Enzymatic Regulation of Drought and Heat Stresses in Maize (Zea mays L.)

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Abstract. Unfavorable environmental conditions, whether towards increase or decrease direction, are a general feature of our planet ecosystem. Stress conditions fall into two categories, biotic including insects and diseases and abiotic including drought, salts, temperature, etc. Drought is described the most limiting factor that determine crop productivity, and under certain condition drought damages cannot be avoided. Plant have evolved a wide range of mechanisms to cope with extreme environmental conditions. However, most of these strategies depend partially or completely on antioxidant defense system through which plants can control the cell content of reactive oxygen and nitrogen species (ROS and RNS). There should be more attention to climate change, not only by developing tolerant species, but also to natural disasters that can be devastating, as happening nowadays.

1. Introduction

Field crops, including maize, suffer from extreme climatic changes, such as high temperatures, water shortage and poor quality, thus the local crop productivity remains low compared to the global production. In the same context, drought phenomenon became more complicated due to the increased risk of global warming, lack of precipitation [1]. Water deficient is a key environmental stresses affecting the productivity and distribution of field crops because of its direct and indirect impact on the physiological and morphological processes that control the growth and plant final yield [2].

Heat stress generally refers to an increase in temperature beyond a critical threshold proper for each plant type for a period of time sufficient to cause irreversible damage to plant growth and development. Being an important cereal crop with a wide ecological range, the yield of maize varies depending on the conditions of the growing season, especially at temperatures above 30°C [2, 3]. When temperature exceed the plant ability to cope with via tolerance and adaptation, many changes occur at the molecular, cellular and physiological levels, which can have negative effects on growth and development and may lead to plant death [4]. However, the effects of high temperature depends on the temperature degree, duration of exposure, plant type and the accompanying stresses (light intensity, drought and salinity), as well as the type of exposure being gradual or sudden, resulting in heat shock [5]. A wide range of molecular activities are affected in response to different environmental stresses. Water channel proteins (WCPs), stress-responsive proteins, transcription factors and cellular signaling pathways are common molecular events contribute to stress in general and dehydration in particular. Such molecules confer plant tolerance by protecting cellular components and regulating gene expression. Stress proteins and water channel proteins are water-soluble that have a crucial
role in drought tolerance, so they give endurance by holding water to cellular contents (Hydration)[6]. Under conditions of water scarcity and high temperatures, many physiological and biochemical changes occur in plants, like photosynthesis, electron transport, and the activity of enzymes. These changes cause excessive production of free radicals (O₂⁻, H₂O₂, Ho, O⁻²) which drive oxidative stress to a higher level that destroys cell components such as nucleic acids, proteins, fats, etc. [7].

Plants are distinguished from other living organisms as they are sessile, therefore they have to adapt to fluctuate environmental conditions. Alternatively, plants have an active defense system against inappropriate environmental conditions, represented by the production of both enzymatic and non-enzymatic antioxidants that suppress the harmful effect of free radicals [8]. The water requirements of maize are reduced in the early stages of growth and gradually increased until reaching their maximum at the reproductive stage, and then begin to decrease again when the crop approaches the final stage of maturity. Maize during the flowering stage usually needs 8-9 mm of water per plant. The most important period in the life cycle of maize is four critical weeks, two weeks before pollination and two weeks after pollination. Being a drought-sensitive crop, maize is affected in all stages of its growth by deficient moisture.

The cessation of metabolic processes followed by the death of the plant resulting from closing the stomata and inhibiting gas exchange occurs in response to the plant's exposure to long periods of moderate drought. Nevertheless, the reproductive stage is the most sensitive stage to drought stress [9,10].

2. Abiotic stresses

Plant species are the main source for feeding human and animals. Considering survival ability, animal species have the ability to change position to avoid threats or unsuitable growth environment, on other side, plants have no such ability simply because they are sessile. That is why, plants need to respond to the surrounding environment via activating certain mechanisms to cope with stressful conditions [11,12]. During their lifetime, plants expose to different environment fluctuations classified into biotic like, diseases and insects and abiotic such as drought, salinity, extreme heat, plant density, pollution and lack of nutrients. These stresses vary and affect significantly during the stages of plant growth and development. Molecularly, stressful conditions could contribute to a wide range of cellular functions like fine tuning of gene expression, transcription rate, protein stability, and ion exchange [13, 14, 15].

Most of these modifications act as a signal triggering specific pathways on specific time in response to different fluctuate environmental conditions via either one of two approaches; activating existing pathway or developing novel approach of response [16]. Plants are sensing biotic or abiotic stresses; however their reacting occurs on different levels. For example, in physical sensing, the contraction of plasma membrane is a common symptom revealed by drought stressed plants. Whereas, re-structured proteins and activation/deactivation of some enzymes considered the main mechanism that plant developed to cope with heat stress through biophysical sensing. During metabolic sensing, the accumulation of byproducts like ROS is detected in high light intensity. Contrarily, the biochemical sensing of heat stress involves special proteins of calcium channels that detect alteration in Ca²⁺ balance. A higher level of stress sense represents by epigenetic sensing, in which DNA and/or RNA is modified without altering base sequence including DNA methylation, histone modification, chromatin remodeling and siRNA. [17, 16]. The described stress-sensing mechanisms can work individually or synergistically to activate signal pathways which in turn induce plant cells to trigger stress-induced defense system helping plant to survive.

2.1. Drought

The challenge of global warming is magnified over the last decade, which portends major climatic transformations that may exceed the ability of many living organisms to cope with,
especially plants. Predictions are clearly pointed to significant changes in the precipitation reflecting in more frequent drought and floods, increase CO₂ and atmospheric temperature, leaching of soil nutrients and a decrease in the availability of fresh water [18]. With imminent global weather change and the urgent need to provide food for the growing populations in the near future, awareness and better understand to the consequences of climate change should be considered especially within long-term strategic plans to preserve crops productivity [19].

Systems of biological stresses involve identifying metabolic pathways of stress signaling, norm of gene action control tolerance responses, and in engineering and breeding more efficiently and improving new crop varieties for adaptable arid and semi-arid regions. The search for the adaptive capacity of crops by increasing their ability to tolerate stress must be expanded to new heights [20]. Responses to drought stress vary with respect to the type of plants involved or dehydration intensity and duration. Plants have three strategies by which plants can grow and develop normally even in water-restricted environments. First, escape drought, which can be witnessed in short season plants where plants complete their production cycle in a relatively short time and before the water shortage becomes severe. Secondly, avoid dehydration, which reduces for example, transpiration or increases water absorption. Third, drought tolerance that involves some protoplasmic tolerance [21, 22, 23].

Longstanding exposure to drought stress hampers growth and leads to great losses in plant production. Although many drought-responsive genes have been recognized and functionally investigated, the mechanisms laying behind responses to drought treatments and water restoration have not been completely explained [24]. Recently, drought-related losses approved to be the main challenge facing grain production. In the last few decades, drought events have become more frequent and stronger and are probably linked to global warming, and off course weather shifting. In respect of weather predictions, this situation tends to get worse, hence the availability of irrigation water is expected to diminish. However, the demand for food is growing expressively that it will be necessary to provide more water for agricultural activities. Regarding this, water use (consumption) and water use efficiency (Water Use Efficiency (WUE) that is, the amount of water consumed to produce a certain amount of biomass) are basic parameters in water-affected areas. In fact, modern agriculture strategies focus on achieving higher crop production combined with less water consumption (“more crops per drop”). In a broad sense, drought is a multidimensional phenomenon, including water deductions not only in the soil but also in the atmosphere [25, 26].

2.2. Heat stress

Global agricultural production is greatly affected by the availability of growth factors such as water, nutrients, light, heat, and etc. Each crop has optimal levels of those inputs in its growth and thus its productivity are negatively affected when the availability of one or more growth factors is below or above those levels, with different Relative to plant species. Accordingly, crop types show a wide range of response to inappropriate growing conditions, and this is determined in principle by their genetic ability to absorb the change in the level of the growth factor during one growing season and between successive seasons such as temperature, which is known as adaptation. There is no doubt that the least that can be described by all growth factors is that they are necessary for the normal growth and development of the plant to complete its life cycle. Unfortunately, human activity deliberately or accidentally caused climate change that was fatal at times for many types of living organisms and threatened their existence on the surface of the globe.

Global warming or global warming is one of the most important features of human activity and its negative impact on the environment. Climate scientists generally agree that a doubling of carbon dioxide levels since the start of the Industrial Revolution will lead to a 5°C increase in Earth's average temperature over the next half century, with significant climate consequences. The average temperature has increased in more than one region of the world, and the last decade was the hottest during the past 1000 years, and 1998 was the warmest
among them [27, 28]. The effects of global warming may not be limited to an increase in average annual temperatures in more than one place in the world, as there will be a significant increase in both the number of high heat waves and the rate of temperature per wave leading to more extreme hot and cold days, which will have profound effects on Natural and agricultural systems.

Plants have a limited ability to adapt to extreme temperatures, and a long-term adaptation will have a pronounced effect on the growth volume and the biomass of plant communities [29, 30, 31], with a relative difference in the timing of emergence Heat stress symptoms according to plant species and their natural growth environment, as when comparing the response of desert plants and that of temperate plants [32]. Temperature is gaining importance as a physical factor that determines the environment and distribution of living organisms as a result of its direct influence on the molecular structure of BIO components such as DNA, proteins, carbohydrates and lipids, or supramolecular such as membranes and chromosomes [33, 34]. Cell organelles display a rapid response to change in ambient temperature, and may involve alteration of biochemical and molecular pathways in all parts of the cells and the formation of an integrated cellular response [33, 35].

3. Stress and gene expression

The negative impact of environmental factors (biotic and abiotic) on plant development and growth ending with the yield and its components, as quantity or a type known collectively as stress [36]. The pattern of plant response to stress conditions that occurs simultaneously and automatically is what gives the plant cell the opportunity to survive, by adapting gene expression to accommodate the change in the growth environment [37]. As the optimal regulation of gene expression provides the plant with a response appropriate to the intensity and duration of stress, and here the different molecular response mechanisms overlap through a network of molecular signals within what is known as “crosstalk”, in which different cells, tissues and organs participate, and heat shock proteins play and regulate the flow of kinase cascades, regulate Transcription Factors (TFs), and small RNAs play a pivotal role, as well as the overproduction of reactive oxygen species (ROS) [38, 39]. Because of the importance of ROS as secondary carriers of cellular signals, its overproduction under the influence of environmental stress leads to a disturbance in the growth of tissues and organs, so it must be regulated by specialized metabolic pathways, and to prevent reaching the stage of DNA damage and entering into a programmed cell death (PCD) [40, 41]. Reactive oxygen species are formed naturally by aerobic metabolic processes such as respiration and photosynthesis, so the major ROS aggregates are produced in plastids, mitochondria, plasma membranes, and apoplasts [42, 43, 44].

The molecular mechanisms through which plants respond to stress conditions in general and to drought and high temperature in particular have not been adequately explained, and many TFs, which regulate the action of genes responsible for the development of the response, have been studied [45]. According to their response to drought conditions, genes are classified into two main groups, which included early response genes and delayed response genes. Within a few minutes, early response genes induced and encode transcription factors that in turn induce and regulate late response genes (AREB, ABF, CBF/DREB, AtMyb, and RD22BP) are induced shortly after plant stress, usually a large group of genes [10].

3.1. Molecular regulation of oxidative stress

When plants are exposed to instant stress, the cell must keep the concentration of reactive oxygen species (ROS) under control, and prevent their accumulation at high levels because this will lead to the destruction of the cell and accelerate the entry of the plant into the aging stage. The rate of ROS accumulation in the plant is often detected by measuring some of the symptoms associated with its increased accumulation, such as increased respiration rate, energy fraction consumption, loss of intracellular ionic balance, or increased rate of lipid oxidation [46, 47]. Therefore, the activation of the genes that encode the enzymes to remove
effective oxygen species is an important indicator to show the efficiency of plants in resisting oxidative stress resulting from exposure to environmental stress in general, and water stress in particular. Thus, increasing the enzymatic activity or increasing the activity of certain genes or both is evidence of the ability of plants to resist oxidative stress. The results of some studies indicate a decrease in the activity of ROS enzymes, while others indicate an increase during exposure to stress conditions, bearing in mind that the genetic or enzymatic response occurs during the response of the plant as a whole [48, 49].

Plants vary in their ability to grow and adapt to stress conditions as a result of genetic, physiological and phenotypic differences that give them different mechanisms to overcome unsuitable growth conditions, as in saline and desert plants. However, most of these mechanisms are usually accompanied by a positive improvement in the ability of plants to get rid of ROS compounds, and this indicates an increase in the activity of enzymes and genes necessary for this. When plants fail to absorb the change in the surrounding environment and continue to grow, this is due to two reasons, the first is the increase in the intensity of stresses so that it exceeds the plants’ ability to endure, and the second reason is that plants by their nature do not bear stresses as most plants are classified as medium endurance, and the plants have in this case, the activity of the removal enzymes and the activity of the genes decrease, not because the plants do not need them, but because they are no longer able to control them, to the degree that the removal enzymes themselves are damaged by stress as the rest of the enzymes that run the vital system of the plant [47, 50, 51].

3.2. Enzymatic activity in the regulation of oxidative stress

If the formation of ROS compounds (superoxide, hydrogen peroxide, and hydroxyl radical) is inevitable, plants in turn have several mechanisms to control the levels of these compounds so that they do not reach the degree of toxicity in cells and protect their living systems. This system consists of a group of enzymatic and non-enzymatic components that work to remove, suppress or scavenging active oxygen species also it may protect membranes such as the chloroplast membrane by precise processes. This scavenging system includes superoxide dismutase, catalase, peroxidase and etc [52].

3.2.1. Superoxide dismutases (SODs)

Superoxide dismutase is the key of enzymatic antioxidants. It is widely found in plants, animals and microorganisms and actively participates in many physiological processes against various adverse conditions. SODs form the front line of defense against injuries caused by reactive oxygen species (ROS) as well as suppress free radicals produced during stressful conditions (O2-).

Superoxide radicals has a major role in the plant protective system because their molecules are impermeable in the plasma membrane, thus, it is necessary to have active SOD to cure the damage of oxidative molecules and turn them into water and get rid of its toxic effect [53]. Mostly, SODs are found in different cell organelles such as chloroplasts, mitochondria, peroxisomes, cytoplasm, nucleus and cavities between the outer membranes. This explains the presence of SOD in all parts of the cell, in the form of four molecular forms depending on the accompanying mineral (CO-Factor). The first form is Mn-SOD, the second form is Cu / Zn-SOD, while the third form includes Fe-SOD, and the fourth form includes Nickel SOD. These minerals are often found in cereals and legumes, all of these forms are distributed unevenly in the biological kingdoms and located in different cellular compartments. In general, Mn-SOD is found in mitochondria and peroxisome, while Fe-SOD is found in chloroplasts, while Cu/Zn-SOD is found in chloroplasts and cytoplasm and may be outside the cell. Nickel SOD is found in the lumen between cell membranes [54].

Mn-SOD and Fe-SOD appear to be highly homologous in both sequence and structure, while Cu/Zn-SOD has no homology to Mn-SOD or Fe-SOD. SODs play a central role in the protection against oxidative damage, because SODs can catalyze the decomposition of O2-.
into hydrogen peroxide or hydrogen peroxide (H₂O₂) and oxygen (O₂), and serve to extinguish the oxidizing power of the superoxide, since O₂⁻ is a relatively medium-reactive radical. It is quickly formed in the cell, but this does not end the threat, as it destroys an effective cleavage and produces another, which quickly attacks proteins that contain thiol groups, so other enzymes are needed to break down hydrogen peroxide [55].

### 3.2.2. Catalase

During photorespiration, hydrogen peroxide is broken down by an enzyme called catalase (CAT). Catalase is present in plant tissues and cells, especially the peroxisome, while all enzymatic and non-enzymatic antioxidants are present within different cell organelles [56]. CAT enzyme works to oxidize the cell and remove the cytotoxic effect. In one second this enzyme can convert 83,000 molecules of hydrogen peroxide into water (H₂O) and O₂. When the concentrations of the bacterial enzyme catalase increase, the ability of plants to tolerate intense light increases when they exposed to drought stress [57, 58]. During the process of photorespiration, H₂O₂ is transferred from the plastids to the peroxisome, due to the presence of the CAT enzyme in the peroxisome, so it breaks down hydrogen peroxide without any catalyst in the peroxisome because it is more available than the plastids, so the peroxisome is more resistant than the plastids [59]. Peroxisomes play vital roles in multiple aspects of plant life by trapping diverse oxidative reactions and increasing the efficiency of photosynthesis and detoxification of ROS. Oxidative pathways present in peroxisomes include fatty acid β-oxidation, which contributes to embryogenesis and plant seed growth [60].

### 3.2.3. Peroxidase (POD)

The other part of the antioxidant enzymes is the peroxidase (POD), which is present in all parts of the plant and works to break down hydrogen peroxide and convert it to H₂O and O₂. In addition to the role of POD in analyzing H₂O₂ and scavenging free radicals, many studies have found that it has other vital functions in the plant life cycle, such as building lignin, which works to strengthen the cell wall and transform cell walls into suberization (corky tissue), [61]. There are other types of peroxidases such as (GP) glutathione peroxidase and (APX) ascorbate peroxidase found inside the plant working to transfer the oxidizing force to a receptor of H₂O₂ and these types break down hydrogen peroxide and turn it into dehydro ascorbate and water, the action of POD is not limited to scavenging free radicals ROS only, but works to control the levels of free radicals and make the ROS build the cell wall [62]. Hassan and Mansoor [63] found significant differences in the activity of the POD enzyme when exposing the plant to the stress of heavy minerals such as cadmium and lead.

### 3.3. The effect of abiotic stress on the viability of reproductive parts

Because the root is anchored, plants can tolerate various inappropriate environmental conditions that negatively affect growth and development [64]. Among the environmental stresses, drought is on the top of abiotic stresses list that eventually leads to a significant loss in crop yield. Maize is a widely cultivated food crop around the world. In the coming years, an increase in its production may be expected to match that of wheat and rice. However, maize productivity is sharply declined through water scarcity conditions. Maize growth stages can be divided into the seed growth, vegetative growth, flowering stage in which the vegetative growth stop and the grain develop and the maturity stage.

During drought stress plants, plant growth is restricted, extending of vegetative growth, root reorientation and alteration of carbohydrate distribution [65]. Short-term water shortage causes a loss of 28-32% of the dry weight during the rapid vegetative growth, while in the flowering phase and ear formation, the loss is magnified up to 66-93% of the dry weight [66]. In many occasions, heat stress is combined with drought stress which create huge pressure on normal plant development particularly in the critical stage of pollination and fertilization. Also, lack of water and the high frequency of ovary abnormalities in the fertilized ear leads to
develop empty rows or deformed seeds. After flowering, maize plants will show a rapid and steady increase in the accumulation of nutrients and dry weight, which continues in reproductive stage [67]. Additionally, prolonged drought (21 days or more) during the pre-flowering phase has been shown to reduce the terminal sizes of some leaves and internodes, delay the emergence of (male) and (female) inflorescences, and cause yield losses estimated at 15 to 25% [68]. During the pollination stage, maize grain formation of parental inbred lines and their hybrids is determined at least or perhaps as early as the stage of silk formation, that is why the grains number will significantly affected. When the plant reaches the stage of pollination and is exposed to water stress estimated at five days, it will produce abnormal embryos, which ultimately affects the plant yield in terms of the ears number, grain weight and grains number per a row.

Basically, a person may notice the presence of a group of poor quality grains in areas apical cornice [10]. Ear leaf may contribute significantly to biomass accumulation due to photosynthesis. When the photosynthesis process is completed, the grains are formed to a large extent by the leaves located above or below the ear. Finally, drought stress in plants leads to a significant decrease in the rate of photosynthesis, which hinders the growth by reducing the source size [69].

4. Conclusion
Plants are exposed to various stresses throughout their lifecycle. Some of these stresses are not avoidable. However, other stress types can be avoided through a specific strategies evolved by plant species on different phenotypic, physiologic and molecular levels.

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