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Exogenous nano-selenium alleviates heat-induced oxidative damage in date palm seedlings by modulating the plant hormones and antioxidant defense

Hussein Jasim SHAREEF [1, 2]

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Abstract: Crops are destroyed by extreme heat, which also limits their growth and yield. The present study sought to determine whether selenium (0, 15, or 30 mg l^{-1}) impacted 'Barhee' date palm seedling's development under heat stress (in the field and canopy temperature). The growth parameters, chlorophyll and relative water content, ascorbic acid, catalase activity, and phytohormones in seedlings were reduced under heat stress. At the same time, ascorbate peroxidase activity, proline, phenols, malondialdehyde, hydrogen peroxide, and abscisic acid in seedlings increased. Enhancing growth features, chlorophyll content, relative water content, ascorbic acid, catalase activity, plant hormones, proline, phenols, and ascorbate peroxidase activity with exogenous nano-Selenium (15 mg l^{-1}) reduced the negative impacts of heat stress. Date palm seedlings can be protected from high temperatures by using nano-selenium. Selenium reverses heat-induced oxidative damage by enhancing the antioxidative mechanism, improving reactive oxygen species scavenging, lowering lipid peroxidation, and modulating plant hormone levels.

Key words: nano-selenium, antioxidant enzymes, ascorbic acid, abscisic acid, malondialdehyde, phytohormones

Dodatek nano selena zmanjšuje od vročine povzročene oksidativne poškodbe v sejankah dateljeve palme s spremembami v rastlinskih hormonih in antioksidativni obrambi

Izvleček: Gojene rastline uničuje ekstremna vročina, ki zmanjšuje njihovo rast in pridelek. V raziskavi se je poskušalo določiti učinek dodatka selena (0, 15, or 30 mg l^{-1}) na razvoj sejank dateljeve palme 'Barhee' v razmerah vročinskega stresa (na prostem in temperaturi krošnje). Rastni parametri kot so vsebnost klorofila, relativna vsebnost vode, vsebnost askorbinske kisline in fitohormonov ter aktivnost katalaze so se v sejankah zmanjšali v razmerah vročinskega stresa. Istočasno so se v sejankah povečali parametri kot so vsebnosti prolina, fenolov, malondialdehida, vodikovega peroksida in abscizinske kisline ter aktivnost askorbat peroksidaze. Povečanje rastnih parametrov, vsebnosti klorofila, relativne vsebnosti vode, vsebnosti askorbinske kisline, prolina, fenolov in povečanje aktivnosti katalaze in askorbat peroksidaze je povzročil dodatek nano selena (15 mg l^{-1}), ki je tako zmanjšal negativne učinke vročinskega stresa. Sejanke dateljeve palme bi tako lahko zaščitili pred visokimi temperaturami z uporabo nano selena. Selen odpravlja od vročine povzročene oksidativne poškodbe s povečanjem antioksidativnih mehanizmov, izboljšanjem nevtralizacije reaktivnih zvrsti kisika, zmanševanjem peroksidacije maščob in uravnavanjem ravni rastlinskih hormonov.

Ključne besede: nano selen, antioksidacijski encimi, askorbinska kislina, abscizinska kislina, malondialdehd, fitohormoni

1 Department of Date Palm Research Center, University of Basrah, Basrah, Iraq
2 Corresponding author, e-mail: hussein.shareef@uobasrah.edu.iq

1 INTRODUCTION

One negative effect of global warming is that plants are predicted to suffer from heat stress (Raza et al., 2019). Although some plants adapt to rising temperatures, temperatures that exceed adaptation cause heat stress, significantly impacting metabolism and production (Hatfield & Prueger, 2015). Plants contain many temperature-sensitive biochemical reactions that depend on temperature and duration of exposure (Missaoui et al., 2017)and to validate the hypothesis that genes underlying stem determinacy might be involved in the mechanism of summer dormancy. Our results suggest that vernalization is an important requirement in the onset of summer dormancy in tall fescue. Non-vernalized tall fescue plants do not exhibit summer dormancy as vernalized plants do and behave more like summer-active types. This is manifested by continuation of shoot growth and high root activity in water uptake during summer months. Therefore, summer dormancy in tall fescue should be tested only in plants that underwent vernalization and are not subjected to water deficit during summer months. Total phenolic concentration in tiller bases (antioxidants. Because reactive oxygen species (ROS) are produced uncontrollably under conditions of high temperature, oxidative stress results (Manafi et al., 2021). Plants have an adequate antioxidant defense system that involves enzymes and non-enzymatic antioxidants that play an essential part in ROS signaling to adjust for the damage caused by ROS (Mohi-ud-din et al., 2021). Non-enzymatic antioxidants include proline, phenolic compounds, ascorbate, and others (Khan et al., 2019). In contrast, enzymatic antioxidants include many enzymes, such as catalase and ascorbate peroxidase, which work according to antioxidant mechanisms to eliminate ROS toxins and defend plant cells against oxidative stress (Awan et al., 2020).

Heat stress causes an imbalance in hormonal levels and decreases growth-promoting plant hormones such as auxins (IAA), cytokinins (CK), and gibberellins (GA) (Al-Zahrani et al., 2022). At the same time, heat stress increases abscisic acid (ABA), which is known as a component of transduction signaling (Ryu & Cho, 2015)as a sessile organism, rely on the endogenous regulators for the modulation of growth and development under severe stress conditions for their survival. Plant hormones have long been considered as essential endogenous molecules involved in regulating plant development and tolerance or susceptibility of diverse stresses including salinity stress. Plants are frequently exposed to numerous adverse environmental factors such as drought, cold, heat and high salinity. Under high salinity, plants rapidly reduce the growth and developmental programs in response to the stress due to either the effects of specific ions on metabolism, or adverse water relations. Recent investigations on the functional roles of plant hormones in response to unfavorable environmental conditions have eventually unravel their potentials in coffering tolerance to such conditions including salinity stress. In this review, we will present recent progress of our understanding to the important role of plant hormones including abscisic acid (ABA. ABA transduction signaling leads to the induction of genes for proteins necessary for the protection of plants under stress conditions (Zhu, 2016).

Young date palms, such as seedlings or tissue culture plantlets, are exposed to leaf dryness and reduced growth in dry regions, especially in the summer, due to high temperatures and rapid moisture loss from the soil and plant (Shareef & Al-Khayri, 2021). Although plant reactions to heat stress are widespread, the full explanation of the mechanisms of heat tolerance is still limited. Botanists are responsible for researching ways to reduce environmental stress. Abiotic stresses can be reduced by using fertilizer or foliar spraying.

It has been reported that selenium (Se) has a beneficial role in plants. Some plant species that have been treated with selenium have also demonstrated increased tolerance to specific abiotic stresses such as salinity and drought (Alharby et al., 2021), temperature extreme (Safari et al., 2018), mineral toxicity (Zhang et al., 2020), and ultraviolet radiation (Banerjee & Roychoudhury, 2019). There is evidence that selenium plays a role in encouraging antioxidant system mechanisms in plants that lead to removing ROS toxins from the plant (Abbas, 2018). Three possible mechanisms are proposed in ROS scanning in response to the Se application. These mechanisms involve superoxide (O^{2-}) breakdown in hydrogen peroxide (H_2O_2), direct cooling of O^{2-} and hydroxide ion (OH^-), and antioxidant activity regulation by various enzymes. (Balal et al., 2016). Data on the interaction of selenium in plant hormone levels under high temperatures are unavailable. Recently Li et al. (2021) reported on the interaction of nano selenium in limiting cadmium damage on pepper plants (*Capsicum annuum* L.) improves plant hormone content, including jasmonic acid (JA), ABA, salicylic Acid (SA), and brassinolide (BL).

Despite numerous studies on Se's role in mitigating the toxicity associated with heavy metals or salinity, there are only a few reports about the mitigating effect of Se in case of heat stress. As a result, treating plants with selenium foliar may adjust oxidative stress and antioxidant metabolism in young date palm plants, enabling them to withstand high temperatures. This research aims to assess selenium's capacity to prevent oxidative damage caused by high temperatures and to encourage the development of date palm seedlings under heat stress.

2 MATERIALS AND METHODS

The experiment was carried out in Date Palm Research Center, University of Basrah. Seeds of 'Barhee' date palm were approved by Date Palm Research Center, Basrah University to conduct the experiments in 2021 and 2022. For germination, seeds were grown in unadulterated sand soil in an incubator for two months at a temperature of 27 ± 2 °C. Seedlings were separately moved to plastic pots (5 kg) filled with sand with particle size 0.7-2.0 mm, and peat moss (White Sphagnum Peat, H 1-3 von post) in a 2:1 proportion. Seedlings were developed in the wooden canopy at 30 ± 2 °C, with a relative humidity of about 20 %, and photoperiod maintained at 12 h d^{-1}. Thirty seedlings were selected to experiment. The development of 15 seedlings in the wooden canopy continued under the previous temperature (27 ± 2 °C), for two consecutive years (2021-2022), while the other fifteen seedlings were transported to the field in 2022. The service process and irrigation are carried out evenly, regularly, and as needed. On 1st May 2021, the experiment used a randomized block design with two factors (heat stress) at two levels (field and canopy temperature) and three concentrations of nano-selenium* (0, 15, and 30 mg l^{-1}). Each of the six treatments had five replications. A year later, in 2022, the selenium treatments were applied again on 1st May 2022. Temperature, humidity, and illumination intensity are measured inside and outside the canopy for five months (Table 1).

Five seedlings per treatment were utilized for growth analysis on 1st October 2022. The number and length of leaves were recorded. The leaves of each seedling were isolated and weighed to establish the fresh weight. Dehydration in an oven at 70 °C for two days defines the dry matter.

2.1 CHLOROPHYLL CONTENT IN LEAVES

According to Lichtenthaler and Wellburn (1983), 100 mg of fresh leaves were squashed in 10 ml $(CH_3)_2CO$ acetone (80 %) and centrifuged at 2000 rpm for five minutes. Chlorophyll content was colorimetry estimated at 663 and 645 nm. According to the following equation: Total chlorophyll (mg l^{-1}) = 20.2 (O.D. 645) + 8.02 (O.D. 663).

2.2 PROLINE CONCENTRATION IN LEAVES

Proline content was assessed according to Bates et al. (1973). The leaf sample (0.5 g) was homogenized with 5 ml of 3 % sulfosalicylic acid. This mixture was separated, and 3 ml was mixed with ninhydrin reagent (3 ml) and glacial acetic acid (3 ml). This mix was warmed in a bubbling water bath for an hour until it reached 90 °C and quickly cooled to 25 °C. A chromophore was shaped by adding 4 ml toluene to the cold solution. The absorbance was measured at 520 nm using the UV-VIS spectrophotometer. Proline solution (0-10 µg ml^{-1}) was used as a standard.

2.3 RELATIVE WATER CONTENT (RWC) IN LEAVES

Plant leaves were weighed (fresh biomass) instantly

Table 1: Light intensity and temperature change during the growing season of 2022

		Months				
Weather elements	location	May	June	July	August	September
Maximum temperature (°C)	Field	43.31	46.86	48.34	47.32	42.23
	Canopy	37.42	42.57	43.61	41.54	36.87
Minimum temperature (°C)	Field	25.73	26.55	32.34	29.94	26.21
	Canopy	19.45	20.45	26.43	23.56	21.34
Relative humidity (%)	Field	21.18	26.34	32.45	34.74	28.54
	canopy	28.54	32.56	38.43	38.43	34.56
the light intensity µmol m^{-2} s^{-1}	Field	341.14	370.832	382.463	389.528	352.547
	canopy	325.832	362.834	365.743	370.264	328.496
Rainfall (mm)	Field	0	0	0	0	0
	canopy	0	0	0	0	0

Selenium nanoparticles, purity: 99.9 %, APS: less than 80 nm, stock no: NS6130-01-171. Nanoshel LLC company, 3422 Old Capitol Suit 1305, Willmington DE – 19808, United States upon harvesting, drenched in distilled water at 25 °C for 24 h to calculate turgid mass, and afterward dried in the oven at 80 °C for 48 h to calculate dry biomass. The following equation was used to calculate *RWC: RWC = (fresh mass-dry mass) / (turgid mass-dry mass)* x 100.

2.4 ASCORBIC ACID CONTENT IN LEAVES

The method of Luwe et al. (1993) was used to measure ascorbic acid (AsA). 10 ml of 6 % trichloroacetic acid was used to homogenize leaves tests (0.5 g). The concentrate was combined with 2 ml of 2 % dinitrophenylhydrazine (pH 5) and one drop of 10 % thiourea (in 70 % ethanol). After bubbling for 15 minutes in a water bath, the mixture cooled at room temperature before mixing with 5 ml of 80 % (v/v) H_2SO_4 at 0 °C. The absorbance was measured at 265 nm. A standard bend plotted with its known focus was used to calculate the ascorbic acid content.

2.5 TOTAL PHENOLIC ASSAY OF LEAVES

The Folin-Ciocalteu technique was used to evaluate the phenol extract (Waterman & Mole, 1994). A 25 µl concentrate (500 µg ml^{-1}) was used, along with 25 µl of (1:1) Folin-Ciocalteu reagent and 100 µl of 7.5 % sodium bicarbonate solution, and hatched at room temperature for 2 hours in dim conditions. The absorbance was measured at 765 nm using a UV-VIS spectrophotometer. Gallic acid from 0 to 100 µg ml^{-1} was used as a standard to calculate the phenol content of the sample.

2.6 MALONDIALDEHYDE (MDA) CONTENT IN LEAVES

Davey et al. (2005) report that leaves (0.2 g) were homogenized in ten quantities of 80 % ethanol on frozen ground and separated by centrifugation at 14000 x.g. for 15 min. The permeate was incubated at 97 °C for 20 minutes with an equivalent amount of 0.70 % (w/v) thiobarbituric acid (TBA) solution that contains 200 percent (w/v) trichloroacetic acid and 0.01 % oxytoluenes. 5 µl of the supernatant was used for HPLC analysis using an ODS column (4.6 mm) acclimatized to 35 % methanol through 60 mM buffer with potassium phosphate (pH = 6.8) after cooling and centrifugation. MDA was measured at 540 nm after being eluted at 1.5 ml min^{-1}. MDA prepared chemically by acid-hydrolysis of tetra ethoxy propane was used for calibration.

2.7 HYDROGEN PEROXIDE (H_2O_2) IN LEAVES

The hydrogen peroxide was extracted to cold acetone using the method described by Tabatabai (1998). The extract was quantitatively mixed with titanium tetrachloride and ammonia to create a peroxide-Ti complex. Centrifugation was used to collect the complex, which was then dissolved in 2 M sulfuric acid. The solution's absorbance was measured at 420 nm, and the H_2O_2 content was calculated using the standard curve.

2.8 ANTIOXIDANT ENZYMES IN LEAVES

Activities of ascorbate peroxidase (APX) and catalases (CAT) were determined using the protocol of Radić et al. (2009) using a spectrophotometer.

2.9 HORMONES ANALYSIS IN LEAVES

To ensure data reliability, indoleacetic acid (IAA), abscisic acid (ABA), gibberellic acid (GA3), and zeatin (ZT) were determined using the same tissue extracts. Date fruit samples were washed and dried with a paper towel. They were immediately placed in liquid nitrogen, and stored at -20 °C for 48 hr. One gram of fresh mass (FM) samples were ground in liquid nitrogen and medium-term extracted with 30 ml of 80 % cold methanol at 4 °C. The concentrate was centrifuged for 15 minutes at 2000 x.g. and 4 °C and the supernatant was collected. At that point, new cold methanol was used to fill the remainder, extracted four times using the methods described above. The all-out methanolic separate was dried in a rotary evaporator and divided into 10 ml aliquots of methanol. According to Tang et al. (2011) IAA, ABA, GA3, and ZT were determined by infusing the concentrate into a turnaround stage HPLC on a switch stage C18 section (250 4.60 mm, 5 microns) in an isocratic elution mode utilizing a portable stage comprised of acetonitrile: water (26:74) with 30 mM phosphoric acid.

2.10 STATISTICAL ANALYSIS

Analysis of variance (ANOVA) was performed on the data using SPSS variant 21.0 (SPSS, Chicago, IL), and the means were separated using the Duncan test at the 5 % significance level.

3 RESULTS

3.1 NANO-SELENIUM PROMOTES THE GROWTH OF DATE PALM SEEDLINGS

Field temperature of heat stress reduced the growth of seedlings by reducing plant height and leaf numbers (Fig. 1). Treatment of selenium 15 mg l^{-1} in canopy and field temperature significantly ($p \leq 0.05$) increased the plant height and leaf numbers. While the control treatment recorded the lowest plant height and number of leaves under the field temperature. No significant differences between the canopy and field temperature under selenium 30 mg l^{-1} in leaf numbers.

3.2 NANO-SELENIUM ENHANCES THE CHLOROPHYLL, RWC, PROLINE, AND ASCORBIC ACID CONTENTS IN DATE PALM SEEDLINGS

The contents of chlorophyll, RWC, and AsA in seedlings were reduced significantly ($p \leq 0.05$) by field temperature. At the same time, proline levels increased (Fig. 2). Selenium spraying improved total Chl, RWC, Pro, and AsA levels in seedlings. Under canopy temperature, selenium increased total Chl and proline. In the field, however, selenium increased total chlorophyll and Pro, RWC, and AsA compared to the control. Under field and canopy temperature, a selenium concentration of 15 mg l^{-1} increases total Chl. There were no significant differences between Se treatments at 15 and 30 mg l^{-1} selenium in RWC in AsA under canopy and temperature.

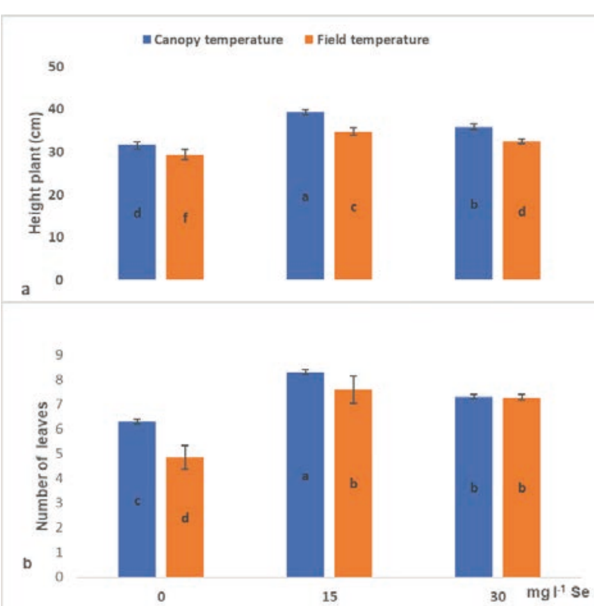

Figure 1: The response of date palm seedlings to nano-selenium in (a) plant height and (b) leaves number under field and canopy temperature (n = 5; means ± SE). Means denoted by different letters differ significantly at $p \leq 0.05$

3.3 NANO-SELENIUM ENHANCES TOTAL PHENOL AND REDUCES OXIDATIVE STRESS IN DATE PALM SEEDLINGS

Phenols, MDA, and H_2O_2 increased significantly ($p \leq 0.05$) under field temperature (Figure 3). Selenium increased total phenols under the canopy and field temperature. Se at 15 mg l^{-1} increased total phenol under field temperature, whereas MDA and H_2O_2 decreased. No significant differences were shown between Se at 15 and 30 mg l^{-1} in the content of MDA and H_2O_2 in field temperature.

3.4 NANO-SELENIUM ENHANCES THE ACTIVITIES OF ENZYMES IN DATE PALM SEEDLINGS

In the field, APX activity increased while CAT activity decreased. Date palm seedlings' responses to selenium spray increased significantly ($p \leq 0.05$) the activity of APX and CAT enzymes (Figure 4). Selenium increased enzyme activity under canopy and field field temperature. Under canopy and field temperature, Se at 30 mg l^{-1} increased enzyme activities compared to 0 mg l^{-1}. In either the canopy or the field temperature, selenium at both concentrations had no significant effect on increasing the activities of the APX enzyme.

3.5 NANO-SELENIUM ENHANCES THE CONTENT OF GROWTH REGULATORS IN DATE PALM SEEDLINGS

High field temperature decreased IAA, GA, and CK while increasing ABA. Selenium spraying increased significantly ($p \leq 0.05$) IAA, GA, CK, and ABA levels in date palm seedlings (Fig. 5). Selenium increased GA, CK, and ABA while decreasing IAA under canopy temperature. In the field, Se at 15 mg l^{-1} increased IAA and GA while decreasing ABA compared to the control. Under field temperature, Se at 30 mg l^{-1} significantly ($p \leq 0.05$) increased CK and ABA levels.

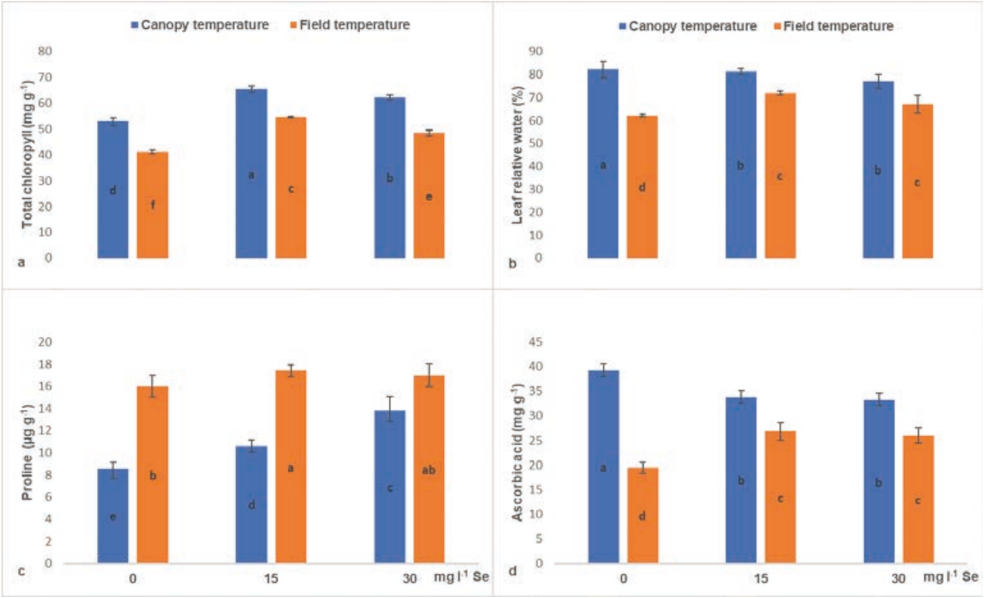

Figure 2: Date palm seedlings respond to nano-selenium in (a) Total chlorophyll, (b) RWC, (c) Proline, and (d) AsA under field and canopy temperature (n = 5; means ± SE). Means denoted by different letters differ significantly at $p \leq 0.05$

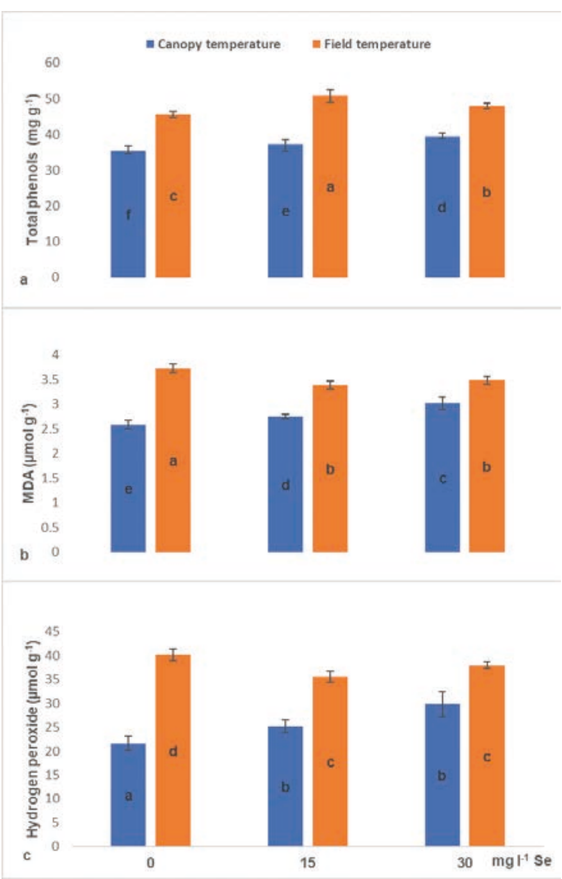

Figure 3: Response of date palm seedlings to nano-selenium in (a) Total phenols, (b) MDA, and (c) H_2O_2 under field and canopy temperature (n = 5; means ± SE). Means denoted by different letters differ significantly at $p \leq 0.05$

4 DISCUSSION

Heat stress is one of the primary causes of plant growth decline because the energy of the photon needed for photosynthesis raises the temperature of the tissues exposed to light (Missaoui et al., 2017)and to validate the hypothesis that genes underlying stem determinacy might be involved in the mechanism of summer dormancy. Our results suggest that vernalization is an important requirement in the onset of summer dormancy in tall fescue. Non-vernalized tall fescue plants do not exhibit summer dormancy as vernalized plants do and behave more like summer-active types. This is manifested by continuation of shoot growth and high root activity in water uptake during summer months. Therefore, summer dormancy in tall fescue should be tested only in plants that underwent vernalization and are not subjected to water deficit during summer months. Total phenolic concentration in tiller bases (antioxidants. The plant is subjected to oxidative stress when subjected to heat stress. Any factor that reduces oxidative stress damage, such as selenium, improves plant growth and tolerance to surrounding conditions (Alharby et al., 2021).

Although the mechanisms associated with the action of Se reduce the effect of specific environmental stresses such as light, high temperatures, drought, heavy metal, and salinity remain incomplete. Several studies have indicated that exogenous nano-Se improves plant growth under stressful or non-stressful conditions (Banerjee & Roychoudhury, 2019).

Several studies have shown an improvement in

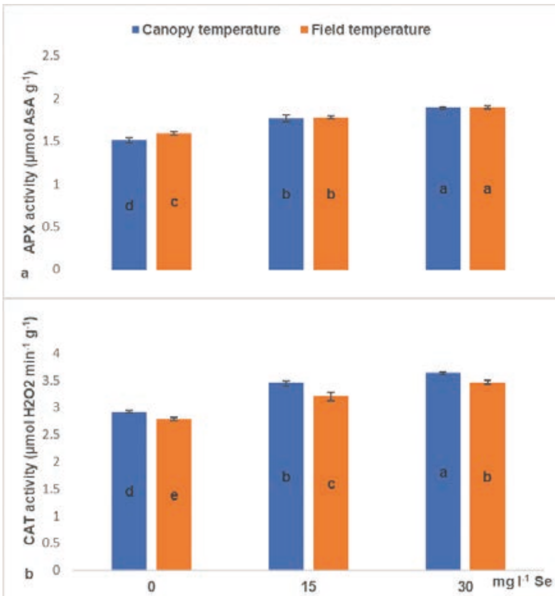

Figure 4: Date palm seedlings respond to nano-selenium in activities of enzymes (a) CAT and (b) APX under field and canopy temperature (n = 5; means ± SE). Means denoted by different letters differ significantly at $p \leq 0.05$

growth in selenium-treated plants such as lettuce (Hawrylak-Nowak et al., 2018), maize (Fernandez et al., 2018) Maize (Zea mays L., potatoes (Somalraju et al., 2022) solutions to manage late blight in organic systems are scarce. This study was undertaken to evaluate the effect of selenium (Se, and soybeans (Alharby et al., 2021) by regulating the water state of the plant or delaying aging. The results showed that nano-Se improved plant growth in both canopy and field temperature (Fig. 1). Our study showed that heat stress decreased the chlorophyll content, whereas Se increased the chlorophyll content in the field and canopy temperature (Figure 2a). Under field temperature, chlorophyll content decreases due to low chlorophyll biosynthesis and disrupted photosystem biochemical reactions (Mathur et al., 2014)ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco. Appropriate selenium levels can reduce chlorophyll damage and increase chlorophyll content in various plants (Missaoui et al., 2017)and to validate the hypothesis that genes underlying stem determinacy might be involved in the mechanism of summer dormancy. Our results suggest that vernalization is an important requirement in the onset

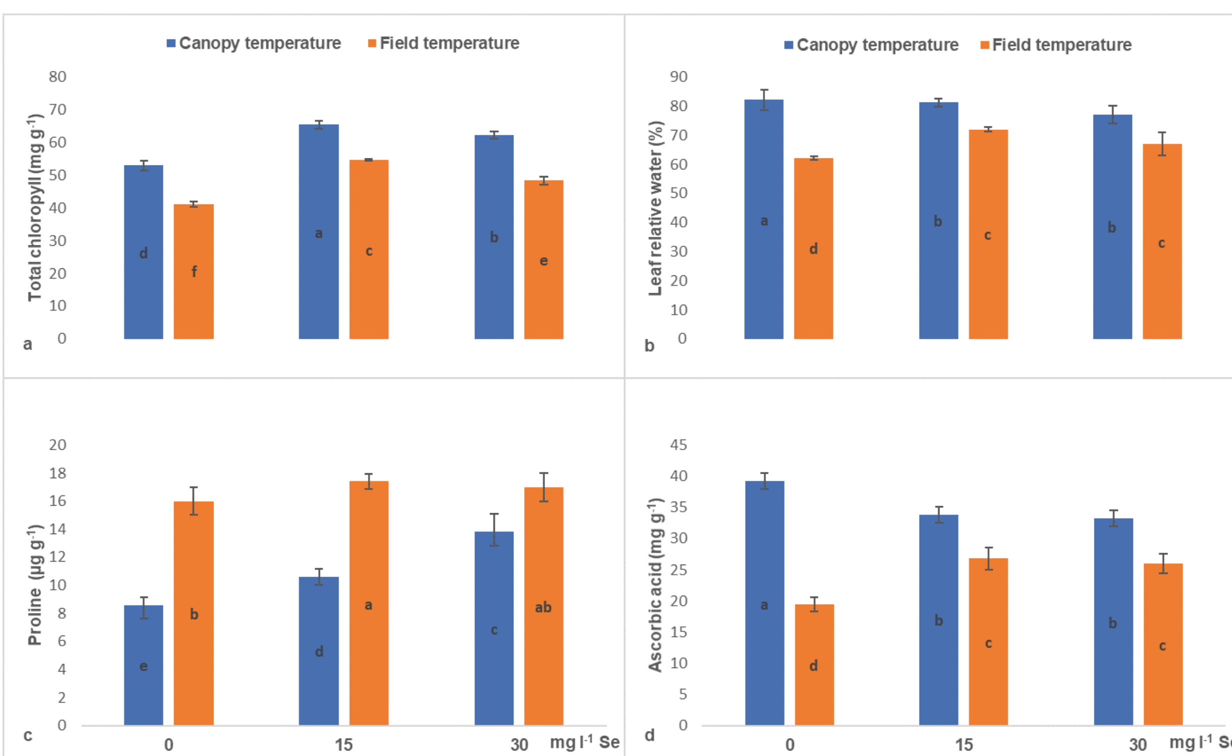

Figure 5: Date palm seedlings respond to nano-selenium in (a) IAA, (b) GA3, (c) CK, and (d) ABA under field and canopy temperature (n = 5; means ± SE). Means denoted by different letters differ significantly at $p \leq 0.05$

of summer dormancy in tall fescue. Non-vernalized tall fescue plants do not exhibit summer dormancy as vernalized plants do and behave more like summer-active types. This is manifested by continuation of shoot growth and high root activity in water uptake during summer months. Therefore, summer dormancy in tall fescue should be tested only in plants that underwent vernalization and are not subjected to water deficit during summer months. Total phenolic concentration in tiller bases (antioxidants. Selenium's beneficial effects on chloroplast enzymes increase the photosynthesis of photosynthetic pigments and, as a result, the chlorophyll content (Jiang et al., 2017)1, 5 and 25 µM Na 2 SeO 3.

Plants absorb less water when exposed to severe environmental conditions, including drought and extreme heat (Hussain et al., 2019). Therefore relative water content is considered an efficient parameter for evaluating plants to withstand those stresses (Pour-Aboughadareh et al., 2019). In our results, field temperature led to a significant reduction in RWC (Figure 2b) which is supported by other studies (Hasanuzzaman et al., 2014; Manafi et al., 2021). Spraying selenium on heat-stressed seedlings conserves cell water and improves water absorption (Malerba & Cerana, 2018). The use of selenium nanoparticles in the treatment of seedlings increased the reinforcement and protective capacity by providing an effective antioxidant system against heat stress. This is because nanoparticles can penetrate the leaves, improving the plant's ability to absorb and use water, which led to the creation of an enzymatic system and enhanced seedling growth (Wang et al., 2021).

Abiotic stresses cause plants to accumulate large amounts of proline, which helps in proteins, reduces hydroxyl radicals, regulates cellular pH, and maintains turgor pressure (Hao et al., 2021). The increase in proline accumulation under heat stress conditions could be attributed to a lack of the enzyme proline oxidase (POX) (Servet et al., 2012)as an initial set of indicators may need further development and refining. This chapter mentions several frameworks that have been proposed to measure the progress of societies. The point is simply that there are potentially useful frameworks that seek to capture the definition and scope of national wellbeing. They provide potential starting points. However, the framework that is most suitable for a given locality, country or group of countries should be determined through a process of deciding and meeting user requirements, which is explored in the chapter. For the wellbeing conceptual framework that the Organisation for Economic Co-operation and Development (OECD. Increased protein decomposition due to high temperatures caused by increased activity of the protease enzyme under stress conditions to release free amino acids, including proline, for storage, transport, or use in the plasmolysis modification (Alahmad et al., 2022). From our results, high field temperature significantly increased the proline content (Figure 2-c). Similar proline levels have been observed in sugarcane (*Saccharum officinarum* L.) under heat stress (Elsheery et al., 2020).

In contrast, selenium spraying increased proline content in heat-stressed plants, related to higher water content and reduced oxidative stress (Balal et al., 2016). The stressful environments lead to increased proline in the plant, which contributes to stress tolerance by preventing cell degradation and maintaining osmotic balance, and stability of membranes, thus preventing electrolyte leakage. Proline accumulation causes ROS concentrations to be placed within normal ranges, thus preventing the explosion of oxidation in plants (Yaish, 2015).

Increased production of phenolics in plants exposed to stress is an adaptation of the plant to the surrounding conditions (Šamec et al., 2021). From our results, field temperature significantly increased the phenol content in the leaves and nano-Se at 15 mg l^{-1} increased total phenol under field temperature (Figure 3a). Saffaryazdi et al. (2012) found that exogenous selenium on spinach (*Spinacia oleracea* L.) increased total phenols in the leaves. Selenium significantly increased phenolic contents by increasing phenylalanine ammonia-lyase activity (PAL) (Walaa et al., 2010).

Ascorbic acid acts as the main store of oxidation and reduction and as a cofactor for enzymes that regulate plant hormones, photosynthesis, antioxidant regeneration, cell division, and growth (Abdellatif & Ibrahim, 2018). By removing ROS, AsA protects cells and organelles from oxidative damage (Al-Zahrani et al., 2022). Heat stress reduced AsA. In turn, selenium increased the contents of ascorbic acid under heat stress (Figure 2, d). AsA is a precursor to the construction of chlorophyll and thus increases photosynthesis leading to the accumulation of various parts of soluble sugars in plant tissues under stress conditions, in addition to possibly mitigating the negative impacts of overheating through the removal of oxidative agents and the prevention of protein oxidation (Paciolla et al., 2019).

H_2O_2 can cause oxidative stress when its level rises in cells and can increase due to heat stress and other abiotic stresses (Hossain et al., 2015). Extreme heat raised MDA and H_2O_2 (Fig. 3, b-c). An ineffective antioxidant defense system under field temperature caused these findings. Due to high levels of antioxidants and the activities of antioxidant enzymes, plants treated with selenium showed lower MDA and H_2O_2 when exposed to heat stress (Gupta & Gupta, 2017). On the other hand, a high concentration of selenium can likely harm plants by stimulating ROS generation (Lehotai et al., 2012).

APX hinders the accumulation of H_2O_2 by lowering it to H_2O in the AsA-GSH cycle as an enzyme's first line of defense (Hasanuzzaman et al., 2014). APX activity increased significantly under heat stress (Fig. 4, a). However, a high dose of selenium (30 mg l^{-1}) increased CAT and APX activity in stressful and non-stressful conditions (Fig. 4). Se increases antioxidant capacity, reduces free radical production, and promotes biomass accumulation under high temperature (Balal et al., 2016). Se-treated seedlings showed higher activity of CAT enzymes in canopy conditions than in field temperature. Catalase plays a vital role in converting H_2O_2 to H_2O. CAT activity reduces oxidative stress (Manafi et al., 2021). Furthermore, selenium nano-treatments significantly increased APX and CAT levels. Under heat stress, selenium is an active ingredient that can stimulate antioxidant enzyme gene expression and protein biosynthesis (Safari et al., 2018). Se increased the transcription of antioxidant defense genes, improving overall enzymatic activity in maize (*Zea mays* L.) (Jiang et al., 2017)1, 5 and 25 μM Na 2 SeO 3.

Thermal stress decreased the content of growth-promoting hormones IAA, GA, and CK and increased ABA (Fig. 5). Se reduced heat stress by increasing nutrient absorption, including nitrogen absorption (Shalaby et al., 2021). Nitrogen works to stimulate and produce auxin. Nitrogen is necessary for constructing the amino acid tryptophan, which forms the basis for the structure of indole acetic acid. IAA encourages the process of cell division and elongation of cells (Labeeuw et al., 2016). Plant hormones, on the other hand, such as IAA, stimulate the activation of the ATPases plasma membrane leading to hyperpolarization of the cell membrane (Zhang et al., 2017). The rise in the content of IAA, GA, and Ck plant hormones after selenium treatment under heat stress conditions could be related to selenium's beneficial role in reducing water loss by transpiration. Increased cell volume leads to plant hormone regulation and growth (Wang & Irving, 2011). Li et al. (2021) reported that the interaction of nano selenium in reducing cadmium damage on pepper plants improves the content of ABA in the roots and leaves. Malheiros et al. (2019) indicated that selenium regulates the development of primary and lateral roots in rice seedlings, resulting in novel patterns of root architecture through changes in auxin and ethylene levels. The high concentration of nano-selenium 30 mg l^{-1} showed a better effect than 15 mg l^{-1} in increased enzyme activities compared to controls.

Although nano-selenium reactive molecules may harm cell membranes and molecules, they may be essential signals in the reaction stages. These reactive molecules can activate cellular defense mechanisms, thereby mitigating the adverse effects of stress. When exposed to abiotic stress, the plants showed a specific response (Ramegowda & Senthil-Kumar, 2015). ABA appears as a chemical indicator sent by roots to the leaves to activate the mechanism of controlling water loss and closing stomata (Saddhe et al., 2017). Hormone results showed that nano-selenium stimulates the biosynthesis of natural hormones by increasing the levels of those substances in the leaves. The presence of abundant plant hormones may be crucial in regulating plant growth.

5 CONCLUSION

The findings showed that low concentrations of nano-selenium significantly improved the enzymatic and non-enzymatic antioxidant defense components in date palm seedlings exposed to heat stress. The enhanced antioxidant defense system shields seedlings from lipid peroxide and H_2O_2 overproduction. Nano-Se affects the metabolism of stressed and non-stressed plants. Nano-selenium improves the antioxidant defense system under heat stress by regulating growth regulators (IAA, GA, CK, and ABA), enzymatic activities, and osmolyte soluble such as proline, ascorbate, and phenols. Nano-selenium can be used to protect seedlings of date palms against high temperatures.

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