MS (gas chromatography-mass spectrometry), and HPLC (high-performance liquid chromatography) with electrochemical detection (HPLC-ECD), or HPLC-MS (high-performance liquid chromatography-mass spectrometry) [8]. It also acts as an antioxidant molecule by scavenging ROS (reactive oxygen species) and RNS (reactive nitrogen species) without depending on any kind of receptors [9]. In animals, MT1 and MT2 are the two major receptors present in the mitochondria, which communicate signaling pathways, whereas, in plants, only one melatonin receptor (CAND2/PMTR1) is identified to date. It modulates stomatal closure *via* H₂O₂ and Ca²⁺ signaling transduction pathway. Besides this, phytomelatonin improves stress tolerance by elevating the expression of antioxidant enzymes, regulating transcription factors involved in various stresses, and modulates photosynthetic rate by interacting with unknown receptors and activating the H₂O₂/NO signaling cascade [10]. The synthesis of

phytomelatonin is regulated in plants in a fashion to cope with any situation or stress.

MELATONIN: SYNTHESIS IN PLANTS

Melatonin is present in almost every living organism like bacteria, yeast, algae, plants, and animals. The production and secretion of melatonin occur in the pineal gland of animals, but there is no such organ in the case of plants. The synthesis pathways in plants are slightly different from animals; they are regulated by various environmental factors (light intensity, heat, cold, drought, and UV light) and developmental factors (fruit maturation, senescence, and leaf development) [4]. In plants, the chloroplast is the primary site of melatonin synthesis. In most plant species, tryptophan is the initiator of melatonin synthesis. The major enzymes are TDC, T5H, SNAT, CAMT, ASMT, and HIOMT, are involved in the biogenesis of melatonin. The first step enzyme involved in melatonin synthesis is TDC (tryptophan decarboxylase) that converts tryptophan into tryptamine. Then, tryptamine converts into serotonin involving T5H (tryptophan 5-hydroxylase). Serotonin is the intermediate molecule of melatonin synthesis [11, 12]. The final synthesis of melatonin from the serotonin involves a two-step mechanism, participation of enzyme SNAT, which converts serotonin into N-acetyl serotonin, and another enzyme ASMT (N-acetyl-serotonin methyltransferase), which converts N-acetyl serotonin into melatonin. Serotonin converts into melatonin by two pathways. In some plants, such as *Hypericum perforatum*, tryptophan 5-hydroxylase (TPH) catalyzes tryptophan into 5-hydroxytryptophan, and then modifies into serotonin by TDC/AADC (aromatic-L-amino-acid decarboxylase). Then the next two steps of conversion of serotonin into melatonin by involving SNAT/AANAT (arylalkylamine N-acetyltransferase) and ASMT/HIOMT (hydroxy indole-O-methyltransferase) is the same in both plants and animals. In some cases, the other pathway involves the conversion of serotonin into 5-methoxy-tryptamine by HIOMT, and finally, SNAT catalyzes 5-methoxy-tryptamine into melatonin [6, 13 - 18]. The enzymes involved in the synthesis are located in chloroplast and cytoplasm. SNAT, whose main function is to modulate the production of melatonin, is located in chloroplast and enzyme ASMT and T5H in the cytoplasm and endoplasmic reticulum, respectively [19]. In these pathways, the production of serotonin and melatonin proves to be useful for plants to cope with various biotic and abiotic stresses. The production of melatonin is enhanced or decreased according to the stress condition. Tryptophan begins to form various secondary metabolites during abiotic stresses in some plants. These secondary metabolites such as phytoalexins, indole glucosinolates, alkaloids, and serotonin provide a defense to plants by their spatial and temporal distribution (Fig. 1) [20].

Phenylpropanoid Biosynthesis and its Protective Effects against Plants Stress

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Abstract: Phenylpropanoids are a class of secondary metabolites in plants that are derived from aromatic amino acids like tyrosine and phenylalanine. It mainly includes stilbenes, monolignols, coumarins, flavonoids, and phenolic acids. These are considered to play a crucial role in protecting the plants against both abiotic and biotic stress by quenching the generation of reactive oxygen species (ROS) through a wide range of mechanisms. Phenylpropanoids are found widely in the plant kingdom and serve an essential role in the development of plant by acting as an important cell wall component, floral pigments to mediate the interactions of plant–pollinator, antibiotics (phytoalexins) against pathogens and herbivores, and protectants against UV radiation and high light. Several phenylpropanoids are helpful for the plant to fight against microbial diseases and thereby show broad-spectrum antimicrobial activity. The biosynthetic pathway of phenylpropanoid is mostly activated under abiotic stress conditions including salinity, heavy metal, ultraviolet radiations, high/low temperature, and drought and results in the accumulation of different phenolic compounds that are helpful in scavenging the deleterious effect of ROS. A series of enzymes involved in the activation of the biosynthetic pathway are reductases, transferases, lyases, oxygenases, and ligases. Among these, many are encoded by superfamilies of genes, like NADPH-dependent reductase gene family, the 2-oxoglutarate dependent dioxygenase (2-ODD) gene family, the cytochrome P450 membrane-bound monooxygenase (P450) gene family, and the type III polyketide synthase (PKS III) gene family. Thus, keeping in view the importance of phenylpropanoids in plant defense, the present book chapter is focused on unraveling the role of these essential compounds in ameliorating the stressful conditions in plants.

Keywords: Antimicrobial, Biosynthetic pathway, Phenylpropanoids, Plant, ROS.

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INTRODUCTION

In continuously evolving environmental conditions, plants are subjected to numerous abiotic stresses that are unfavorable for growth and development. Such abiotic stresses include water stress (drought and flooding), heavy metals, salinity, nutritional surplus or deficiency, high and low temperature (chilling and freezing), intense light periods (high and low), radiations (UV-A and UV-B ultraviolet), ozone layer depletion, sulfur dioxide and other mild plant stressors [4]. The adaptive response of plants to the abiotic stresses is the synthesis and accumulation of the phenolic compounds in plant tissues [1]. Plant synthesizes an enormous number of primary as well as secondary metabolites that is an essential adaptation strategy adopted by plants to their immediate environment. Primary metabolites are distributed everywhere in plants and are required by the plants for growth and development. These include sugars, nucleic acids, fatty acids, and amino acids. Secondary metabolites are not essential for the plants as such but are synthesized in the changing environment scenario. Moreover, secondary metabolites are structurally and chemically more diverse than primary metabolites [2]. Other than polyphenols, the most widely occurring group of secondary metabolites with substantial physiological and morphological importance in plants are the phenylpropanoids. The phenylpropanoid pathway is an essential and ubiquitous pathway in plants leading to the bio-synthesis of many important signaling and protection molecules.

In agricultural crops, the losses due to the abiotic stresses are manifold, and hence an adequate understanding of the plant adaptation to the environment is necessary to minimize the yield losses in crop plants [3]. Hence the understanding of the bio-synthesis of phenylpropanoids is of utmost importance for the selection of useful cultivars with the ability to survive under harmful conditions and for improving the nutraceutical value of food. Since the secondary metabolites of plants like phenylpropanoids also have antioxidant properties, hence their accumulation in the fruits is a health benefit for humans. To neutralize the environmental stresses and environmental irritants, phenylpropanoid pathways are one of the main defense mechanisms employed by the plants. Phenylpropanoid compounds are basically phenols with one or more hydroxyl groups. Cinnamic acid is the precursor molecule for the phenylpropanoids having a simple carbon skeleton with C₃-C₆ carbon chain and complex carbon skeleton with C₇-C₂₂ carbon chain [5]. The precursor molecule of the phenylpropanoid pathway, cinnamic acid, is synthesized from phenylalanine by undergoing acylation, cyclization, condensation, glycosylation, methylation, hydroxylation, prenylation, and dehydration reactions. The various enzymes involved in the synthesis of phenylpropanoids are phenylalanine ammonia lyase, chalcone synthase, chalcone isomerase, dihydroflavonol reductase, and anthocyanidin synthase [6].

The main compounds belonging to the phenylpropanoids are benzoquinones, naphthoquinones, acetophenones, coumaric acids, flavonoids, bioflavonoids, isoflavonoids, and tannins. The biosynthesis of all the above-mentioned compounds under abiotic and biotic stress conditions in plants is essential to ameliorate the reactive oxygen species (ROS) synthesized by the plants in response to the external stress. The oxidation and reduction potential of the bio-compounds synthesized in phenylpropanoid pathway is known to have excellent scavenging potential for the reactive oxygen species [7]. This chapter will explore the protective role of phenylpropanoids in the growth and development of plants.

SECONDARY METABOLITES PRODUCED BY PHENYLPROPANOID METABOLISM

Phenylpropanoid pathway is considered as the major frequently evaluated metabolic route for the synthesis of the secondary metabolites. Certain simple phenylpropanoids (having basic carbon skeleton C6-C3 of phenylalanine) are generated from cinnamate *via* a series of methylation, hydroxylation, and dehydration reactions, including caffeic, sinapic acids, p-coumaric, ferulic acid, and simple coumarins [8]. The phenylpropanoid pathway products are involved in several aspects of plant growth and response to the stimuli associated inextricably with the land life as well as structural support. They play a key role in stress response upon mineral shortage [9], light variations [10] as well as act as crucial mediators of the interactions of plants with other organisms [11, 12].

The diverse aromatic metabolites produced by the phenylpropanoid biosynthesis in plants play a number of important biological functions. The shikimate pathway of the plant is the entry to the phenylpropanoids biosynthesis [13]. The general metabolism of phenylpropanoid produces a wide array of secondary metabolites based on the few shikimate pathway intermediates as the core unit. The three enzymatic activities of the central phenylpropanoid pathway is defined by (i) the deamination of phenylalanine to the trans-cinnamic acid by phenylalanine ammonia-lyase (PAL), (ii) the hydroxylation of trans-cinnamic acid by the activity of cinnamic acid 4-hydroxylase (C4H) activity to the 4-coumarate, and finally (iii) the 4-coumarate conversion by 4-coumarate-CoA ligase (4CL) to the 4-coumaroyl-CoA [14]. Phenylpropanoid-based polymers such as condensed tannins, suberin, and lignin substantially contribute to the robustness and stability of angiosperms and gymnosperms towards environmental or mechanical damage like wounding or drought. When plants are stressed under sub-optimal photosynthesis, the low concentration of shikimate pathway intermediates may redirect the whole phenylpropanoid pathway for the formation of anthocyanins, volatiles, phytoalexins, flavonoids, and de novo proteins synthesis [15, 16].

Role of Plant Growth Regulators in Abiotic Stress Tolerance

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Abstract: Plants are frequently exposed to different types of stressful environmental conditions, which have adverse effects on their growth, development, and productivity. These conditions, such as salinity, drought, floods, chilling, freezing, UV exposure, pollution, nutritional deficiencies, metal toxicity, *etc.*, are collectively known as abiotic stressors and hinder plants from fully expressing their genetic potential. With advancements in scientific fields such as genetics and molecular biology, it has become easier to understand that under abiotic stress, a myriad of responses are triggered in plants. These changes include alterations in gene expression to changes in cell metabolism to avoid or tolerate the stress. The intensity of these plant responses depends on affected tissue, age of the plant, type of stress posed, duration and severity of stress, *etc.* It has been observed that plant growth regulators such as auxins, abscisic acid, cytokinins, ethylene, gibberellins, jasmonic acid, brassinosteroids, salicylic acid, polyamines, strigolactones, *etc.*, which influence the growth and differentiation in plants, also have very important roles in regulating the stress tolerance in plants. This chapter is a comprehensive account of literature based on the role of different plant growth regulators in the regulation of tolerance of plants towards abiotic stressors. The contents of this chapter include a brief discussion about different types of abiotic stressors, their effects on plants, and responses developed in plants against them. There is also a detailed discussion about plant growth regulators, their role in the normal functioning of plants, followed by their contribution and underlying mechanisms in building abiotic stress tolerance in plants.

Keywords: Abscisic acid, Auxins, Brassinosteroids, Cytokinins, Ethylene, Gibberellins, Jasmonic acid, Polyamines, Salicylic acid, Strigolactones.

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INTRODUCTION

Abiotic stress in plants is a cumulative adverse effect of non-living environmental factors on the plants affecting their physiology, growth, development, and yield [1]. Plants are immobile in nature and highly susceptible to ever-changing environmental and climatic conditions, including abiotic stress. The latter results in the malfunctioning of different physiological, biochemical, and molecular mechanisms, ultimately decreasing the plant yield [2]. Demand for agricultural products has increased enormously with the increasing human population. However, the impact of different abiotic stressors has posed a grave danger to plant growth, fertility, and agricultural productivity [3].

Abiotic stress on plants is posed by environmental conditions (physical or chemical) such as drought, water logging, UV exposure, excessive heat, chilling, frosting, soil salinity, mineral toxicity, pollution, and nutritional deficiencies (Fig. 1) [1, 4, 5]. Plants have developed various methods to adapt to these abiotic stressors, in turn causing a reduction in biomass and yield [3]. It is extremely important to manage abiotic stress on plants as it limits the agricultural yield affecting the economy of countries, and the livelihood of the farmers is dependent on these crops [5]. To improve the yield and sustainability of crops, it is necessary to understand the factors and mechanisms behind plant responses to different abiotic stressors [3]. The severity of plant responses is dependant on different factors such as affected part or tissue of the plant, growth stage, and conditions of plant, species or ecotypes of plant, type and intensity of abiotic stress, duration of exposure to the stress, *etc* [6, 7].

Plants are able to perceive even minor unfavourable alterations in environmental conditions and start a myriad of stress responses for coping with the situation and establishing homeostasis [8, 9]. Plant growth regulators, which play a vital role in the regulation of the growth and development of plants, are instrumental in tolerating and combating abiotic stress in plants [10]. Different plant growth regulators, such as abscisic acid, cytokinins, auxins, gibberellins, ethylene, jasmonic acid, salicylic acid, brassinosteroids, polyamines, strigolactones, *etc.* have an extensive role in developing and maintaining abiotic stress tolerance in plants [11 - 13]. Considering these facts, this chapter has been written as an attempt to compile information on adverse effects of different types of abiotic stressors on plants, responses of plants to these stressors, the role of different plant growth regulators in stress tolerance and crop improvement, regulation of tolerance of plants towards abiotic stressors.



Fig. (1). Different types of abiotic stressors posed to plants [Based on: 1, 4, 5].

ABIOTIC STRESS AND PLANT RESPONSES

Abiotic stress is a major factor responsible for hindrance in the full expression of the genetic potential of plants. It triggers a variety of responses in plants, including changes in cellular metabolism, signal transduction, gene expression, plant growth rate, and crop yield [14]. According to Onaga and Wydra, under abiotic stress, membrane receptors in plants perceive different initial stress signals such as changes in membrane fluidity and osmotic effects. Then, signal transduction triggers transcription, which is controlled by different hormones, miRNAs, transcription factor binding proteins, and transcription factors. It activates defense response in plant cells to fix damaged membranes and proteins and to reinstate ion homeostasis in cells (Fig 2) [15]. This defense response includes various changes such as:

CHAPTER 11

Genomics, Proteomics and Metabolic Approaches against Abiotic Stress**Harjit Kaur Bajwa¹ and Hina Khan^{1,*}**¹ *Department of Botany and Environmental Science, Sri Guru Granth Sahib World University, Fatehgarh Sahib, Punjab 140407, India*

Abstract: Abiotic stressors such as drought, salination, flooding, cold, heat, ultraviolet radiation, heavy metals, *etc.*, are the paramount cause that reduce crop yield and weaken universal food security as they strongly affect plant growth, physiology, and metabolism. Plants frequently face a large number of environmental stressors and usually generate common responses to deal with these unfavorable conditions. However, crop improvement against abiotic stressors is one of the urgent priorities that need undivided attention, while a huge increase in demand for various plant-derived products will rise in the near future owing to the rising human population. As conventional methods for crop enhancement have limitations, therefore an epoch of omic research has shot up with new and encouraging perspectives in breeding to improve the crops against abiotic stress. In this light, the genomic, proteomic, and metabolomic approaches are emerging as powerful tools for the identification and description of cellular networks through which stress perception, signal transduction, and defensive responses are exhibited. Further advances in omic techniques have permitted a comprehensive investigation of crop genomes and have magnified the perception of convolution of the mechanisms controlling abiotic stressor tolerance and the adaptation to mitigate them. This chapter will give an overview of genomics, proteomics, and metabolic approaches and their usage to enhance the possibility of producing abiotic stressor tolerant crops.

Keywords: Abiotic stress, Genome, Metabolome, Proteome.

INTRODUCTION

Flora are often subjected to numerous abiotic stressors forever that limit their yield and productivity. Tolerance to abiotic stressors can halt yield loss in crops for maintaining agricultural productivity. Abiotic stressors such as high/low temperature, salinization or alkalinity, and drought are the major challenges fac-

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ing agriculture. 50% of the total yield loss in essential crops worldwide is only due to these abiotic stressors [1]. Approximately 45% of the world's agricultural land is affected by drought, while 19% is considered saline [2].

Thus, the production of sustainable and high-yielding crops with improved tolerance to different abiotic stressors is a precondition to meet the increasing universal food supply. The conventional methods such as plant breeding and genetic engineering played a vital role during the last century for enhancing abiotic stress tolerance but have limited success due to the genetic complexity of stress responses [3]. Consequently, agriculture has to adopt novel approaches to produce plants with natural ability of enhanced level of tolerance to environmental stressors. Plant reaction to abiotic stressors is a complicated process because abiotic stressors could initiate multigene response which comprises a number of events including stress signaling, stress transduction, and gene expression. This whole process leads to the agglomeration of transcription factors, stress-related proteins, enzymes, and metabolites. Therefore, genomics, proteomics, and metabolomic techniques are playing a key role in studying plants' reactions to different stimuli [4]. Genomics had disclosed the gene and protein sequences while proteomics and metabolomics uncover the biological function of the gene product. The "omic" technologies are high-throughput innovations that allow a system biology approach toward understanding the compound interactions between genes, proteins, and metabolites within the resulting expression [5]. Furthermore, recent advances in molecular biology and omic technology allowed explaining functions of many key genes, proteins, and metabolites involved in plant responses to abiotic stressors [6]. The purpose of this book chapter is to provide an overview of genomics, proteomics, and metabolic approaches and their usage in developing abiotic stress-tolerant crops.

GENOMICS APPROACHES AGAINST ABIOTIC STRESS

Genomics studies the sequence, organization, and functions of DNA of an organism using a wide variety of experimental, computational and statistical methodologies. Genomics has been substantially expedited in the last few years with the emergence of next-generation sequencing technologies, high-density molecular assays, and advances in computational biology and biostatistics [7]. Sequencing the complete genome is the most effective method to accelerate molecular research and provided the ground for the production of simple sequence repeat (SSR) and single nucleotide polymorphism markers. This approach has already been successfully used in several crops and also has high scope in the future [8].

Arabidopsis thaliana was the initial plant to be completely sequenced. It was asserted that the *Arabidopsis* genome sequence helped in the extensive perception of plant development, environmental responses, genome organization, regulation, and evolution [9]. However, these days, crops like rice and maize have gained importance. Abiotic stressor reactions in plants are controlled by different transcription factors and signaling mechanisms. The relevant factors in each mechanism have been identified and addressed using responsive genes as markers [10]. Many stress-regulated genes are respondent to diverse stressors and stimuli; for instance, KNAT3, KNAT4, SEN1, DIN9, DIN10, and ACP4 are drought-regulated genes but also respond to light [11]. Similarly, ERD14, RD29B, and COR47 are responsive to duo stressors, water deprivation as well as cold. In *Arabidopsis*, 67 genes have been identified which exhibited responses to the cold, osmotic stressor, salinity, drought, genotoxic stressor, UV light, oxidative stressor, osmotic, injury, and high temperature [12]. The approaches based on genomics provide ingress to agronomically desirable alleles present at quantitative trait loci (QTLs), thus enabling the improvement of abiotic stressor tolerant plants. Recently, marker-assisted selection (MAS), multi-parallel analysis of transcript levels, microarray analysis, RT-qPCR, serial analysis of gene expression (SAGE), massive parallel signature sequencing (MPSS), and oligoarray techniques are used to evaluate abiotic stressor responses in plants. Moreover, various bioinformatic tools, ESTs, and subtractive cDNA have added more to the area of genomics in order to get to the bottom of the genetics of stress tolerance [13]. The main goals of genomics is to assemble the genomic sequence of organisms, search out the position of the genes for analyzing the relationships, interpret the gene set, learn gene functions, and compare gene protein profiles among distinct organisms.

Structural Genomics

The main purpose of structural genomics is to identify the structure of the genome. It can be very useful in manipulating genes and DNA segments in particular plant species. The initial step in structural genomics is the assignment of genes and markers to individual chromosomes, then the mapping of these genes and markers within a chromosome, and finally the preparation of a physical map culminating in sequencing [14].

Molecular Markers

The first low-throughput markers were discovered using restriction fragment length polymorphism (RFLP) method. The restriction fragment length polymorphism subsequently improved by coupling PCR that gave rise to random

CRISPR/CAS9 Technologies to Enhance Tolerance to Abiotic Stress in Crop Plants

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Abstract: Plants can identify and cope up with biotic and abiotic stressors, thus resulting in a reduction in agricultural production significantly. Currently, the primary goal of plant breeders is to develop the ability to tolerate multiple stress conditions without lowering the productivity of the crop. However, numerous attempts have failed to release these plants due to persistent divergence between growth and resistance to stressors. Such strategies are not appropriate to effectively enhance characteristics and ensure potential environmental impact. Genome editing approaches and RNA interference (RNAi) technique has been used to develop plants resistant to different environmental changes. Newly developed approaches such as CRISPR are used to grow new varieties that have external stressors tolerance and reduce a vital yield loss. This chapter addresses the use of CRISPR/CAS techniques to improve the stress tolerating ability of the plants

Keywords: Abiotic, CRISPR, Plant breeding, RNA interference (RNAi), Stress.

INTRODUCTION

The global reduction in crop yield is caused by abiotic stress, which further restricts the average cultivation of all agricultural crops [1]. It also restricts plant efficiency, growth [2], and overproduction of toxic reactive oxygen (ROS) species [3]. These plants immediately evolve different mechanisms for reducing environmental stress-related toxic effects [1]. Plants establish specific immune responses during growth and development with metabolic alterations [4]. Besides

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the management of genetic strategies in plant growth, development, and productivity [5], the mechanism for plant defense needs in-depth investigations of stress-sensitive messengers and signals, including protein interaction, transcription factors (TFs), and promoters. Also, a broad spectrum of abiotic stressors, including temperatures, light, heavy metals, osmotic stressors, and a range of biotic factors, can over-produce highly toxic ROS [1]. The plant may establish a defensive mechanism by which the damage is either prevented or even alleviated. This process triggers the highly stressors-responsive, antioxidant gene expressions [1]. Besides this, these genes will function through metabolic modifications in the plant cell.

IMPORTANCE OF BREEDING TOWARDS ABIOTIC STRESS TOLERANCE

Drought, salinity, heavy metals, and extreme temperatures are significant abiotic stress factors that thrive in modern farming. The decrease in yield in the primary cultivation of plants is approximately 50 percent, although the degree of yield losses depends on the nature and intensity of stressors during plants' growing cycle [6]. Different abiotic stressors-triggered gene reduced expression of genes responsible for biochemical and phenotypic modifications, thereby reducing photosynthetic capacity, lowered nitrogen assimilation capability, altered behaviors of several core molecules, plasma membrane properties, and cellular biochemical changes. Plants are affected in many ways, *e.g.*, by altering growth and development of plants, membrane diffusion, contraction of stomata, chlorophyll loss, osmotic fluctuations, photosynthetic activity declined and decreased the CO₂ assimilation. In contrast, the level of abscisic acid (ABA), osmolytes, proline, sorbitol, mannitol, radical scavenging molecules (glutathione, ascorbate) enhanced [7]. Plant breeders should implement new genetic engineering approaches to improve the breeding system. Breeders might concentrate on strengthening the resistance of plants towards environmental stressors [8]. Research and development of crop varieties with improved response to stressors *via* the use of genetic techniques helps in the development of several specific characteristics and reduces time and complexity under stressors conditions. Based on the continuously rising world population, it is very difficult to meet the demands of the increasing supply of food production. The biological response of crops may help to develop systems or prototypes that can better understand a range of plant responses to diverse abiotic stressors. These strategies help to identify the adequate technology for the development of enhanced abiotic-resistant crop species. The advancement of plant science in systems biology is inevitable. It would help to coordinate different high-level “omic” networks such as transcriptome, proteome, metabolome, and genomics to recognize plant responses and regulatory mechanisms and their interactions [9].

GENETIC APPROACHES TO ENHANCE ABIOTIC STRESS TOLERANCES IN PLANTS

Classic breeding techniques have become a standard protocol in the study of the genetic diversity of crop varieties. Most of the crop cultivars introduced several conventional breeding programmes, such as wheat [10, 11,], rice [12, 13], soybeans [14], and maize [15]. Hence, traditional breeding technology has strengthened crop quality at anatomical, biochemical, and molecular levels against numerous abiotic stressors. However, the limitations of this technology are noteworthy because it is arduous, time-consuming, complicated, and expensive. Therefore, the development of a new cultivar with improved features by this technology takes several years. Besides, several of the genes with inconsequential or undesired traits might also be introduced, which may be challenging to eliminate. The availability of germplasm or low genetic variations can also be a significant selection factor [16]. The complexity of adapting the plant under stress makes breeding difficult. This suggests that several traits can combine and help to improve the tolerance of plants towards various abiotic stressors. Therefore, the tremendous progress in genomics science led to a deeper understanding, *e.g.*, gene expression, communication, and the interaction toward stressors tolerance. Qualitative biotechnology and strategies, particularly, the high-throughput study of expressed sequence tags, targeted or random mutagenesis, large-scale simultaneous genomic study, the identification of innovative genes, and their trends of expression in response to abiotic stressors help in improving knowledge of their functions in stressors tolerance [17].

RNA INTERFERENCE (RNAI) APPROACHES TO RESIST ABIOTIC STRESSORS

The next-generation sequence has quickly resolved the weaknesses of DNA-mutagen dependent screening by mapping mutations using transposons and phages, rather than synthetic mutagens, to recognise sequences of insertions that are conducive to sequencing-based research [18, 19]. Besides, new genetic disruption methods for gene knock-down using the RNAi pathway, and forward genetic predictions were revolutionized [20]. Small RNAs (sRNAs) emerged as outstanding alternatives for promoting crop development and quantitative biotechnology experiments these days. siRNA and miRNA are core regulators in the growth and development of both the post-transcriptional and transcriptional stages. Different studies have indicated RNA silencing technology as an epigenetic regulation, which results in double-stranded post-transcriptional gene silencing (dsRNAs) to hinder the expression of specific genes [21]. RNAi technology is reliable, competent and is one of the main strategies for the improvement of crops, as well as for modifications relevant molecular

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