



HEAT STRESS IN CITRUS: A MOLECULAR FUNCTIONAL AND BIOCHEMICAL PERCEPTION

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Abstract Misfortunes caused by high temperatures compel us to more readily comprehend the physiological, hormonal, and sub-atomic systems of reactions, particularly in humid and subhumid yields such as citrus organic products that are accustomed to specific conditions. Heat stress is accustomed to drought and many other environmental factors affecting Citriculture. We observe the role of Rubisco, antioxidant enzymes, HSPs, physiological changes in plasma membranes, and the role of ABA and SA under heat stress in citrus. Notwithstanding essential exploration, developing and utilizing new and well-developed citrus rootstocks is an essential element for the regulation, according to ecological circumstances. Rootstocks are essential in controlling how plants react to changing environmental factors, such as heat stress. They transfer beneficial features and increase stress tolerance, which helps citrus plants be more resilient overall. The duration of growth, yield, fruit quality, and tolerance to biotic and abiotic challenges are only a few of the characteristics of citrus horticulture that can be significantly improved using the right rootstocks. Enhancing citrus fruits' resistance to unfavorable environmental circumstances is urgently needed due to climate change. We can learn more about how different rootstocks affect the scion's capacity to withstand abiotic pressures by examining the metabolic responses caused by those rootstocks. Because of its increased antioxidant capacity, improved stomatal control, and storage of protective proteins, Carrizo citrange, for instance, demonstrates superior resilience to heat stress when compared to Cleopatra mandarin. The combined impacts of heat and drought on citrus vegetation differ from the effects of each stress alone. Specific metabolic changes occur, which agree with findings from other plant research looking at the combined impacts of stress on physiology, transcriptome, proteome, and metabolome. When using rootstocks like Sunki Maravilha mandarin under drought stress, important metabolites such as galactinol, raffinose, and SA can be enhanced in scions through grafting. On the other hand, the Cleopatra rootstock alters the metabolism of the scion, resulting in lower quantities of the amino acids galactinol, raffinose, proline, phenylalanine, and tryptophan, which could lead to undesired characteristics. These results highlight the value of continued research to solve the problems brought on by climate change and provide light on the role of rootstocks in citriculture.

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1. Introduction

1.1. Citriculture and heat stress

The citrus family of fruit tree crops has been acknowledged as having significant value for the world's agriculture industry. Most of the citrus horticultural zones are in the "citrus belt," a sub-humid zone distinct by the 40° north-south latitudes, at which the temperature hardly lowers to freezing levels (Baldwin et al., 1993). Oranges, mandarins (tangerine, mandarin, clementine, satsuma), lemons, and limes comprise citrus merchandise. Brazil, with 17 million tons, and China, with 10 million tons, were

the major growers of oranges in 2018. Spain holds the top place for exporting 50% of its production, securing second place after China with 2 million tons of mandarin production. Interconnected molecular responses are activated by salinity, heat, and drought stress to provide the plant with an acclimation effect and begin signaling cascades to assist in relieving the stress syndromes that have already occurred (Pardo, 2010). The extensive use of rootstocks in citriculture is a crucial component of the entire effort to sustain and endure the heat and drought stress. One of the important techniques for the survival and tiling of

many annual and arboreal species is grafting. Grafting combines aerial parts and roots of two different species or varieties. This method copes with many issues, such as biotic and abiotic stresses, less yield, or changes in harvest season for economical prices. Citrus trees have been the subject of extensive research, which has revealed that trees can withstand insufficiency of water using rootstocks that modulate the biological activity of the tree through changes in plant stomatal conductivity, leaf water potential, and water conduction (Rodríguez-Gamir et al., 2011; Romero et al., 2006).

The major plant activities that are affected by grafting are water conduction, hormonal discharge, and movement of proteins, mRNAs, and sRNAs (Warschefsky et al., 2016). When rootstock and different varieties work together, several properties that are thought advantageous for the variety are transmitted by the rootstock. For instance, the resilient rootstock to tristeza virus (CTV); and Poncirus trifoliata, when grafted with a variety that is vulnerable to the virus, can transmit tolerance capability (Albrecht et al., 2012; Castle, 2010). Briefly, rootstocks are used to transfer a desired trait desired by the farmers, and these abilities will vary based on the rootstock/variety combination chosen (Forner-Giner et al., 2009; Martínez-Cuenca et al., 2019; Morales et al., 2021; Primo-Capella, Martínez-Cuenca, Gil-Muñoz, et al., 2021).

From a physiological perspective, it is simple to show these graft-induced advantages. Still, explaining the molecular activities and the relationship that a rootstock has with a variety is crucial. Due to advanced techniques such as RNA sequencing, the movement of transcripts between rootstocks and variety has been studied, which results in the communication and remote control of many features (Kim et al., 2001; Kudo & Harada, 2007; Thieme et al., 2015). The ability to transmit genomes between plants allows us to comprehend current issues and find solutions. Using the right rootstock, the scion can benefit from crucial enhancements such as a shorter juvenility period, higher yields, better fruit eminence, uniform plant manner, defense against pathogens, and optimal resistance to abiotic stress. (Albacete et al., 2015; Han et al., 2019; Rasool et al., 2020). It is shown that under drought stress, the buildup of hormones and antioxidant substances in the scion was modified by the Sunki Maravilha mandarin citrus rootstock (Santana-Vieira et al., 2016).

Carrizo citrange is more resistant to heat stress than Cleopatra mandarin, partly attributable to its higher antioxidant capacity, improved stomatal control, and storage of protective proteins. (Balfagón et al., 2018; Zandalinas et al., 2017; Zandalinas, Rivero, et al., 2016). In the current study, we proposed that plant tolerance to harmful conditions could be determined by the rootstock's effect on the scion's metabolic response to stress. The rootstock controls the accretion of vital stress-responsive metabolites, which

are important for managing the scion's abiotic stresses, leading to mutual grafting between Carrizo and Cleopatra (Balfagón et al., 2022).

One study applied solo or combination stress conditions to the Carrizo orange and Cleopatra mandarin leaves. The individual stress effects of heat and drought were different than combined stress effects. The plant response to the combined pressures was neither a combination of the effects of the individual stresses nor an amplified response to one of the distinct stresses. To adapt to the abiotic stresses applied, the combination of heat and drought stresses affected Carrizo and Cleopatra vegetation, which triggered unique metabolic alterations that are constant with results from other plant studies when investigating the effect of combined stress on the physiology, transcriptome, proteome, and metabolome of plants. (Balfagón et al., 2019; Lopez-Delacalle et al., 2021).

The results of this study suggest that the separate metabolic responses of abiotic factors differed from the combined effect of heat and drought stress combination (Figure 1). Also, abiotic stress is reported to induce a change in the scion's metabolic processes, which can intensify or reduce the plant's resistance to such detrimental conditions. Carrizo citrange, in particular, had a favorable effect on scions when utilized as rootstock by levitating stages of important metabolites (galactinol, raffinose, and SA) in response to stress. Cleopatra rootstock modified the metabolism to create less galactinol, raffinose, proline, phenylalanine, or tryptophan, which conveyed undesirable features to scions. Finally, this study sheds light on the significance of rootstocks in enhancing citrus plants' resistance to the unfavorable environmental conditions that frequently arise due to climate change.

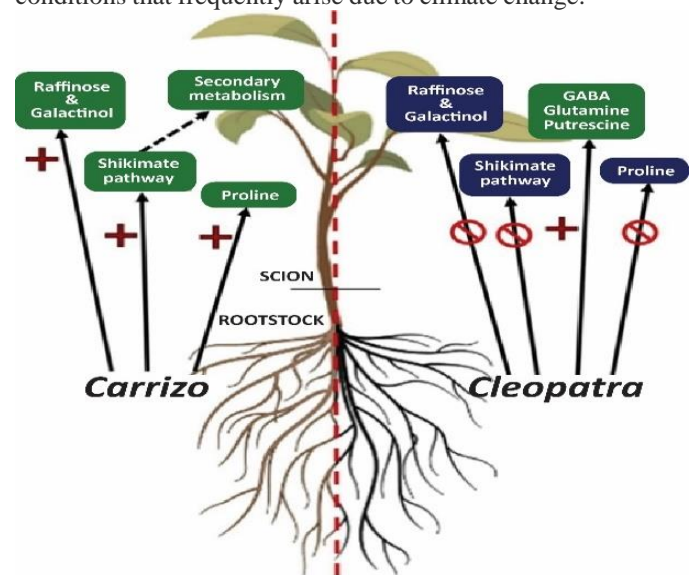


Figure 1 : Diagrammatically representation of the combined effect of heat and drought stress on the resistant (Carrizo citrange) and susceptible (Cleopatra mandarin) citrus rootstocks.

Ion exclusion techniques are also used in citrus trees to implement salt resistance. The movement of small quantities of harmful salts by roots and/or basal stem tissues to the actively photosynthesizing leaves is known as salt exclusion. The ploidy level affects rootstocks' salt tolerance as tetraploid citrus plantlets exhibit stronger salt resistance than the diploid genotype (Ruiz et al., 2016). The salt omission process in citrus plants is a genetic trait discovered through in-depth molecular analysis. This finding prompted the establishment of numerous improvement curricula aimed at creating citrus hybrids that omit salt ions effectively and perform healthily compared to the parent genotypes. (Forner-Giner et al., 2009)

2. Heat stress

When exposed to an environment with unique characteristics, plants enter an ideal steady state where their metabolism is adapted. Adaptation and adjustment must be made in the activity and amount of enzymes and metabolites for the metabolism to operate as effectively as possible in the circumstances in which it is found. Many reactions may develop over time in plants exposed to a changing environment (Primo-Capella, Martínez-Cuenca, & Forner-Giner, 2021)

2.1. Molecular response at high temperature

The major goals of thermal damage in plants are the role of Rubisco in carbon fixation, the water splitting complex also called the oxygen-evolving complex, and its associated cofactors in photosystem II (PSII), and the ATP production method. Current research on the collective effects of heat stress and light intensity suggests that moderately alleviated temperatures do not seriously harm PSII prevent PSII from being repaired. The de novo protein synthesis is entailed by the latter, especially the D1 protein, encoded by the *psbA* gene, which is harmed due to ROS generation, which disturbs electron flow and reduces carbon fixation and oxygen expansion. The PSII repair system, unlike PSII reaction center (RC), is most affected by ROS attacks during mild heat stress. Moreover, heat stress causes the RC proteins to cleave and aggregate; however, the mechanisms behind these actions are still unknown.

In contrast, it appears that sensors linked to membranes cause the neighborhood around PSII membranes to accumulate compatible solutes like glycine betaine. Moreover, they act as a source of the manifestation of stress proteins, facilitating the acclimatization process by reducing the repair of damaged photosynthetic mechanisms aided by ROS. In this review, we also summarize recent developments in the research on the effects of heat stress on the molecular structure of photosynthetic systems, particularly in PSII (Allakhverdiev et al., 2008). Stress can impede photosynthesis in two main, according to the literature:

- First, direct damage brought on by the stressor

- second, reactive oxygen species' suppression of de novo protein synthesis (ROS).

Other stresses that prevent PSII and/or PSI from being repaired are those caused by CO₂ restriction, drought, cold, or salinity (Mohanty et al., 2007; Nishiyama et al., 2005, 2006; Nishiyama et al., 2001). The fluidity of thylakoid membranes serves as a detector for practical alterations brought on by temperature (Horváth et al., 1998). The PM is damaged at multiple stages by both low and high temperatures, although the initial phases of damage brought on by cold and heat appear to vary. The Calvin-Benson cycle's enzymes are heat labile. Because of this, the mechanism that assimilates carbon is sensitive to high temperatures and is severely hindered by mild thermal stress (Berry & Bjorkman, 1980; Sharkey, 2005; Urs et al., 1998). Rubisco is a heat-stable enzyme, but Rubisco activase causes the enzyme to lose function at high temperatures because of its sensitivity to high temperatures. (Salvucci & Crafts-Brandner, 2004; Sharkey, 2005). Early consequences of thermal stress include changes in the Chl-protein complex structure brought on by heat and the inactivation of enzyme function. Heat exposure also changes the chloroplast's macroscopic structure (Kreslavski et al., 2008; Semenova, 2004; Vani et al., 2001). A reduced membrane stacking and an overall rearrangement of the thylakoid membranes are seen at temperatures in the range of 35-45°C. The ion seepage from heat stricken leaves also reflects these structural changes (Wahid & Shabbir, 2005) and modifications in the energy balance between the two photosystems (Mohanty et al., 2002). Elevated temperatures also affect energy transfer from Light Harvesting Complex-II to PSII's antenna complex. Even at 35°C, the light balance system in two photosystems is effected when the migration of LHClI from the intergranal region towards PSI is stopped (Pastenes & Horton, 1996). When temperatures rise over 40–42°C, the electron acceptor site of PSII becomes inactive, and the rate of electron transfer in the chloroplasts is reduced, contributing to the loss of photosynthetic activity. At alleviated temperatures, ROS, including hydroxyl radicals, superoxide radicals, and hydrogen peroxide at the PSII RC (SOD) are scavenged by superoxide dismutase and other antioxidants, including thermal stress at elevated climate (Bukhov & Mohanty, 1999). Organisms are protected by Antioxidant enzymes by reducing the production of singlet oxygen, formed under intense light (Krieger-Liszkay, 2005).

2.1.1. Post stress recovery

Recent research on wheat plants on the recovery from heat stress revealed the intricacy of the procedure. Mitochondrial phosphorylation determined the carbon assimilation and PSII performance recovery in the dark, whereas temperature and light intensity regulated recovery in light (Kreslavski et al., 2008). Chloroplasts isolated from leaves of damaged and

recovered plants were used to evaluate cyclic and non-cyclic photophosphorylation, which showed that recovered plants displayed the augmentation of Cyclic electron flow in chloroplasts. These findings support earlier hypotheses that repeated electron flow shields PSII from photoinhibition and might supply additional ATP needed for protein synthesis (Allakhverdiev et al., 2005). The protein biosynthetic machinery is required for post-temperature stress recovery to repair PSII's thermal or photo-damage (Takahashi et al., 2004; Yang et al., 2007).

The discharge of 33, 23, and 17 kDa proteins from chloroplasts under thermal stress resulted in the loss of cofactors. As heat stress persists, loss of cofactors, particularly PsbU, causes PSII to become inactive, D1 protein to become thermally damaged, and ROS to be produced both in the light and in the dark. Since PsbU mutants had improved detoxification systems for exogenously administered H₂O₂, it has been hypothesized that PsbU protects against ROS (Balint et al., 2006).

To protect cells from heat and other stresses, heat-shock proteins (HSPs) are crucial (Barua et al., 2003). Experiments in living organisms showed that PSII's O₂ evolution and OEC proteins are protected against high temperatures by tiny HSPs when they connect with thylakoids. In tomato species, proof was shown to support the importance of HSPs in chloroplasts for tolerance against heat stress (Heckathorn et al., 2002). It is believed that chloroplast HSPs serve to avoid damage rather than assist in restoring cell damage by heat stress (Allakhverdiev et al., 2008).

Molecular disturbance and motion within a lipid bilayer is called "The fluidity of the membrane" (Los & Murata, 2004). Studies that examined the impact of low temperatures made it abundantly evident that membrane fluidity declines as temperature falls (Los & Murata, 2004). The fluidity of membranes is enhanced at high temperatures, which can cause them to break down. The heat resistance of photosynthetic oxygen evolution did not affect the fatty acid unsaturation. On the other hand, based on recent observations of the changes in the temperature necessity and heat sensitivity of PSII in *Synechocystis* sp. grown at 25 and 35° C, it was suggested that the foremost bases of the change in the heat-sensitivity of PSII are the lipid desaturation levels of plasma and thylakoid membranes (Aminaka et al., 2006).

2.1.2. Atomic reaction in citrus in heat stress

The stress hormone abscisic acid (ABA) appears to play a significant role under conditions of drought or high salinity (Bartels & Sunkar, 2005; Finkelstein, 2013), responsible for controlling stomata closure, producing suitable osmolytes, and activating genes that trigger adaptive responses. The enzyme 9-

neoxanthin cis-epoxycarotenoid dioxygenase (NCED) increases in response to an increase in ABA levels and is a blockage in ABA production. The defence mechanisms against biotrophic infections have been linked to salicylic acid (SA) (Vlot et al., 2009). Recent research has proposed that SA also contributes to stress tolerance due to abiotic conditions and induces heat resistance in many plants, such as citrus (Clarke et al., 2009; Larkindale & Huang, 2005). Research on *Arabidopsis* mutants reveals that the SA-signaling mechanisms promoting basal thermos-tolerance and those responding to biotic stressors overlap. In this way, genes related to pathogenesis (PR) are activated in response to biotic stressors and high temperatures (Clarke et al., 2004). SA accumulation due to stress, external use, or genome changes has played a positive response against high temperature in a variety of plant species, including poplar (Xue et al., 2013), *Agrostis stolonifera* (Larkindale et al., 2005), *Avena sativa* (Sánchez-Martín et al., 2015) and grapevine. The positive impact of SA accumulation is related to a surge in antioxidant mechanism and the resistance of the photosynthetic apparatus against electron escape (M. I. R. Khan et al., 2015).

2.2. Physiological Responses

Heat stress alters the development, variations, functional mechanisms (Figure 2), and yields in various ways that are frequently detrimental (Hasanuzzaman et al., 2012; Vahdati & Leslie, 2013). The oxidative stress caused by extreme production of reactive oxygen species (ROS) is one of the major effects of heat stress (Hasanuzzaman et al., 2012; Vahdati & Leslie, 2013). Plants modify their working in many ways in response to heat stress. Most particularly, plants create suitable solutes that can assemble proteins and organelles, uphold cell turgidity by water balance, alter the antioxidant system, and maintain homeostasis (Janská et al., 2010; Munns & Tester, 2008; Valliyodan & Nguyen, 2006). At the atomic level, the expression and activation of genes involved in resistance against heat stress change at alleviated temperature (Chinnusamy et al., 2007; Shinozaki & Yamaguchi-Shinozaki, 2007). They include the genes that produce transporters, regulatory proteins, detoxifying enzymes, and osmoprotectants (Krasensky & Jonak, 2012; Semenov & Halford, 2009). In situations like high temperatures, heat tolerance is developed by altering the gene expression of functional and biological mechanisms as acclimation or, in the best-case scenario, adaption (Mirza et al., 2010; Moreno & Orellana, 2011). Plants respond to HT in a variety of ways, depending on the extreme and duration, as well as the types of plants present and other environmental conditions in the environment.

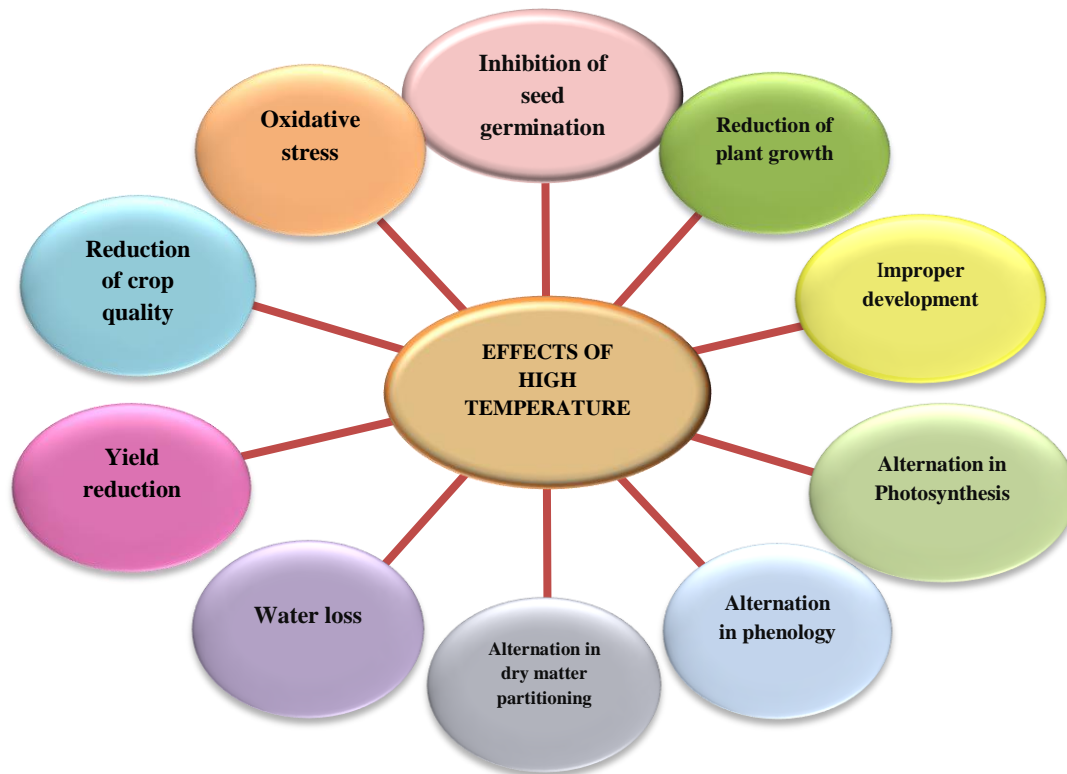


Figure 2 : Physiological effects of temperature on plant

2.2.1. Citrus membrane alterations in response to heat stress

Heat resistance is a complex biological characteristic dependent upon the operation of numerous biological mechanisms, the individual cells' physiological state, and plant's overall health. These heat resistance characteristics and the group of genes contributing to high-temperature resistance have been reviewed in the latest studies ([Allakhverdiev et al., 2008](#)). The factors providing heat resistance to plants, including QTL, are:

- (1) HSPs
- (2) antioxidants or carotenoid
- (3) unsaturation of lipid in membrane
- (4) gene expression
- (5) stability of membrane protein
- (6) solutes accumulation

We already know these factors greatly tolerate PSII and OEC ([Allakhverdiev et al., 2007](#)). The combination of heat and light causes plasma membrane inhibition. They frequently occur simultaneously, and their interaction may cause their harmful effects on the PM to either lessen or strengthen. In particular, low or weak light repairs the photosystems to activate enzymes and protein photophosphorylation. The repair depends on light to switch the Plasma membrane's adaption mechanisms to thermos-stress. Besides, exposure to intense light magnifies the PM inhibition by heat stress with the production of additional ROS during heat-induced photoinhibition ([Kreslavski et al., 2008](#)). According to several studies, plants are better able to adapt to strong light when exposed to temperatures greater

than their growth temperature ([Kreslavski et al., 2008](#)). Cross-tolerance is the term used to describe this kind of resistance to stress stimuli. Rubisco activase, which is necessary for Rubisco's function, is known to be inactive at high temperatures. In these circumstances, limitation in the fixation of CO₂ may not repair the damaged PSII, which could speed up photoinhibition. This theory summarizes that high temperature prevents the restoration of damaged PSII and promotes photoinhibition ([Yang et al., 2007](#)). Although these diverse stressors may prevent the repair of photodamaged PSII, their restricted effects may also be partly due to the Calvin cycle's limited ability to fix CO₂ ([Takahashi & Murata, 2008](#)).

2.2.2. Photosynthesis and heat

Frequent high temperatures (HT), a major environmental danger brought on by global warming, severely restrict plant growth, metabolism, and productivity ([Raza et al., 2019](#)). Earth's temperature may rise till the mid-century to 1.5°C, which could result in a decline of 2.5-16% in crop production. (IPCC 2021). Plants are particularly vulnerable to HT stress regarding their most crucial physiological and metabolic systems ([Mittler et al., 2012](#)). According to Zhang and his colleagues, "HT stress significantly reduces the plant leaves' capacity for photosynthesis and content of photosynthetic pigments, ([Zhang et al., 2005](#)) reducing the rate of photosynthesis in plants". Heat stress is linked to non-stomatal and stomatal limitations, such as closure. Some restrictions in the photosynthetic system include membrane deterioration, damage to chloroplast ultrastructure and degradation, and enzymatic protein degradation

(Song et al., 2014). The oxidative damage in plant cells is also caused by the unwarranted formation of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and hydroxyl radicals, in the presence of heat stress. (OH), superoxide (O_2^-), and other substances, etc. cause RNA and DNA damage, lipid peroxidation in cell membranes, coloration of pigments, protein inactivation, and eventually plant death (Muhlemann et al., 2018). By monitoring basic enzymatic and non-enzymatic antioxidant barricades, plants have built-in defensive systems to retaliate against oxidative stress. Elimination of excess ROS and preventing ROS-induced damage to plants can stabilize the generation and foraging of ROS by enzymatic antioxidants such as superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX) (Chung et al., 2006; Xu et al., 2020). Consequently, their photosynthetic and antioxidant capacities are crucial for plants to grow and develop normally under HT stress. Ascorbic acid (AsA), glutathione (GSH), phenolic compounds, and tocopherols are non-enzymatic antioxidants that can control the activities of major cells and help remove ROS produced by stressed plants (Chao et al., 2022).

Photoinhibition

Decreased photosynthetic activity, primarily due to the CO_2 absorption brought on by too much light, is called photoinhibition. It (inhibition) refers to suppressing photosystem II (PSII) metabolism; nevertheless, reduced light acclimatization may be subjected to damage to some components of the photosynthetic metabolism (Figure 3). As light is the energy that powers photosynthesis, photoinhibition occurs if the light output exceeds the rate of photosynthetic activity. Yet, the balance between photodamage and PSII repair determines how much photoinhibition occurs (Primo-Capella, Martínez-Cuenca, & Forner-Giner, 2021). Models of leaves, plants, or land surfaces have not been able to adequately incorporate plant physiological responses to stress and the ensuing damage. A lack of water causes a variety of responses in plants: stomatal closure reduces evaporative cooling and raises leaf temperature, which might impact photosynthetic energy absorption, especially under conditions of high irradiance. The Farquhar-von Caemmerer-Berry (FvCB) model of photosynthesis does not often account for photoinhibition, which results from excessive energy absorbed by PSII (Bambach & Gilbert, 2020). Summertime in tropical and subtropical areas is typically characterized by moderate heat stress and erratic lighting. Photosystems I and II may sustain photodamage due to this type of stress. As wild-type plants are constantly provided with high light, PSI is typically resistant to photoinhibition at normal growth temperatures. When additional environmental factors like dryness accompany high PPFD, photoinhibition happens. Yet, even during the day, due to high PPFD at midday, an increase in air temperature and a

decrease in RH% can occur (Pimentel, 2014). When light intensity suddenly rises, electron flow from PSII to PSI increases quickly (Tan et al., 2020). Stomata opening and CO_2 fixation have substantially slower kinetics. As a result, the main metabolism cannot promptly use the electrons transferred from PSII to PSI, which causes an excessive reduction in PSI electron carriers (Yamamoto & Shikanai, 2019). As a result, the peripheral PSI component is attacked by the resultant superoxide and singlet oxygen, which leads to PSI photoinhibition. Photosynthetic electron flows, such as linear electron flow (LEF) and cyclic electron flow (CEF), are suppressed as soon as PSI photoinhibition takes place, which hinders CO_2 uptake and plant growth (Lima-Melo et al., 2019). Moreover, PSI recovery takes far longer than PSII restoration (Zivcak et al., 2015) because the PSI core complex has fully deteriorated rather than been healed. Fluctuating light is a significant physical stressor which affects crop progression and efficiency.

Citrus leaves exposed to temperatures higher than $0^\circ C$ don't exhibit any symptoms. However, damage to photosystem II is found when their photosystems are examined using the CFI (chlorophyll fluorescence imaging) method.

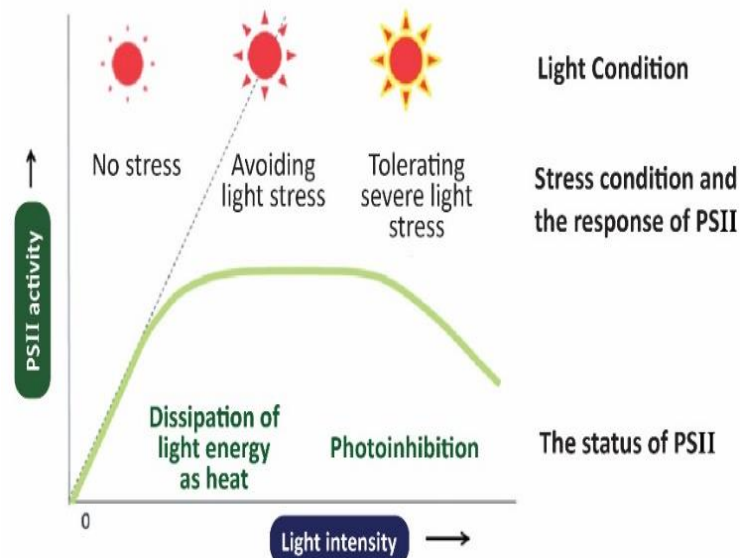


Figure 3: Photo tolerance of thylakoids and activity curve of for PSII activity under such condition. PSII activity increases under low light. Under high light intensity, photoinhibition of PSII becomes obvious and permanent accumulation of PSII proteins which brings out irreparable photoinhibition of PSII.

2.2.3. Photosynthesis and photoinhibition in citrus under heat stress

Perennial (sub)tropical fruit plantations frequently experience problems due to the suddenly rising springtime sun after a long, gloomy winter (Table 1). While each investigated Citrus species responded differently to photoinhibition, they were all sensitive

to it. Photoinhibition has an especially negative impact on citrus.

Table 1: Reasons why citrus is particularly prone to photoinhibition

Sr. #	the causes of citrus's heightened photoinhibition susceptibility
1.	tropical origin, but widely cultivated in an arid environment with a quick increase in light in the spring
2.	Low photosynthetic potential and insufficient light for photosynthesis are characteristics of C ₃ plants.
3.	lower rate of photosynthesis than other C-type trees and plants
4.	Evergreen leaves exposed to shortened daytime sunshine in winter

Citrus is a plant that uses C₃ photosynthesis and leaves lasting two to three years. Citrus has a low light compensation point of 30 to 50 micro mol PAR m⁻²s⁻¹, a mature leaf's maximum photosynthetic rate is typically 7 to 10, and its low light saturation ranges from 700 to 900 micro mol PAR m⁻²s⁻¹ under atmospheric CO₂ concentration (Veste et al., 1999).

2.3. Water potential

Water balance is crucial for metabolic processes to occur in plants. In a plant, there are three paths to move water.

1. Apoplastic transport is the passage of water movement through the cell walls of prevailing cells and water transport from root hair to the xylem following this pathway.

2. Simplest pathway is the transport between cells through plasmodesmata;

3. Transcellular transport uses cell membrane transport. Plants must choose between using water for CO₂ fixation purposes, water photolysis, and transpiration in the context of abiotic stress or ceasing resource intake to halt growth (Primo-Capella, Martínez-Cuenca, & Forner-Giner, 2021).

PIP and high-temperature stress

Aquaporins play a major role in transcellular water transfer. Aquaporins are included in a family of high membrane-conserved membrane-binding called MIP (major intrinsic protein). Thirty-five genes in Arabidopsis code for the vast protein family known as aquaporins (Johanson et al., 2001; Maurel et al., 2015), 31 in maize (Chaumont et al., 2001), 33 in rice (Sakurai et al., 2005), 47 in tomato (Reuscher et al., 2013), 55 in poplar (Gupta & Sankaramakrishnan, 2009).

2.4. Osmotic stability in citrus under heat stress

Plants grown in the field are subject to harmful environmental circumstances, which frequently happen simultaneously. Recent research on two genotypes of citrus plants has examined how drought and high temperatures interact: Carrizo citrange (Poncirus trifoliata L. Raf. × Citrus sinensis L. Osb.) and Cleopatra mandarin (Citrus reshni Hort. ex Tan.)

(Balfagón et al., 2018; Zandalinas, Balfagón, et al., 2016). Heat and drought impact the physiology and morphology of citrus plants, resulting in stomatal closure and a sharp decline in photosynthesis. In the end, this causes losses in fruit yield and quality. Above-optimal temperatures and a water deficit in the soil negatively impact stomatal conductance and net assimilation rate, two types of gas exchange. Stomatal closure inhibits transpiration than CO₂ diffusion into the leaf tissues (Shafiqat et al., 2021).

2.5. Biochemical response

Osmoprotectants, by definition, are tiny organic molecules with low toxicity and a neutral electrical charge. They disturb the already imbalanced cells when stress is present and builds up in cells in vast numbers in the extracellular environment. They are also known as suitable solutes due to their excellent solubility and minimal interference with metabolic processes (Primo-Capella, Martínez-Cuenca, & Forner-Giner, 2021).

2.5.1. Osmoprotectants and high temperature

Compounds produced during osmotic stress conditions for maintaining normal plant metabolism are compatible solutes or osmoprotectants. They are minute, chemically inert composites crucial for the regulation of proteins and the stability and defense of membranes against abiotic stressors (Yancey, 2020). Three major groups of osmoprotectants are: (1) amino acids (2) polyol/sugars, and (3) quaternary amines (M. S. Khan et al., 2015).

The catalyzation of biosynthesis pathways of osmoprotectants is important due to the plethora of transcriptome study statistics related to abiotic stress produced in many plant species. Thus, these osmoprotectants shield plants in many respects from the detrimental effects of subordinate stresses like osmotic and ionic stresses.

Proline

Many plant varieties have been observed to accumulate proline under various abiotic stressors (Hayat et al., 2012). Proline buildup has been observed in plants under osmotic stress brought on by salt and drought stressors (Delauney & Verma, 1993). Proline's main job in plants is maintaining the protein arrangements and foraging free radicals metabolism to neutralize the repercussions of osmotic pressure (Biedermannova et al., 2008; Smirnov & Cumbes, 1989). The precursor chemical glutamate aids in the beginning point of the proline biosynthesis pathway. Yet, the primary mechanism for proline production under osmotic stress is the glutamate pathway. In Arabidopsis thaliana, the proline biosynthesis route has been thoroughly investigated (M. I. R. Khan et al., 2015; M. S. Khan et al., 2015). Proline buildup in plant cells under saline, scarcity, and osmotic stress has revealed that it plays a significant role in their response to those conditions. The ability to genetically modify plants to overexpress proline biosynthesis genes has made it possible to combat osmotic stress brought on by salt and drought conditions. Kishor and

his colleagues ([Kishor et al., 1995](#)) observed proline overproduction in transgenic tobacco, and the transgenic plants underwent water stress caused by increased root biomass. Proline was thought to serve a potential role in ROS scavenging in addition to safeguarding essential proteins ([Smirnov & Cumbes, 1989](#)). More proline was accumulated by transgenic plants, which led to drought resistance for 14 days. Due to high proline accumulation under heat and salt stress, the transgenic plants exhibited greater development, additional chlorophyll and relative water content, and lower levels of lipid peroxidation under salt stress as a product of their relatively high proline accumulation.

The research implies that proline production in transgenic plants significantly protects against osmotic stress from saline, heat, and scarcity circumstances ([M. I. R. Khan et al., 2015](#); [M. S. Khan et al., 2015](#)). On day 24 of the water and heat stress, proline levels in the Gada dahi citrus rootstock were measured and compared to resistant rootstocks. The data gathered presented that proline buildup was higher in vulnerable genotypes than in tolerant genotypes due to increased stress. Proline accumulation was less due to proline's protective role in eliminating radicals, maintaining the redox equilibrium, and minimizing cell damage ([Naliwajski & Skłodowska, 2014](#)).

Protein

In various pathways that alter plant metabolism as a reaction to stress and trigger the plant's defense signal, proteins are involved ([Sarkar et al., 2020](#); [Zandalinas et al., 2017](#)). In general, drought-tolerant genotypes have higher protein content than drought-sensitive genotypes. A genotype known as Carrizo Citrange has remarkable soluble proteins in its leaves and roots ([Chakraborty & Pradhan, 2012](#); [Mathews et al., 1992](#)).

2.5.2. Polyols

The accretion of polyols in different plant species induces high resistance to salt and drought stress ([Bohnert & Jensen, 1996](#)). Polyols primarily work with glutathione-ascorbate cycle enzymes to resist reactive oxygen species (ROS) to protect membranes and enzyme complexes. This group of substances consists of fructose, sucrose, trehalose, mannitol, and D-ononitol.

2.6. Hormones and stresses in high temperature

2.6.1. ABA and heat

Distinctive approbatory reactions of plants to combinations of stresses can't be inferred immediately from each type of stress applied separately ([Pandey et al., 2015](#); [Suzuki et al., 2014](#)). It has long been recognized that abscisic acid (ABA) is critical to respond to abiotic stressors in plants ([Mittler & Blumwald, 2015](#)). For instance, in salt and water stress conditions, ABA acts as a crucial controller of stomatal functioning, such as closure to avert unnecessary osmotic damage via transpiration

([Boursiac et al., 2013](#)). Moreover, regulatory genes necessary for plants adaptation to abiotic stressors such as **late embryogenesis-abundant** class genes are activated by ABA through signaling pathways ([Shinozaki & Yamaguchi-Shinozaki, 2007](#)). According to recent studies, ABA is involved in how specifically plants respond to drought and heat stress combined as well as either one administered alone. For instance, it has been demonstrated that poplar plants gradually increase their production of 9-cis epoxy-carotenoid dioxygenase (NCED), a protein required for the production of ABA, in response to high temperature or drought stress ([Li et al., 2014](#)). The latest study showed that Arabidopsis mutants with deficiencies in ABA production had trouble adapting to the combination of heat stress and drought ([Zandalinas, Balfagón, et al., 2016](#)). It's possible that the absence of a stomata closure mechanism does not cause the susceptibility of these mutants to stress combination. Stomata of the *abi1-1* variant were more opened than those of water stress plants in periods of drought. Still, the stomatal aperture reduced to a level equivalent to water stress (WT) plants on the combination of drought and heat stress. The results imply that managing stomatal mobility in the presence of combined effects of drought and heat stress may include alternative signalling pathways other than ABA signalling. Indeed, *abi1-1* mutant accumulated more ROS and jasmonic acid (JA) than WT under this condition, which may help in stomatal closure ([Murata et al., 2015](#)).

2.6.2. ABA and SA

ABA is considerably aggregated due to individual drought applications in citrus ([Zandalinas, Balfagón, et al., 2016](#)). In contrast, to ABA, the combination of heat and drought stress play a role in increasing Salicylic acid (SA) accumulation compared to the independent action of these stresses, which is involved in the pathway opposing ABA ([Moeder et al., 2010](#)). Under these individual and combined conditions, citrus gave different configurations of ABA and SA accretion than Arabidopsis, which had the maximum or lowest degree of ABA or SA accumulation, respectively ([Zandalinas, Rivero, et al., 2016](#)).

2.7. Agronomic management

The negative effects of heat and drought stress are reduced by using effective orchard management techniques. The management comprises choosing the best cultivars and rootstocks resilient to various pressures, nutrition and irrigation methods based on the trees' needs, and organic and synthetic mulches ([Khan & Khan, 2021](#)). For the proper production of citrus in climate change, resistant rootstocks are required to tolerate biotic and abiotic stresses. Environmental factors and newly discovered illnesses encourage the development of novel citrus rootstocks in response to market needs ([Yang et al., 2015](#)). Citrus rootstocks like Volkamer lemon (*C. volkameriana*), Rangpur lime (*C. limonia*), and Rough lemon (*C.*

jambhiri Lush.) are resistant to drought and escalate the production of implanted cultivars. PGRs such as cytokinins, ABA, and salicylic acid play a role in drought and heat resistance. When PGRs are used, citrus trees have more chlorophyll and water potential (Zhang et al., 2004). Mulching is frequently utilized as a water conservation method underneath trees (Chakraborty et al., 2008). Mulches regulate evaporation, soil temperature, and high soil moisture levels (Yang et al., 2015); hence reducing irrigation requirements in growing seasons.

2.8. Breeding strategies

Citrus rootstock breeding projects aim to develop novel rootstocks that combine biotic and abiotic tolerance/resistance. An innovative standard variety in the citrus sector typically takes at least 15 years to emerge from a breeding program. Therefore, it might be challenging to spot a sexual hybrid in its early stages. When identifying sexual hybrids at the seedling stage, trifoliolate leaves (a morphological identifier) are utilized as the male parent and unifoliolate leaves as the female (Carlos de Oliveira et al., 2002).

2.9. Biotechnological interventions

An important technique for plant production, somatic approaches to hybridization combine (completely or partially) chosen cultivars of vegetative cells, species, or genera, leading to novel genomic combinations. The interspecific mixtures between *C. reticulata* and *C. maxima* are under emphasis to address the unique requirements of the citrus industry in somatic hybridization in addition to intergenerational mixing (Ananthkrishnan et al., 2006; Shen et al., 2011). Poncirus is more sensitive to droughts than Citrange C-35. The rootstock 4475 citrumelo has the greatest capacity for environmental adaptation of all of them. Citrus improvement approaches that are key tissue culture methods include in vitro chromosomal mutation (mutagenesis) and somaclonal variation. The salt-resistant cultivars *C. sinensis* cv. "Shamouti" (Ben-Hayyim & Kochba, 1982), *C. limonium*, and *Troyer citrange* are success stories of cell lines. Aminoglycoside phosphotransferase II gene is being aimed to be expressed in sweet orange (*Citrus sinensis* Osbeck) protoplasts with the help of a direct DNA transfer method called PEG-mediated genetic transformation of citrus fruits (Shafqat et al., 2021).

3. Conclusion

The adaptability of plants is regarded as an impressive tactic to mitigate changing environmental conditions. The two most significant global warming impacts are temperature variations and droughts, which negatively impact plant composition, structure, osmotic balance, biochemistry, anatomy, genetics, and yield. This research has been conducted to expand our knowledge about abiotic stresses such as heat and droughts impact on citrus. Thus, it should be a top priority to design production systems that are more water-efficient and can adapt to the high temperature and dry climatic circumstances utilizing agronomic

practices. It is vital to create citrus rootstocks and scion resistant to climate change using genetics and biotechnology. By learning more about the interactions between rootstocks and varieties, we have improved our grafting methods and ability to choose plants from plant breeding programs. Examples of reactions without a clear cause include photosynthesis inhibition, water aptitude, chlorophyll breakdown, and the production of suitable solutes. The analysis of transcriptomes and genomes illuminates the knowledge gap. With model organisms like *Arabidopsis thaliana*, the fastest reaction to high temperatures has just come to light. This aspect makes it much easier to extrapolate our results to other arboreal or horticultural species that aren't present in a large number of gain- and loss-of-function mutants (Primo-Capella, Martínez-Cuenca, & Forner-Giner, 2021).

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Author's contributions

SN, AS, MZH, and MHA wrote the initial draft of manuscript. MDJ, AK, MIA, MM and QA edit the manuscript for final submission. All authors have read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.



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