Chapter 3

Role of Osmolytes and Antioxidant Enzymes for Drought Tolerance in Wheat

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Additional information is available at the end of the chapter

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Abstract

Plants are vital to life as their presence maintains ecosystem on this living globe. Environmental stresses trigger multiple responses initiated by plant cells to save plant life, from altered gene expression up to changes in cellular metabolism to regulate plant growth rates, which lead to better crop yield. The production of different osmoprotectants like proline, glycine-betaine (GB), trehalose, and antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase has shown a promising role to keep away cells from immediate cellular damage. Root-to-shoot ratio was enhanced in the drought-affected genotypes, while osmolytes and antioxidant enzymes take up the role to overcome drought situation in wheat germplasm. PEG-induced protocol was used to find out the production of osmolytes (proline, glycine-betaine, and trehalose) and antioxidant enzymes (SOD, CAT, and APX) biochemically. The levels of antioxidant enzymes and osmolytes were enhanced significantly in all germplasms indicating the defensive measures of plant cells in drought situation. DNA fingerprinting results have shown that the different wheat germplasms have an association with the levels of osmoprotectants and antioxidant enzymes during drought stress.

Keywords: drought tolerance, PEG 8000, osmolytes, antioxidant enzymes, wheat germplasm

1. Introduction

Global wheat production in the key production areas is being threatened by recurrent drought situation which is predicted to increase with climate change. Drought-tolerant wheat varieties are the ultimate solution of safeguarding the crop against adverse effects of drought [1]. Plants are frequently exposed to environmental stresses both due to some natural cause and rough agricultural practices. Various types of both biotic and abiotic stresses may result in limited plant productivity. Plant stresses like oxidative, chemical toxicity, drought and salinity, extreme temperatures along with the attack of insects, pests, and plant pathogens result in significant crop losses which are a serious threat to agriculture [2].

Drought is significantly damaging the plant that further limits the crop productivity, and most countries are facing this big disaster. Plant productivity is greatly inclined due to stressful conditions that affect almost every aspect of plant growth. All plants develop a unique pattern of biochemical and molecular mechanisms to manage odd situations linked with stress tolerance. Environmental stresses like drought, high salinity, and low temperature initiate gene expression that raise osmolytes and antioxidant enzyme levels in plant cells to tolerate stress responses.

Due to ever-increasing population around the globe, food crop productivity is highly enviable, and it is the need of the hour to expand measures for maximum produce. Wheat occupies an important place as staple food and the yield improvement of the wheat germplasm under different stresses and agro-climatic situations [3] have an essential task for researchers to deal within the present scenario. Human food comprises many valuable produce like rice, pulses, and meats but wheat is among the most important for human consumption worldwide. A part of the total wheat crop production is also used as a feed for livestock. *Triticum aestivum* is common bread wheat, but the other two species of wheat are of commercial importance as *T. durum* is pasta product wheat and *T. compactum* is pastry flour wheat. About 35% of the human population consumes wheat as food, covering 29% of caloric intake. Wheat shares the largest cereal market due to its global production at more than 651. 4 million metric tons per annum [4]. The high nutritive value (>10% protein, 2.4% lipids, and 79% carbohydrates) of wheat is based largely on its ingredients and the versatility of its use in the production of a wide range of food products [5]. The global climate change is facing adverse effects, while some other areas that have adopted the effects of climate change have shown benefits to crop system. Wheat is grown in the regions where rainfall ranges 30–113 cm [6]. Plant productivity is hampered by environmental stresses [7], and water shortage definitely limits plant growth and productivity even more than any other environmental factor [8]. Elevated levels of stress hormone viz. ABA has been associated with water stress tolerance in crop plants. Heat regulates stomatal conductance and water loss under desiccation that results in the accumulation of osmolytes like proline, mannitol, glycine-betaine, and soluble sugars like trehalose which lower the osmotic potential of the cell sap and thus prevent the movement of water out of the cell [9].

Pakistan has faced recently a big problem of wheat shortage due to drought situation and had to import wheat to fulfill the need of the country. Our globe is affected by drought: about 45% of the land area mostly of Africa (Ethiopia) and its surroundings, most of the Mediterranean, Mexico, Australia, and some parts of Middle East, India and Sindh province area of Pakistan. Irrigated land is only 15% of total cultivated land which yields twice as much as rain-fed land and producing one-third of the world's food [10]. Plant stress also plays a major role in determining the distribution of plant species due to soil texture and climate limitations. To maintain a gradient of water flow into the plant, the soil water potential is much important but its reduction may lead to increased soil solutes that make it increasingly difficult to establish osmotic pressure. The resulting osmotic stress leads to stomatal closure in some plant species [11] and a reduced rate of photosynthesis [12].

2. Plant stresses

Environmental stresses are the main cause of limited crop production in the world. The land is affected by mineral stress about 20%, by drought stress about 26%, and 15% by freezing stress [13]. Environmental stresses are of two types: biotic stresses and abiotic stresses. Biotic stresses include infection and competition by other organisms. Abiotic stresses include light, temperature, water (drought), excess (flooding), radiation, and salinity stress. The capacity of plants to cope with unfavorable environments is known as stress resistance. Plant adaptations to tolerate stress depend upon genetically modified resistance genes that improve resistance as a result of prior exposure of a plant to stress. The mechanisms of drought resistance may fluctuate with climate change and soil conditions. Leaf expansion is restricted by water stress as one of the earliest responses occurring when decreases in turgor resulting from water deficit reduce or eliminate the driving force for cell and leaf expansion. Leaf abscission mechanisms start due to water stress and root extension into deeper, wetter soil, and stomatal closure as a response of water deficiency. Water deficit leads to the gene expression involved in acclimation and adaptation to the stress. The sensing and activation of signal transduction cascades mediating these changes in gene expression involve both an ABA-dependent pathway and ABA-independent pathways [9]. Anjum et al. [14] have studied the drought-induced changes in growth, osmolytes accumulation, and antioxidant metabolisms of maize hybrids. Drought stress in crop production system is much more precarious than any abiotic stress due to climatic changes. According to them, physio-biochemical regulation of plants under drought stress can be used as markers for drought tolerance in selection and breeding purposes. The maize growth and yield responses were highly related to ROS production, osmolytes accumulation, and activation of antioxidative defense system under drought situation.

3. Water-use efficiency

The water-limited productivity of plants depends on the total amount of water available and on the water-use efficiency of the plant. Any plant capable of acquiring more water or that has a higher water-use efficiency will resist drought better. When water shortage develops slowly, it is sufficient to permit changes in developmental process as water stress has more than a few adverse effects on plant growth. In this situation, compatible solutes like proline, glycine-betaine, and trehalose are produced to counter the unfavorable cellular conditions. Osmotic adjustment (OA) is a net increase in these solute contents per cell, and it develops slowly in response to tissue dehydration and maintains turgor and osmotic pressure of effected plant species. Osmotic potential fluctuation by the soil solution creates the stress in plants by water ultimately leading to plant death as a result of growth arrest and molecular damage. Osmotic adjustment in plant cells helps to maintain plant water balance to carry on regular life processes [9].

Most plants take up CO_2 from atmosphere while limiting water loss. The cuticle covers exposed plant surfaces as an effective barrier to water loss that protects the plant from desiccation. These plants cannot prevent outward diffusion of water without excluding CO_2 from the leaf. The concentration gradient of CO_2 uptake is much smaller than the concentration gradient that drives water loss. If water reservoir is higher than usual, this triggers regulation of stomatal apertures at day time and remained close at night. There is no photosynthesis in the night, so no demand for CO_2 inside the leaf; therefore, stomatal apertures are kept small, preventing unnecessary water loss. When water supply is abundant on a sunny morning, the solar radiation incident on the leaf favors a high photosynthetic activity, the demand for CO_2 inside the leaf is large, and the stomatal pores are wide open to claim more CO_2. Through transpiration, water loss is substantial under these conditions, but since the H_2O is plentiful, it is beneficial for the plant to trade water, the product of photosynthesis, which is essential for growth and reproduction. Mild water deficits also affect the development of the root system. Root-to-shoot biomass ratio appears to be governed by a functional balance between water uptake by the root and photosynthesis by the shoot. A shoot will grow with maximum water uptake by the roots and becomes limiting to promote growth until their demand for photosynthate from the shoot equals the supply. This functional balance is shifted if the water supply decreases. On the other hand, when soil water is less abundant, the stomata will open less or even remain closed on a sunny morning. Thus, plants avoid dehydration by keeping its stomata closed in dry conditions [9].

Many organisms accumulate intracellular low-molecular-weight compounds due to water deficit to maintain equal water potential with the external conditions. Osmotic adjustment is contributed by many compounds which besides providing protection to macromolecules such as enzymes, proteins, electrolytes and temperature. Plant cells generally accumulate the inorganic ions mostly present in the soil environment, but in high concentration these become harmful to cellular integrity [15]. The organisms usually accumulate specific types of organic molecules called as compatible solutes required for maintaining the cytoplasm osmotically balanced. The main function of formed osmolytes is to maintain osmotic balance within the cell, and even their high concentrations may not impair the normal physiological function of the cell. As plant life savers, organic osmolytes facilitate osmotic adjustment normally to maintain cellular milieu.

3.1. Root-shoot ratio

Crops of tomorrow are expected to grow under huge levels of atmospheric CO_2. Basic crop growth parameters will be affected and major among those is carbon allocation. The ratio of root to shoot is dependent upon the separation of photosynthate which might be influenced by environmental stimuli. The upper layer of the soil gets dry without water, but the root growth started more to qualify moist zones under the soil. Deeper root growth into wet soil can be considered a second line of defense against drought. Better root growth into moist soil zones during stress requires osmolytes indirectly to maintain osmotic potential in order. During water shortage, the root growth is less prominent in reproductive plants as compared to vegetative plants. Therefore, the plants are more sensitive to water stress during reproduction period.

According to Iqbal et al. [16], the water deficit situation in wheat germplasm has shown detrimental developmental processes which effects plant growth ultimately. To counter this situation, compatible solutes like proline, glycine-betaine, and trehalose protect cellular milieu from dehydration. An increase in root growth in different plants under drought stress was also shown by Tahir et al. [17] and Jaleel et al. [18]. Plants with a higher proportion of roots can compete more effectively for soil nutrients, while those with a higher proportion of shoots can collect more light energy and perform function accordingly. Root length is a better measure than the surface area of the absorbing ability of roots. Water moves slowly in soil so that a small root is almost as effective as a larger one in absorbing water and nutrients. According to Fahad et al. [19], the large proportions of shoot production are characteristic of vegetation in early succession phases, while high proportions of root production are characteristic of climax vegetation phases. Except for injury to the roots, a reduction in the root-shoot ratio is almost always in response to more favorable growing conditions. An increase in the root-shoot ratio would indicate that a plant was probably growing under less favorable conditions.

4. Drought stress

Drought is the lack of inadequate moisture level in soil, leading toward water stress which adversely affects crop productivity. Indeed, it is hypothesized that differences in drought and salt tolerance arise because of changes in the regulation of a basic set of drought and salt tolerance genes. Attempts to improve the drought tolerance of crops by conventional breeding programs have shown very limited success because of the complexity of the trait. Drought tolerance is a complex process genetically and physiologically [20]. The components of drought resistance in plants include both avoidance and tolerance to water stress and desiccation. Early maturity mechanism helps drought resistance in wheat before the period of drought, deeper root system to efficiently utilize the available moisture, and prolonged closing of stomata during drought stress to decrease water loss. The development of wheat cultivars for drought stress tolerance commonly has narrow leaves and lower shoot/root ratios and may have a low yield potential than varieties developed for irrigated areas. Jaleel et al. [21] has reviewed the drought stress as a changed physiological situation caused by the trend to disturb equilibrium. The damage in physical and chemical change shaped the stresses in plants exposed to drought, oxidative stress, low and high temperature, salt, flooding, and heavy metal toxicity. Drought stress tolerance is observed in most of the plants but its extent varies from species to species and even within species. Water deficit and salt stresses are global issues to ensure the survival of agricultural crops and sustainable food production. A ramified root system is established during drought tolerance and high biomass production primarily due to its ability to extract more water from soil and its transport to above ground parts for photosynthesis. When the beginning of stress is in rapid state or the plant has reached its full leaf area before initiation of stress while on the other side, protective mechanisms started in the plant against immediate desiccation. Under these sub-normal conditions, stomata closure reduces evaporation from the leaf surface area. At this stage, the stomatal closure is considered to be an important line of defense against drought. Uptake and loss of water in guard cells change their turgor and modulate stomatal opening and closing. The guard cells are located in the leaf epidermis which can drop turgor pressure as a result of a direct water loss by evaporation to the atmosphere. Hydropassive closure of stomata is due to the decrease in turgor that operates in air of low humidity and when direct water loss from the guard cells is too rapid to from adjacent epidermal cells. Secondly, hydroactive closure mechanism closes the stomata when the whole leaf or the roots are dehydrated and depends on metabolic processes in the guard cells. The reduced solute contents in the guard cells results in water loss and decreased turgor for closing stomata. The hydraulic mechanism of hydroactive closure is a reversal of the mechanism of stomatal opening. The loss of solutes from guard cells can be activated by a decreased water content of the leaf where abscisic acid (ABA) plays an important role in this process. Abscisic acid is synthesized continuously at a low rate in mesophyll cells and tends to accumulate in the chloroplasts. When the mesophyll becomes mildly dehydrated, firstly the ABA stored in the chloroplasts is released to the apoplast (the cell wall space) of the mesophyll cell [22]. The pH gradients redistribute ABA molecule within the leaf, making it possible for the transpiration stream to carry some of the ABA to the guard cells. Secondly, leaf apoplast is saturated with ABA synthesized at a higher rate, and this higher ABA concentration appears to enhance or prolong the initial closing effect of the stored ABA, leading to the mechanism of ABA-induced stomatal closure. Leaf dehydration can vary widely both within and across species due to stomatal responses. The drought tolerant species like cowpea (*Vigna unguiculata*) and cassava (*Manihot esculenta*) are more responsive to stomatal conductance and leaf water potential may remain nearly constant during drought due to less transpiration activity. Chemical signals from the root system may affect the stomatal responses to water stress. The stomatal conductance is more closely related to soil water status than to leaf water status because the average root system is directly affected by soil water status. In fact, dehydrating only part of the root system may cause stomatal closure even if the well-watered portion of the root system still delivers ample water to the shoots.

4.1. Osmotic adjustment

Water plays a crucial role in plants' life as approximately 500 g of water is absorbed by the roots for every gram of organic matter made by plant. Imbalance in water flow can cause water shortage that lead to malfunctioning of major cellular processes. The balancing of water uptake and loss is a crucial challenge for photosynthetic plants to utilize CO_2 from atmosphere, and by doing so, plant exposes to water loss and the next threat of dehydration. A main difference between plant and animal cells that affects almost all aspects of their relation with water is the existence in plants of the cell wall. The internal hydrostatic turgor pressure is a result of their normal water balance inside the cell wall. Turgor pressure is essential for many physiological processes including cell enlargement, gases exchange in the leaves, transport in the phloem, and various transport processes across membrane. Turgor pressure also contributes to the rigidity and mechanical stability of non-lignified plant tissues.

Water is essential to land plants to avoid lethal desiccation by water loss to the atmosphere. The large surface area of leaves, their high radiant-energy gain, and their need to have an open pathway for CO_2 uptake may aggravate water loss. Water conservation and the need for CO_2 assimilation are a constant situation in plants for survival. Water makes up most of the mass of the plant cells, as each cell contains large water-filled vacuole whereas water typically constitutes 80–95% of the mass of the growing plant tissues. Seeds with a water content of 5–15% are among the driest of plant tissues that also absorb a considerable amount of water before germination. Plants continuously absorb and lose water during transpiration means and dissipate heat because the escaped water molecules have higher than average energy, breaking the bonds holding them in a liquid form. The transport of water bulk flow from the soil through the plant body to the atmosphere includes diffusion and osmosis. The plant water can be considered incessant hydraulic system connecting through water in the soil with the water vapors in the atmosphere. Guard cells regulate transpiration through the control of stomatal pore size to meet the photosynthetic demand for CO_2 uptake while limiting water loss to the atmosphere. Large negative pressures (or tensions) in the apoplastic water is generated by water evaporation from the cell walls of the leaf mesophyll cells. Xylem conduits hold these negative pressures, but when transpiration is high, negative pressures in the xylem water may cause cavitations (embolisms) in the xylem that can block water transport and lead to severe water deficits in the leaf. Water deficit plants adapt responses that modify the physiology and development of plants. Water move through soils by bulk flow driven by a pressure gradient, and plants absorb water from soil through roots. The rate of water flow in soils depends upon the factors like pressure gradient through soil and hydraulic conductivity of the soil. As the water content of the soil decreases, the hydraulic conductivity decreases drastically. In very dry soil, water potential may fall below the permanent wilting point. At this point, the water potential of the soil is so low that plant cannot regain turgor pressure which means that the water potential is less than or equal to the osmotic potential of the plant. Different plant species behave differently in soil, and the permanent wilting point is clearly not a unique property of the soil. Water uptake decreases when roots are subjected to low temperature or anaerobic conditions, or treated with respiratory inhibitors like cyanide. The anaerobic roots transport less water to the shoots which suffer net water loss and begin to wilt [15].

4.2. Polyethylene glycol application

Polyethylene glycol (PEG) is a polymer used to modify the osmotic potential of nutrient solutions cultures to induce plant water deficit in experimental protocols. PEG 8000 (18%) is used as an osmoticum to induce drought in wheat genotypes after 1 week of plantation. Water stress greatly suppresses cell expansion and cell growth due to low turgor pressure. Osmotic regulation can enable the maintenance of cellular turgor for plant survival. The reduction in plant height was associated with a decline in the cell enlargement and more leaf senescence. The plants grown in nutrient culture containing PEG suffered from hypoxia, and such system should be oxygenated for running drought-stress experiments [16].

5. Osmolytes

In plants, there are effective mechanisms of osmotic adjustment based on the synthesis of osmolytes which are low-molecular-weight compatible solutes. Osmolytes are frequently used by cells to accommodate osmotic pressure within the effected cells to avoid cellular injury due to oxidation phenomenon. They are highly soluble organic molecules that are synthesized in many organisms in response to different environmental conditions leading to osmotic stress [7]. They accumulate in the cytosol without interfering with the cellular metabolism even at high concentrations. Osmolytes have additional functions during the stress response and act as osmoprotectants by directly stabilizing protein and membrane structures under dehydration conditions. They have a diverse chemical nature, and apart from contributing to maintain osmotic balance, they do protect cell against oxidative stress as scavengers of "reactive oxygen species" (ROS) [23, 24].

The osmoregulators such as protein, sugars, amino acids, and compounds of quaternary ammonium play a vital role in adjusting the osmotic pressure and stabilizing of plant cells and tissues [25]. Drought stress causes osmotic stress in plants which causes a reduction in growth, imbalance ion transport, and a decrease in transpiration rate and an increase in membrane permeability. Such effects result in less water-absorbing capacity of crop plants, and different plant species and genotypes within a species respond differently to adverse environmental conditions. In order to counteract unfavorable environmental conditions, plants accumulate different types of organic and inorganic solutes in cytosol to decrease osmotic potential by which they can maintain cell turgor.

Plant cells lose water and decrease turgor pressure under water-stress conditions. There is an increase in different plant hormones in case of water stress like abscisic acid, which has important roles in the tolerance of plants to drought, high salinity, and cold. Abiotic stresses, which cause depletion of cellular water, are responsible for the greatest agricultural losses. Upon exposure to these prevalent stresses, the accumulation of osmoprotectants is in sufficient quantity to facilitate osmotic adjustment. The increase in cellular osmolarity due to these compatible solutes is accompanied by the influx of water into the cells, providing the turgor necessary for cell expansion [7]. Water deficit develops slowly enough to allow changes in developmental processes as water stress has several adverse effects on plant growth. In this situation, compatible solutes like proline, glycine-betaine, and trehalose produce to counter the unfavorable cellular conditions. The osmotic potential fluctuation of soil solution creating a water stress in plants ultimately leads to plant death due to growth arrest and molecular damage. Osmotic adjustment of cells helps to maintain plant water balance to establish internal milieu [9].

5.1. Proline

Proline is the most extensively studied osmolyte because of its great importance in stress tolerance [26]. The exogenous application of proline can increase its endogenous levels in plant tissues subjected to water-stress conditions which help maintain osmotic adjustment in plant tissues. It may be a good source of minimizing the adverse effects of water stress on plants, and triggering their growth also depends upon the type of plant species and its concentration [27].

The production of proline is widely present in higher plants and normally accumulates in large quantities in response to environmental stresses [28]. For osmotic adjustment, proline contributes to stabilizing subcellular structures (e.g., membranes and proteins), scavenging free radicals, and buffering cellular redox potential under stress conditions. A rapid breakdown of proline upon relief of stress may provide sufficient reducing agents that support mitochondrial oxidative phosphorylation and generation of ATP for recovery from stress and repairing of stress-induced damages [29]. Iqbal et al. [30] have reported that the accumulation of proline in drought-tolerant and drought-sensitive cultivars has revealed the significance of this osmolyte. The role of proline in induced PEG experiment gave evidence that the higher levels of proline are due to the emergent need of stressed plant. This osmolyte is able to control the osmotic regulation of the cellular environment because of its high water solubility and its accumulation in the leaves of many halophytic higher plants grown in saline environment. Proline protects membranes against adverse effects of high concentration of inorganic ions and temperature extremes. It is also functional as a protein-compatible hydrotope and as a hydroxyl radical scavenger [31].

5.2. Proline biosynthesis

Proline biosynthesis in plants is initiated with the ATP-dependent phosphorylation of the carboxy group of glutamate by glutamyl kinase (GK).The resulting glutamyl phosphate (GP) is reduced to glutamic semi-aldehyde (GSA) by GSA dehydrogenase and glutamyl kinase which forms obligatory enzyme complex [32]. The accumulation of proline under stress in many plant species has been correlated with stress tolerance, and its concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants. In wheat, an assessment of the effects of drought stress on proline accumulation in a drought-tolerant and a drought-sensitive cultivar revealed that the rate of proline accumulation and utilization was significantly higher in the drought-tolerant cultivar [33]. Furthermore, in *B. juncea* plants grown under stress conditions, activities of proline biosynthetic enzymes P5CR and ornithine-aminotransferase (OAT) increased mainly intolerant lines though the activity of proline-degrading enzyme “proline oxidase” decreased in all lines.

5.3. Trehalose

Trehalose is a vital soluble sugar osmolyte frequently used by cells to accommodate osmotic pressure within the effected cells to avoid cellular injury due to oxidation phenomenon. According to recent research, sugar-signaling mechanism plays a vital role in accelerating the photosynthetic performance of plants to its maximum rate in association with trehalose metabolism. These positive effects of trehalose on gas exchange parameters are due to its role in osmoregulation which may affect the stomatal opening. It can be concluded that improvement in growth in wheat cultivars under water-stressed condition with trehalose application may have been due to the role of trehalose in osmotic adjustment. Different plant species respond differently on exogenous application of trehalose and proline. The plant development may be hampered by the external application of these compounds resulting in growth inhibition or yield reduction. The beneficial applications of these osmolytes on crop stress tolerance must carefully be determined for appropriate plant developmental stages.

In plants, trehalose increased the biomass production in shoots and roots in all wheat cultivars under water-stressed conditions as an osmoprotectant under adverse environmental conditions. Exogenous applications of trehalose and proline to plants during or after stress exposure and the increase in the internal levels of these compounds generally enhance plant growth and final crop yield under stress conditions [30].

5.4. Glycine-betaine

Among the many quaternary ammonium compounds known in plants, glycine-betaine (GB) occurs most abundantly in response to dehydration stress. GB is abundant mainly in chloroplast where it plays a vital role in adjustment and protection of thylakoid membrane, thereby maintaining a photosynthetic efficiency. In higher plants, GB is synthesized in chloroplast from serine via ethanolamine, choline, and betaine aldehyde [34]. The accumulation of two valuable osmolytes like glycine-betaine and proline in different plant species in response to environmental stresses such as drought, salinity, extreme temperatures, UV radiations, and some heavy metals. The role of these compounds has positive effects on enzymes and membrane integrity along with adaptive ways for osmotic adjustment in plants grown under stress conditions.

Cellular responses to stress include changes in the cell cycle and cell division, changes in the endomembrane system and vacuolization of cells, and changes in cell wall architecture, all leading to enhanced stress tolerance of cells. Plants alter metabolism in various ways to accommodate environmental stresses at a biochemical level by producing osmoregulatory compounds such as proline and glycine-betaine. The molecular events linking the perception of a stress signal with the genomic responses leading to tolerance have been intensively investigated in recent years. Certain plants accumulate significant amounts of glycine-betaine [35] in response to high salinity, cold, and drought stress. This quaternary amine has protective functions for macro-components of plant cells such as protein complexes and membranes under stress. GB is known to accumulate in response to stress in many crop plants, including sugar beet (*Beta vulgaris*), spinach (*Spinacia oleracea*), barley (*Hordeum vulgare*), wheat (*T. aestivum*), and sorghum (*Sorghum bicolor*). In these species, tolerant genotypes normally accumulate more GB than sensitive genotypes in response to stress. The relationship between GB accumulation and stress tolerance is species or even genotype specific [36]. The increased biosynthesis of GB from choline in stress-sensitive plants is capable of synthesizing this protective solute as drought-stress management.

All plant species are not equally capable of natural production or accumulation of osmolytes in response to stress. Tolerance to abiotic stresses is very complex at the whole plant and cellular levels. The complexity of interactions between stress factors and various molecular, biochemical, and physiological phenomena affects plant growth and development eventually [24]. Exogenous application of proline as pre-sowing seed treatments significantly affected the shoot and root K^+, Ca^{2+}, P, and N contents of root while this effect of proline on shoot N contents was inconsistent [37]. Similarly, Cuin et al. [38] reported that compatible solutes such as glycine-betaine, proline, and trehalose have explanatory effects on K+ efflux in Arabidopsis under stressed condition.

6. Antioxidant enzymes

The enzymatic and non-enzymatic mechanisms are available for scavenging of reactive oxygen species (ROS) in plants. The biochemical adaptive function of osmoprotectants to scavenge these harmful ROS by-products of hyperosmotic and ionic stresses causes membrane dysfunction and cell death ultimately. These active oxygen species are superoxide (O_2−), hydrogen peroxide (H_2O_2), hydroxyl radical (√OH), and singlet oxygen ($1O_2$) which is produced through oxidation phenomenon. Many plants have the ability to eliminate superoxide with the help of superoxide dismutase (SOD), which catalyzes the superoxide into H_2O_2 and O_2. Thylakoid membrane has potential enzyme ascorbate peroxidase (APX) to eliminate hydrogen peroxide to save cell membrane from severe damage.

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6.1. Superoxide dismutase

SOD concentrations typically increase with the degree of stress conditions as the compartmentalization of different forms of SOD throughout the plant makes them counteract stress very effectively. There are three classes of SOD metallic coenzymes that exist in plants that act to control increased levels of oxidative stress. SOD's role as a free radical scavenger is established, and those genotypes have higher levels indicating a higher level of stress tolerance in wheat. The availability of different forms of SOD in plants show a maximum stress tolerance in affected crops, giving protection to the plant. The trends observed in the present research might be due to the reasons discussed earlier.

6.2. Catalase

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen and catalyzes the decomposition of H_2O_2 to water and oxygen. The highest turnover by catalase molecule could convert millions of molecules of H_2O_2 to water and oxygen in each second. Hydrogen peroxide is a toxic by-product of many regular metabolic processes. It must be quickly converted into less toxic substances to prevent most cellular damage and tissue injuries.

6.3. Ascorbate peroxidase

Ascorbate peroxidases (APXs) are the enzymes that detoxify hydrogen peroxide using ascorbate as a substrate. It is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules. Higher plants produce active oxygen species during metabolic processes including mitochondrial, chloroplastic, and plasma membrane-linked electron transport system. Due to biotic and abiotic stress, conditions can give rise to excess concentration of these active oxygen species resulting in oxidative damage at a cellular level. At this stage, the antioxidant enzymes have to function to interrupt the cascades of uncontrolled oxidation in each organelle. Ascorbate peroxidase (APX) exists as isoenzymes that play an important role in the metabolism of H_2O_2 in higher plants. APX activities generally increase along with activities of other antioxidant enzymes like catalase, SOD, and GSH reductase in response to various environmental stress factors regulating the components of ROS-scavenging systems. APX has been identified in many higher plants and comprises a family of isoenzymes with different characteristics.

Photosynthetic organisms including higher plants and eukaryotic algae have developed AOS-scavenging systems, including APX isoenzymes. AOS-scavenging system also established in prokaryotic cyanobacteria has an H_2O_2 tolerance system of the Calvin cycle and an H_2O_2 diffusion system. The distinct regulatory mechanisms are expressed by APX isoenzymes in response to various environmental stresses or cell conditions and play a cooperative role to protect each organelle and minimize tissue injury. The action of antioxidant systems under drought has been investigated by many authors in several crops, such as spinach, pea, sorghum and sunflower, and wheat [39]. Richard et al. [40] have studied the responses to abiotic stresses and activities of superoxide dismutase, catalase, and peroxidase, as well as malondialdehyde (MDA) contents and solute potentials in seedlings of seven wheat (*Triticum*) species (nine genotypes representing three ploidy levels: hexaploid, tetraploid, and diploid) subjected to water stress for 4, 8, and 12 days by withholding water. In most species, the activities of superoxide dismutase and catalase showed an increase in the early phase of drought and then a decrease with further increase in water stress.

The enzymatic activities partly recovered and malondialdehyde contents decreased with re-watering. Under drought situation, hexaploid wheat had higher peroxidase activities and MDA contents than tetraploid and diploid wheat. The solute potentials and the activity of SOD and CAT were similar among three groups. Conventional breeding techniques have been unsuccessful in transferring the drought tolerance trait to the target species [2]. The basic biotechnology tools can be employed to manage stress tolerance, hence improving yield stability. Different genetic markers were identified as linked to different traits of interest to determine polymorphism among a variety of wheat genotypes. Richard et al. [40] have studied the responses of growth and primary metabolism of water-stressed barley roots to rehydration.

The assessment of the quantity of variety detected with microsatellite exposes additional polymorphism among different genotypes. The individuality and the value of microsatellites started their multi-allelic nature, co-dominant transmission, wide genome treatment, and requirement for a small amount of starting DNA. Genetic diversity among adapted cultivars or elite-breeding materials has a considerable impact on the improvement of crop plants. Molecular markers can determine genetic diversity from pedigree analysis or morphological traits, and they can offer the best estimate of genetic diversity since they are independent of the perplexing effects of environmental factors. Several molecular markers like RAPD and SSRs are available to assess the variability and diversity at a molecular level [41].

7. Conclusion

Drought stress causes osmotic stress in plants which causes reduction in growth, imbalance ion transport, and a decrease in transpiration rate and an increase in membrane permeability. Such effects result in less water-absorbing capacity of crop plants, and different plant species and genotypes within a species respond differently to adverse environmental conditions. In order to counteract unfavorable environmental conditions, plants accumulate different types of organic and inorganic solutes in cytosol to decrease osmotic potential by which they can maintain cell turgor. PEG induced severe stress in the selected wheat germplasm, and root-to-shoot ratio was enhanced in the drought-affected germplasm. The levels of antioxidant enzymes were enhanced significantly in all genotypes while glycine-betaine, proline, and trehalose have shown association and positive effects during drought stress. The safety and survival of the plants depends on the coordination of these vital osmoprotectants with antioxidant enzymes.

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