Chapter

Amelioration of Drought Stress on Plants under Biostimulant Sources

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Abstract

Water stress is one of the most important environmental factors inducing physiological changes in plants, such as decreasing water potential of the cells and the stomatal closure, resulting in reduced CO₂ availability for the plants and inhibiting photosynthesis. One common feature of these stress conditions is the development of oxidative processes mediated by reactive oxygen species (ROS). ROS accumulate in the cells and cause damage in important cellular components, such as thylakoids and chloroplasts. Plants have antioxidant defense systems to cope with ROS. Antioxidants enzymes superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) are efficient scavengers of ROS: superoxide, hydroxyl radicals, and singlet oxygen. The activities of antioxidant enzymes in plants are normally favored when plants are subjected to some kind of improvement in the conditions in which they are grown. In this sense, biostimulants cause changes in vital and structural processes in order to influence plant growth through improved tolerance to abiotic stresses by increasing the antioxidant activity in plants.

Keywords: reactive oxygen species, antioxidant enzymes, plant drought resistance, humic substances, seaweed extracts, hormones

1. Introduction

Water availability is one of the most important environmental factors for plant growth and development. The water deficit caused by drought or salinity in soils is one of the most serious environmental problems that limit agricultural production in various regions of the world. According to [1], water deficit occurs when all water content in the cell is below the highest water content displayed in the state of greatest hydration.

Plants experience a water deficit when water supply to the roots becomes difficult or when the rate of evapotranspiration becomes very high. These two conditions generally coincide in regions with an arid and semiarid climate and affect plants to a greater or lesser extent according to the tolerance that species have [2].

Plant response to biotic and abiotic stresses is a complex network of reactions, which involves different physiological pathways of the primary and secondary metabolism. At the cellular level, membranes and proteins can be damaged by a reduction in hydration and an increase in reactive oxygen species (ROS) [3]. ROS derive from oxidative processes such as photosynthesis and respiration, and, in normal conditions, they are produced in low concentration without any negative consequences for the plants. In stressful conditions (biotic or abiotic), ROS levels
increase as an index of the oxidative burst induced by the stress agent [4]. When ROS become toxic, they can result in a series of damages to plant metabolism, such as deterioration of photosynthetic components, inactivation of proteins and enzymes, and destruction of the structure and permeability of the cell membrane by lipid peroxidation [5, 6].

Antioxidants and their role in the plant defense system have received a lot of attention in scientific research. Many results suggest that the effects of environmental stresses, such as salinity, drought, low temperatures, and herbicide residues, damage plants directly or indirectly by increasing endogenous ROS [7].

Plant cells are protected against the damaging effects of ROS by a complex antioxidant system composed of enzymatic antioxidants, such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) [8]. The close relationship between antioxidant activity and stress tolerance has been identified in many crops such as maize (Zea mays L.) [7], tobacco (Nicotiana tabacum) [9], and grasses [10].

Biostimulants are extracts obtained from organic raw materials containing bioactive compounds. The most common components of the biostimulants are mineral elements, humic substances (HSS), vitamins, and amino acids [6]. Seaweed extracts have been used in agriculture as soil conditioners or as plant stimulators. They are applied as foliar spray and enhance plant growth; freezing, drought, and salt tolerance; photosynthetic activity; and resistance to fungi, bacteria, and virus, improving the yield and productivity of many crops [11, 12]. Seaweeds used for biostimulant production contain cytokinins and auxins or other hormone-like substances [13]. From a legal point of view, the biostimulants can contain traces of natural plant hormones, but their biological action should not be ascribed to them; otherwise they should be registered as plant growth regulators [6].

Humic acids have been used in the composition of many commercial products because they have phytohormones [14] that favor protection against oxidative damage in plants caused by environmental stresses. Thus, the use of biostimulants in agriculture has been emphasized, which are products that contain active ingredient or organic agent free of pesticides, capable of acting, directly or indirectly, on all or part of the cultivated plants, increasing their productivity [15].

The components of biostimulants can change the hormonal status of the plant and have a great influence on its development and health. Seaweed, humic acids, and vitamins are commonly present in biostimulants and are important in improving plant development and hormonal activity [16]. In addition, these products increase the antioxidant activity in plants, especially when they are under water stress, severe temperatures, and herbicide action, among others [7].

Several studies have shown results in improving the resistance of plants to water stress when subjected to the application of biostimulants. The activity levels of the antioxidant enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) have been determined. In general, increases in these antioxidant enzymes have been observed with the use of biostimulants [16]. Another parameter that has been improved in the plant with the application of biostimulants is the photochemical efficiency [17].

Thus, the objective of this chapter was to approach the role of biostimulants in plants submitted to water supply deficit, by affecting the activities of enzymatic antioxidants.

2. Use of biostimulants in plants

Biostimulants are components that produce responses in plant growth by improving tolerance to abiotic stresses. Many of the effects of these products are
based on their ability to influence the hormonal activity of plants. Phytohormones are chemical messengers that regulate the normal development of plants by growing roots and shoots, in addition to regulating responses to the environment where they are located [18].

Many statements about biostimulants also refer to the improvements they provide in the tolerance of plants to water stress, a limiting factor in the management of the crops. Water stress affects many metabolic functions in plants, specifically photosynthesis. The application of biostimulants increases the defense system of the plant by increasing its level of antioxidant enzymes [15].

The components of biostimulants can alter the plant’s hormonal status and have a major influence on its growth and health. Seaweed, humic acids and vitamins are commonly present in biostimulants and are important in improving plant development and hormonal activity [19]. In addition, these products increase the antioxidant activity in plants, especially when they are under water stress, severe temperatures and herbicide action, among others [20].

However, the composition of biostimulants is partly unknown; the complexity of the extracts and the wide range of molecules contained in the solution make it very difficult to understand which the most active compounds are. Moreover, the isolation and study of a single component present in a biostimulant can produce unreliable results because the effects on plants are often due to the combination and synergistic action of different compounds. In addition, the mechanisms activated by biostimulants are difficult to identify and still under investigation [6].

Plants usually thrive when the environment is favorable. Under these conditions, the effects of biostimulants may not be easily identified. However, when plants are stressed and undergo treatment with biostimulants, they develop better, as their defense system becomes more efficient due to the increase in their levels of antioxidants [20]. Besides, many of the active substances of biostimulants can be present in very low concentrations, sometimes below the levels detectable with commonly available technologies, but can provide strong biological effects [6].

Biostimulants and humic substances have shown an influence on many metabolic processes in plants, such as respiration, photosynthesis, synthesis of nucleic acids, and ion absorption. Within the cell, humic substances can increase the chlorophyll content resulting in greener leaves and reduction of some problems in plants, such as leaf chlorosis, since humic substances improve the capacity of nutrient uptake by the roots [21]. Beyond humic substances, various raw materials have been used in biostimulant compositions, such as hormones, algae extracts, and plant growth-promoting bacteria [22].

3. Water stress in plants

Water availability is one of the most limiting environmental factors that affect crop productivity. In the semiarid tropics, the occurrence of drought or water deficit in the soil is quite common, despite the fact that crops in regions of tropical and temperate climate suffer seasonal periods of water deficit, especially during the summer [23].

Drought is a prevalent stress factor especially in arid and semiarid areas and can affect different aspects of plant growth, development, and metabolism. Drought is a multidimensional stress factor, and hence its effects on plants are complex. Its effects on plants can occur on a molecular level up to a whole-plant level. There are several reasons for drought in nature, including low rainfall, salinity, high temperature, and high intensity of light, among others [24].
Some of the plants’ first responses to stress appear to be mediated by biophysical events, rather than changes in chemical reactions resulting from dehydration. The closing of stomata, the reduction of photosynthesis, and osmotic adjustments are the responses of some plants to the first stage of water deficit [25]. As the water content of the plant decreases, the cells shrink, and the cell walls relax. With this, the solutes increase their concentration in the cells, and the plasma membrane becomes thicker and more compressed, as it covered a smaller area than before [1]. Cell expansion occurs when the turgor pressure is greater than the growth of the cell wall. Water stress greatly decreases cell expansion and plant growth due to low turgor pressure [26].

Stomata provide the main mechanism for controlling the rate of water loss. However, the site of water loss is also the site of carbon gain by the plant, so a reduction in water loss by stomatal control also results in a reduction in assimilation with consequent effects on productivity and the accumulation of reactive oxygen species [27]. These responses hinder the supply of CO₂ for photosynthesis and expose chloroplasts to excess energy excitation, especially under high light intensity [25].

The low potentials in the soil and in the plant inhibit their growth, reduce the development activities of cells and tissues, decrease the uptake of nutrients, and cause morphological and biochemical changes [28]. To maintain water uptake, the roots have to grow deeper or increase their density. A characteristic of drought-resistant species is that they have a large proportion of their total mass consisting of roots and a deep-rooted habit. A high root/shoot ratio does not indicate in itself great ability to absorb water: water deficiency invariably increases the root/shoot ratio, but this is due to the loss of plant shoot weight without loss of root mass [1].

Photosynthesis is the driving force of plant productivity. The ability to maintain the rate of photosynthetic carbon dioxide and the assimilation of nitrate under environmental stresses is fundamental for the maintenance of plant growth and production. It is known that when water stress becomes extreme, non-stomatal factors can become even more limiting for photosynthesis [17].

The water deficit often decreases the number of photons captured by the leaves because withered leaves are at a more acute angle to the sun’s rays. Changes in the absorption characteristics of the leaves occur due to the shrinkage of the cells. However, changes in chloroplasts and thylakoid during light capture and energy transfer centers are relatively small under water deficit conditions [29].

### 3.1 Reactive oxygen species and water stress

The diatomic oxygen (O₂) molecules in the Earth’s atmosphere are the major promoters of reactions in cells. Except for those organisms that are specially adapted to live under anaerobic conditions, all animals and plants require oxygen for efficient energy production [30].

Aerobic organisms use diatomic oxygen as a terminal electron receptor, providing a high-energy field compared to fermentation and anaerobic respiration. In this base stage, molecular oxygen is relatively nonreactive, but it is capable of giving rise to excited reactive and lethal states, such as free radicals and their derivatives [31].

Superoxide, produced by electron transport to oxygen, is not compatible with cellular metabolism; hence, all organisms that are involved in aerobic environments must have an efficient mechanism capable of removing or neutralizing free radicals from cellular components. The balance between oxidative and antioxidant capabilities determines the fate of the plant [32]. Without this defense mechanism, plants may not efficiently convert solar energy into chemical energy [33].

The formation of reactive oxygen species occurs primarily through the superoxide radical (O₂⁻), which can be dismutated into hydrogen peroxide (H₂O₂), or even through catalytic action, by the action of the superoxide dismutase (SOD) enzyme.
Antioxidant systems in plants act as mechanisms of resistance to stress by protecting the membranes against damage caused by these oxygen species produced under conditions of environmental and xenobiotic stress [34].

The fate of cells under stressful environments is determined by the duration of the stress, as well as the plant’s protective capacity. Reactive oxygen species (ROS) play a crucial role in causing cellular damage to plants under stress. The sequence of events in plant tissues subjected to stress is increased production of ROS; increased levels of antioxidants; and increase in the capacity to “sweep” ROS, resulting in the plant’s tolerance against water stress [35].

The detoxification mechanisms of ROS exist in all plants and can be categorized into enzymatic (superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX, among others) and nonenzymatic (carotenoids, ascorbic acid, among others). The degree to which the amount and activities of antioxidant enzymes increases under water stress is extremely variable between many plant species and even between two cultivars of the same species. The level of response depends on the species, the development of the plant, as well as the duration and intensity of the stress [35].

The superoxide produced by the thylakoid can spontaneously be dismutated into molecular oxygen and hydrogen peroxide. In chloroplasts, this reaction is catalyzed enzymatically via superoxide dismutase (SOD). Chloroplasts also contain large amounts of ascorbic acid, which can efficiently reduce superoxide to hydrogen peroxide via ascorbate peroxidase [4].

Plants have the superoxide dismutase enzyme containing Cu and Zn, Fe, or Mn as prosthetic metals. Zn is found in superoxide dismutase present in chloroplasts and cytosol, while Mn is found in superoxide dismutase in mitochondria and Fe in superoxide dismutase is present in chloroplasts and mitochondria [36].

Reactive oxygen species can react with unsaturated fatty acids, causing the peroxidation of essential lipid membranes in plasmalemma or intracellular organelles [33]. The damage caused by the peroxidation of plasmalemma leads to extravasation of cellular content and rapid dissection and cell death. The damaged intracellular membrane affects the respiratory activity in the mitochondria, in addition to depigmentation and loss of the ability to fix carbon in chloroplasts [34].

Under normal conditions, antioxidant systems eliminate or slow the reaction of reactive oxygen, preventing its transformation into products more toxic to cells. Photosynthetic cells can tolerate high levels of oxygen because endogenous mechanisms sweep and remove toxic products before cell damage occurs [32]. However, oxidative damage is evident under conditions where the rate of production of ROS is high and the removal ability is low [37].

Water stress conditions can trigger an increase in the production of various forms of reactive oxygen, which can explain the damage to chloroplasts, lipids, and proteins and the alteration of the structural integrity of cell membranes. During the reduction of water inside the plant, the superoxide radical (O₂⁻) can also react nonenzymatically with hydrogen peroxide (H₂O₂), giving rise to products such as hydroxyl radicals (OH⁻) and singlet oxygen (¹O₂), which are more reactive than the superoxide radical (O₂⁻) [32].

Although a number of regulatory mechanisms have been evolved within the plant cell to limit the production of these toxic molecules, oxidative damage remains a potential problem, as it causes disturbances in metabolism, such as loss of coordination between production processes (source) and energy use (drain) during photosynthesis on green leaves under stressful environments [38].

When plants are under stress, free radicals or ROS damage plant cells, and antioxidants decrease the toxicity of these radicals. Plants with high levels of antioxidants produce better root and shoot growth, maintaining a high water content in
the leaves and low incidence of disease, both occurring when they are under ideal growing conditions and under environmental stress [18].

### 3.2 Biostimulants and reactive oxygen species

The use of biostimulants in plant breeding could change the activity of enzymes and antioxidant properties. Lycopene, ascorbic acid, phenolic compounds, and others have antioxidant properties. Antioxidant compounds (e.g., phenols, ascorbic acid) and enzymes (e.g., catalase, peroxidase, superoxide dismutase) detoxify reactive oxygen molecules [20].

Biostimulants stimulate root production and growth when applied to seeds or early plant development, especially in soils with low fertility and low water availability. Biostimulants act in accelerating the recovery of the seedlings in unfavorable conditions, such as water deficit. In addition, biostimulants reduce the need of fertilizers to the plants and increase their productivity and resistance to water stress, since they act as a hormonal and nutritional increment [15].

The application of humic acid extracts seems to be beneficial for field crop monocots. In a study conducted by [39], extracts from vermicompost applied to rice (*Oryza sativa* L.) played a role in activating antioxidative enzymatic function and increased ROS-scavenging enzymes. These enzymes are required to inactivate toxic-free oxygen radicals produced in plants under drought stress. Humic acid extracts may stimulate plant growth by improving nutrient uptake by exerting hormone-like effects as auxins, stimulating shoot elongation and increasing leaf nutrient accumulation and chlorophyll biosynthesis [40].

According to [41], humic acids improve root and shoot growth by increasing the concentrations of antioxidants in tall fescue (*Festuca arundinacea*) and creeping bent grass (*Agrostis palustris*) grown under conditions of low water availability. The authors also claim that exogenous applications of seaweed extracts together with humic acids promote root and shoot growth through the action of antioxidants in plants under water stress conditions.

A study carried out using a biostimulant based on salicylic acid and chitosan nanoparticles had an effect on the enzyme and antioxidant activity in maize leaves under water shortage [42]. The enzyme activity in leaves treated with chitosan, salicylic acid, and a control was comparable, and the activity of superoxide dismutase and peroxidase activity in plants treated with a biostimulant was 7.7 (after 2 days) and 5.2 (after 3 days) times higher than for plants treated with only salicylic acid.

The activities of antioxidant enzymes in plants are normally favored when plants are subjected to some kind of improvement in the conditions in which they are grown. The superoxide dismutase (SOD) antioxidant enzyme is the first line of defense against ROS caused by environmental stresses. Increases in SOD values provide an increase in plant resistance when subjected to environmental stresses [43].

In an experiment with Kentucky bluegrass (*Poa pratensis*) subjected to water stress and humic acid applications, [44] observed an increase in superoxide dismutase activities related to the applied doses of humic acids. However, a decrease in the activity of superoxide dismutase related to soil moisture content was observed. The authors justify this decrease by the increase in nonenzymatic antioxidants favored by the action of humic acids, which caused a decrease in the reactive oxygen species present in the cells.

The activity of superoxide dismutase responds differently to water deficit in different experiments and species: it can be increased [45] or decreased [46], or it cannot be altered [45]. Due to the presence of multiple enzymatic forms of the superoxide dismutase enzyme [33], only the investigation of the responses of each
of its enzymatic forms can provide more information about the behavior of this enzyme in plants subjected to water stress.

Some authors mention that catalase activity has little affinity for hydrogen peroxide, a reason why it is common not to have a significant increase in its activity when evaluated in plants under stress [7]. [47] examined the activity of catalase in rice seedlings (Oryza sativa) under water stress and found that the increase of this enzyme in plants was not significant. Likewise, [48] did not find a significant increase for catalase in tomato plants (Lycopersicon esculentum Mill. cv. Nikita) submitted to three different levels of water stress. However, the extract of Moringa oleifera used as a biostimulant in rocket plants (Eruca vesicaria subsp. sativa) under water stress presented a decrease in the activity of the antioxidant enzymes (catalase, peroxidase, and superoxide dismutase) [49].

Several seaweed species influence ROS-scavenging systems in the plant tissue. Seaweed extracts controlled oxidative stress under drought conditions, by reducing lipid peroxidation, increasing total phenolic content, and enhancing superoxide dismutase, catalase, and ascorbate peroxidase activity in green bean (Phaseolus vulgaris) [50]. Extracts from Sargassum and Ulva, applied as seed presoaking, activated antioxidant systems by enhancing catalase and peroxidase activities, increasing ascorbic acid content, and therefore alleviating stress symptoms in wheat grown under drought conditions [51]. Ascophyllum nodosum extract applied to roots increased the total phenolic and flavonoid content and total antioxidant activity in spinach (Spinacia oleracea) [52]. In tall fescue (Festuca arundinacea), A. nodosum extract increased the activity of superoxide dismutase and in another study additionally enhanced glutathione reductase and ascorbate peroxidase activities [36]. Similarly applied seaweed extract increased the antioxidant capacity and enhanced flavonoid and tannin content in plant leaves of the ornamental hybrid Calibrachoa x hybrida under normal conditions [53].

Seaweed extracts have also been applied in combination with other compounds to enhance antioxidant activity in plants under water stress, such as a mixture of seaweed extracts from A. nodosum, Fucus spp., and Laminaria spp. with zinc and manganese and A. nodosum extract with free amino acids. These mixtures increased superoxide dismutase activity in shoots and roots of maize (Zea mays) and soybean (Glycine max). Collectively, these studies demonstrate that seaweed extracts enhance antioxidant activity, indicating their potential to scavenge damaging ROS molecules and improve plant stress tolerance [54].

Humic acids have also been shown to alleviate water deficit stress. Faba bean (Vicia faba) plants were protected from lead-induced oxidative damage by fulvic acids, which reduced lipid peroxidation, hydrogen peroxide, and pigment content [55]. The foliar application of fulvic acid ameliorated drought stress symptoms of reduced chlorophyll content, gas exchange, and yield while enhancing activities of superoxide dismutase, peroxidase, and catalase and increasing proline content in a study with maize [56]. Humic and fulvic acid based biostimulants, applied to the soil, enhanced superoxide dismutase, ascorbate peroxidase, and catalase activities in leaves of maize grown under well-watered and drought conditions. However, the effect of these biostimulants was less pronounced in soybeans [7].

Humic substances can also increase activity of antioxidant enzymes. Activity of superoxide dismutase, peroxidase, and catalase was higher after foliar application of fulvic acid in maize grown under drought conditions. Biostimulant containing humic and fulvic acids and amino acids increased activity of antioxidant enzymes, specifically superoxide dismutase and ascorbate peroxidase in maize subjected to drought stress, but did not affect catalase activity [7].
4. Conclusions

The composition of biostimulants should present a variety of organic materials such as humic substances, seaweed extracts, organic matter, and amino acids in order to improve stress tolerance. The literature on biostimulants have been reporting an increase in enzyme activities involved in antioxidant functions, especially under stress conditions.

Investigations on the role of biostimulants in the physiological mode of action in plants subjected to drought stress should be continued, since considerable researches remain to be completed to gain a clearer understanding of how these products increase the physiological health of plants under water stress.
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