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Drought Stress in Citrus and Its Related Genera : A Review

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ABSTRACT

Citrus is one of the major world horticultural commodities grown in semiarid conditions and is affected by biotic as well as abiotic stress including drought, extreme temperature, salinity, citrus canker, citrus tristeza virus, and Huanglongbing (citrus greening). Drought is one of the most disparaging challenges that negatively affects the growth and productivity of crops and can be detrimental even at mild levels. Drought affects the productivity by posing negative impact on the morpho- physiological parameters viz., relative water content, shoot length along with total soluble proteins and photosynthetic pigments (Chlorophyll and carotenoid). Drought also leads to enhanced production of reactive oxygen species (ROS) that is generally scavenged through the antioxidative defense system of citrus plants. Besides, several studies on the response of *Citrus* sp. towards drought stress, no proper defense system involvement has been elucidated. The understanding of citrus response towards drought and other harsh environmental circumstances relies heavily on the stress alleviation mechanism studies and intervention of biotechnological tools.

Keywords Abiotic stress, Citrus, Drought stress, Defense mechanism, Stress alleviation.

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INTRODUCTION

With the intimidating climate change conditions, drought and heat stress has become one of the most escalating deleterious abiotic factors impairing growth, development, crop productivity, water relations and water use efficiency in plants (Ziogas *et al.* 2021). Severe drought can have an adverse effect on plant growth, physiology, and reproduction resulting in significant reduction in crop productivity. It has been estimated that up to 45% of world agricultural lands are subjected to drought. Drought stress gives negative impact on most of the physiological processes and significant yield losses have been reported in many crops (Fahad *et al.* 2017). Duration and severity of drought condition have significant negative impact on the reproductive phase of plants. The fruit growth and flowering stages are the most critical period in relation to water deficiency and yield losses, whereas, fruit quality and yield reduction mostly depends on the duration and severity of the stress (Syvertsen and Garcia 2013). Moderate drought condition may lead to growth retardation, inferior fruit quality and low juice content in fruit crops (Carr 2012).

Citrus, a member of family Rutaceae are evergreen sub-tropical trees which can grow in humid and sub-humid regions of tropical, subtropical and temperate regions of the world (Khonglah *et al.* 2019). The genus includes many commercially important fruits viz., Mandarin (*Citrus reticulata*), sweet orange (*Citrus sinensis)*, grapefruit (*Citrus paradisi*), Lemon (*Citrus limon*), and lime (*Citrus aurantifolia*) (Hynniewta *et al.* 2014). Citrus is one of the major world horticultural commodity, and to maintain good economic production heat stress and water deficit problem must be avoided. The water scarcity not only affects the overall growth of tress and fruit quality but also affects the post harvest handling due to reduced reed thickness, resulting in economic loss due to handling and transportation damage (Carr 2012). However, the physiological and biochemical responses of citrus against drought stress has not been well studied, thus it is of paramount importance to understand the underlying biochemical and physiological principles. The scope of this review is to strap up the physiological and biochemical mechanisms involved in drought stress response of citrus as well as to understand the effect, mechanism and alleviating defense mechanism of citrus against drought stress.

Effect of drought on citrus production

Various effects on plants due to drought stress is given below in (Fig. 1). Scarcity of water is a severe limitation which encounters several cultivation problems in citrus (Gimeno *et al.* 2014). Drought stress poses severe negative impact on growth in length, dry and fresh weight of shoots and roots, tissue water content and germination percentage (Zaher-Ara *et al.* 2016) leading to direct impact on crop productivity.

Leaf relative water content (RWC) is an imperative marker of water status in plants that indicates water supply and transpiration rate balance within the plant system. Drought stress has a direct impact on the water retention potential and relative water content of plant (Sarkar *et al.* 2016). Drought tress induced injury in plants leads to reduction of relative water content in leaves (Zandalinas *et al.* 2016). A decline in leaf relative water content results in degradation of chlorophyll, impairment of LHC (light harvesting complex) function, enhanced ROS production and accumulation of osmoprotectant such as proline (Hayat *et al.* 2012). Water stress condition has negative impact on canopy growth, root and shoot growth. However, inhibition of root growth by water stress is also a common feature. The drought signal in plants is first perceived and transmitted by roots, resulting in adjustment of ethylene level and enhanced production of ABA in roots and leaves (Syvertsen and Garcia 2013). The enhanced ethylene and ABA level in leaves result in reduction of transpiration rate provoking the leaf abscission (Syvertsen and Garcia, 2013). The reduced transpiration rate due to stomatal closure also affects the photosynthetic process and hence results in reduction of productivity. In field-grown trees, leaf abscission is also associated with leaf water potential. Severe water stress results in destruction of photosynthetic apparatus leading to reduction of chlorophyll a and chlorophyll b content that negatively affects the photosynthetic rate and hence crop production. Ion transporters are associated with water loss-derived secondary stresses and several developmental processes including embryogenesis and fruit development involve cellular dehydration. Hence, the drought stress imposes a heavy negative impact on production of citrus.

Drought tolerance mechanism in citrus

On imposition of drought the plants directly affects rates of photosynthesis due to less availability of Carbon dioxide as stomata closes under drought stress to conserve water and impaired the plant growth . An increase in leaf soluble sugar and sucrose content and decrease in starch content is observed during water stress. Sucrose is the main photosynthetic product in plants and is involved in various abiotic stresses responses and helps plant growth and development under adverse stress conditions (Yanli *et al*. 2020). Plants accumulate osmolytes like proline which is the most common compatible osmolytes under drought

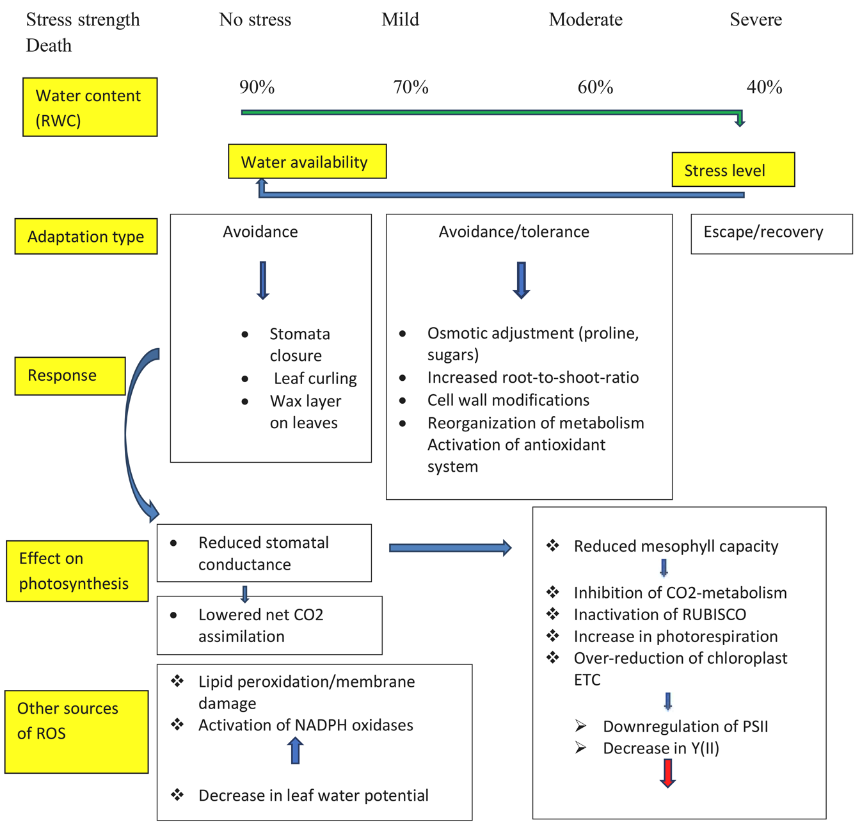

Fig. 1.. Drought stress response mechanism.

stress. Plants also started accumulating proteins under drought stress to cope up against the stress conditions. There are many type of proteins, among them heat shock proteins (HSP) is the most commonly found (Balfagon *et al.* 2018). Their protective role is to prevent other proteins from denaturalization during stress conditions regulating refolding, localization and accumulation as well as preventing agglomeration and degradation.

According to study, it takes two to five years from planting for a citrus tree to reach a stage when it can produce flowers. In subtropical areas, flowering takes place after a period of bud quiescence as a result of exposure to low temperatures in winter. In the tropics, and in areas with a dry season, rehydration after a period of water stress triggers the flowering process. The inflorescence may be leafless or leafy both vegetative and flower buds present on the same shoot. The degree of fruit set is dependent on the type of inflorescence formed. In general, leafless inflorescences emerge first and low setting of fruit. In contrast, flowers in leafy inflorescences are commonly associated with a better fruit set.

In most *Citrus* spp. grown in the subtropics, flowering takes place in the spring and the subsequent formation of fruit can extend, depending on the cultivar, until mid-winter. Citrus fruits are a special type of berry (known as a hesperidium), with two morphologically distinct regions: The pericarp and the endocarp (the juicy pulp). Growth and development of a fruit follows a typical sigmoid curve and can be divided into three phases. The initial phase following anthesis: Two-month period of cell division and slow growth. The second phase: Four- to six-month period of rapid growth as the cells enlarge and require irrigation. Finally, there is the maturation period, a non-climacteric process in citrus. The control of fruit set and abscission in citrus done by genetic, metabolic and environmental factors but not yet understood fully.

Withholding water during the spring (flowering and fruit set phase) and rehydration during summer increase fruit and flower drop. This also results in more off-season flowering in the second and third flushes, which led to 10% off-season fruit. The abscission of leaves and fruits occurs suddenly after rehydration. The content of Abscisic acid (ABA) in the roots and the subsequent accumulation of ethylene increases in the aerial parts of the plant when sudden rehydration followed drought conditions. Series of field trials have been conducted to check on the expansion of fruit size on drought conditions or water availability in Israel. Fruits are found that even at drought stress conditions, fruits continued to accumulate dry matter when subjected to water stress even when the fruits can no longer increased in volume, indeed even when shrinkage occurred. Upon re-watering, fruits on droughted trees expanded faster than those fruits on regularly watered plants. Water stress resulted in decline of fruit water potential as a result of loss of water through transpiration resulted in a decline in the fruit water potential as a result of the loss of water from the fruit to transpiring leaves with an increase in the soluble solid content of the fruit juice. There was also evidence of cell wall loosening in the fruit skin, which caused loss of turgor. As a result of these changes in water potential, the fruits expanded faster in withholding and re-watering plants than those in the well-watered treatment (Carr 2012). Titratable acidity was found higher in fruit under drought stress conditions although acids consistently decreased.

Drought leads to increased generation of ROS as a result of alteration in environmental conditions (Suzuki *et al.* 2012) which are generally proves as a toxic substances affecting the cellular metabolism and photosynthetic processes in plant cells (Choudhury *et al.* 2017) through disturbance in electron transport system and other metabolic processes occurring in chloroplast, microbodies, and mitochondria. These ROS inhibit metabolic enzyme activities, damaging nucleic acids and causing cell death. These ROS are used as important signaling molecules (Baxter *et al.* 2014) because its increase in accumulation results in stomatal closure and reduce carbon dioxide availability. To avoid the negative effects of ROS in plants, different ROS-scavenging enzymes are produced by the antioxidant defense machinery. Under drought stress, plants increase antioxidant enzymatic activities including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), as well as accumulate non-enzymatic antioxidant compounds such as glutathione and ascorbate (Pyngrope *et al.* 2013) to reduce oxidative damage. These enzymatic activities help in detoxifying the excessive ROS under water stress conditions (Pyngrope *et al.* 2013).

Depending on the duration, course and severity of drought stress fruit quality of Citrus increased or decreased. Long term stress leads to reductions of fruit quality and yield while mild drought stress helps in increasing fruit quality. Fruit abscission increases, mature fruit of low juice content, inferior fruit quality was observed under moderate stress (Carr 2012). Salinity and drought stress caused similar physiological disorders to plants. The interlinked molecular responses are then activated under salinity and drought stress to provide defense to the plant. The plant responses to facilitate the alleviation of the occurred stress syndromes are complex and can be different in terms of response to each individual depending upon the duration and intensity of stress syndrome (Ziogas *et al.* 2021). In saline stressed plants, their leaves are rich in Cl^- and Na^+ concentrations helping in control of stomatal closure such that leaf water content (RWC) was maintained during drought stress.

Mechanism of drought stress

There is a need to understand the mechanisms underlying plant abiotic stress acclimation that will help us to develop new fruitful agricultural strategies. Plants cope with water deficit via two general mechanisms;

i) *Stress avoidance:* By creating specialized morphological adaptations, such as leaf surfaces that are less susceptible to transpiration losses, reduced leaf area, sunken stomata, and increased root length and density (Fig. 1).

ii) *Stress tolerance*: Is defined as a set of coordinated physiological and biochemical changes at the cellular and molecular levels, such as the accumulation of late embryogenesis-abundant (LEA) proteins linked to cell antioxidant activity (Fig. 1).

To study the plant water status relative to soil water content, it is time consuming and can't be automated. Therefore, a technique is used which acts as a reliable indicator for soil water deficit. The amount of water needed by irrigation can be easily determined by assessing the canopy temperature using infrared thermometry. The canopy temperature usually increases with a decrease in soil moisture but when plants are well watered, the leaf temperature lowers due to the dissipation of energy in the form of latent heat (Viera and Ferrarezi 2021). As currently available techniques can't detect water stress at early stages, a sensor for early detection of environmental stress is required for rapid control measures and management. Nowadays several studies focus on using thermal cameras to monitor overall canopy temperature, identify water stress and estimate stomatal conductance by (Viera and Ferrarezi 2021). Natural drought tolerant plants prevent themselves from the detrimental effects of drought stress using varieties of metabolites and low molecular weight proteins. Dehydrin (DHN) proteins are one such class of proteins that accumulate in plants during drought and associated stress conditions. These proteins are highly hydrophilic and perform different roles in the protection of plant cells during drought stress conditions. Evidence gathered over the years suggests that DHN proteins impart drought stress tolerance by enhancing the water retention capacity, elevating chlorophyll content, maintaining photosynthetic machinery, activating ROS detoxification, and promoting the accumulation of compatible solutes, among others (Riyazuddin *et al*. 2022). Plants are capable of inducing some stress "memory" or "stress imprinting" following stress exposure leading to acclimation to a later abiotic stress. Priming phenomena have previously been described widely under biotic stress (Conrath *et al.* 2015) but the mechanisms of long-lasting priming are still unclear under abiotic stress. (Jiménez-Areas *et al*. 2015) showed that Arabidopsis seed-based priming against salt stress involves epigenetic changes (DNA hypomethylation) in genes controlling proline metabolism. In this regard, proposal has been made that priming stimulates salicylic acid (SA), abscissic acid (ABA), and jasmonic acid (JA) signaling that could facilitate the transcriptional induction of defense genes and epigenetic changes; this trans-generational induced resistance is obtained by the RNA-directed DNA methylation (RdDM) pathway, which triggers heritable changes in DNA methylation leading to direction of priming-inducing chromatin modifications at defense gene promoters (Pastor *et al.* 2013).

It has also been highly evident that RNS (in the form of nitric oxide, NO) and ROS (in the form of H_2O_2) play important roles in priming phenomena in various annual plants (Del 2015). Almost all abiotic stressors generate reactive oxygen species (ROS) and reactive nitrogen species (RNS), which results in oxidative and nitrosative stress, in plants. ROS-triggered signal transduction via a MAPK-based cascades induces the expression of detoxification and stress protection genes, such as heat shock proteins (HSP), glutathione-S-transferases (GST), peroxidases (POD), superoxide-dismutases (SOD) and pathogenesis-related (PR) proteins, protecting the plant from damage. Under drought stress, the content of proline, Ascorbic acid (AsA) and the activity of SOD and POD were significantly increased by the treatment of exogenous Methyl jasmonate (MeJA). So, this treatment could induce drought stress tolerance by increasing the photosynthesis, osmotic adjustment substances and antioxidant activities in Citrus cultivar (Xiong *et al.* 2020). Glomalin-related soil protein (GRSP) as the most abundant compound in crude extracts from soil (Wu *et al.* 2013) and under soil water deficit stress, it was found as a coating on the fungal hyphae to prevent loss of water.

Genes involved in drought stress in citrus or its related genera: *PtADC* gene from *P. trifoliata* which is tolerant to low temperature and drought. Those transgenic plants expressing *PtADC* have longer primary roots and smaller stomatal density. These two morphological traits are of valuable significance for plants to combat water deficit. These two genes are responsible for drought tolerance in transgenic plants (Fig. 2).

Alleviation Method

Irrigation: The most effective way of irrigating a citrus orchard is with a micro-irrigation system (drip or

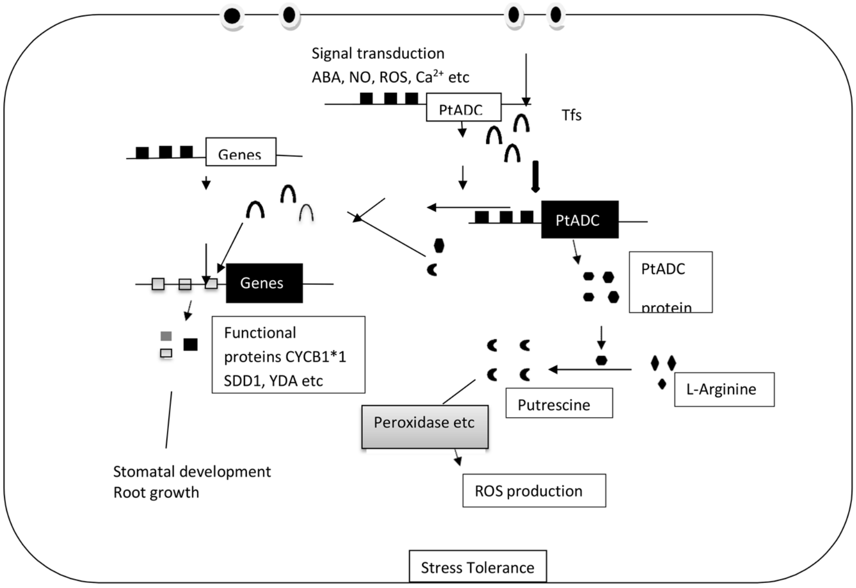

Fig. 2. Model of action of *PtADC* gene in drought tolerance by mediating the physiological, morphological or molecular alterations. Source: Xiao–Quing Gong and Ji- Hong Liu, (2012).

microsprinklers). Citrus trees need for water depends on tree age, tree size, *Citrus* species, climate, and soil type. As a general guide, research studies suggest that mature citrus (orange) trees need about 4000–5000 m^3 of water per hectare and year. The irrigation schedule should start according to the soil moisture, which can be determined by soil samples with an auger or soil moisture sensors/tensiometers. The installation of tensiometers following the irrigation line in the middle point between two emitters (0.20 m from the emitter, 1 m from the trunk) is recommended for mature citrus trees. In the case of a micro-sprinkler, the installation of tensiometers at a distance of 0.5 m from the mini sprinkler or 1 m from the sprinkler is recommended. Citrus has a relatively shallow root system. Thus, it is important to apply irrigation at the effective root zone, minimizing the deep percolation of water (Ziogas *et al.* 2021).

Rootstock and interstock: In citrus, the choice of the rootstock plays a crucial role in the water relations of the tree with the soil and determines its overall response to abiotic stresses. The genetic characteristics of the rootstock determine the robustness of the scion and its survival under water stress. Use of Cleopatra's mandarin rootstock and hybrids orange varieties as interstocks (Ziogas *et al.* 2021).

Plant growth promoting microbes (arbuscular mycorrhizal fungi): A promising agricultural practice is the use of compounds enriched with plant-growth-promoting microbes (PGPM). The long term use of chemical fertilizers and pesticides has caused a severe decline in soil quality, hence, a proper agricultural techniques that will sustain agricultural production is necessary. Towards this goal, it has been reported that Arbuscular Mycorrhizal induced water deficit tolerance of roots of Trifoliate orange by regulating polyamine homeostasis. AMF, Arbuscular mycorrhizal fungi shows altering plant water relations in many experiments and prevents drought stress under certain conditions. An improvement can be obtained in drought tolerance from indirect mycorrhizal effects such as an improved nutrient status, hormonal regulation of stomata, and better osmotic adjustment in AM plants and increased antioxidant levels in AM plants (Wu *et al.* 2013). Drought stress conditions affects the germination of AM fungal spore but the efficacy is that some AMF species can quickly adapt to the DS conditions.

Hydrogels: Research studies indicate that hydrogels may minimize the adverse effects of salinity by reducing the levels of salt ions in citrus tissues. Hydrogels protect trees under drought conditions by supplying water and nutrients when the soil surrounding starts drying. Hydrogel materials cause a reduction in irrigation amount as well as intervals by 50%. In addition, it has been proved that hydrogels can increase soil's waterholding capacity up to four times, ensuring safe soil moisture levels as well as nutrients under drought conditions. There are three forms of hydrogel composites containing natural polymers (polysaccharide derivatives), semi synthetic polymers (cellulosic primitive derivatives), and synthetic polymers. Synthetic polymers indicate higher stability under different environmental conditions than shown by natural ones (Ziogas *et al.* 2021).

Genetic transformation: Gene isolation and functional characterization in Citrus and its related genera has been incorporated into citrus cultivar for many years. But more understanding of the gene networks involved in stress response and gene cloning still lags far behind in Citrus.

CONCLUSION

Drought is another constraint that has a negative impact on crop growth and output. Even at low levels, drought can cause productivity losses. Drought stress is a significant abiotic stress that slows plant growth and stunts plant growth. It is more common in plants that grow in saline soil with little rainfall. Drought and heat stress diminish plant metabolism, which also impacts overall plant chlorophyll and carotenoid concentration in times of scarcity and salinity.

Citrus plants have been found to have relevant parameters associated to plant response to water deficit and salinity stress. Rootstocks that efficiently combine stress avoidance and tolerance mechanisms to enhance plant production under adverse environmental conditions and efficient crop recovery after stress should be given high emphasis. The fact that a stress-tolerant microbial consortium consisting of PGPM strains, mycorrhizal fungi, biostimulant compounds, and hydrogels improves plant growth under abiotic stress conditions identifies them as key factors that can help solve future food security issues while also maintaining soil fertility and plant health.

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