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Enhanced water regulation, antioxidant capacity, and resilience of *Abelmoschus esculentus* (L.) Moench in drought stress

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Abstract
The discovery of plants that are capable of tolerating severe abiotic pressures is vital for the development of environmentally friendly agriculture. This is because a variety of metabolic functions, especially photosynthesis, are inhibited as a result of high heat and drought. *Abelmoschus esculentus* (L.) Moench, more generally known to as okra, is a member of the Malvaceae family. It is resistant to harsh climatic circumstances, mainly drought and heat, and its leaves and berries continue to offer a substantial quality of nutrients. The current investigation was conducted to assess the effects of drought stress on the growth, physiology, enzyme activity, and water consumption efficiency of *Abelmoschus esculentus* (L.) Moench. Twenty-five potting pots, one of which acted as a control, were utilized in the experiment, which was developed following a pattern called Completely Randomized Block Design. Plant height, leaf number, stem girth, root length, shoot length, shoot weight, relative water content, and leaf area ratio were all shown to decrease when the plant was exposed to drought stress. Additionally, the efficiency with which water was utilized also dropped. The length of time that the stress treatment was delivered, as well as the activity of antioxidant enzymes including the enzyme superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), were all elevated. Nevertheless, both the stress tolerance index and the chlorophyll levels were lower than they were previously. The plants that were treated to drought displayed improved antioxidant capacity, water regulation, and drought stress resistance when compared to the group that acted as the control. Underscoring the adaptability of *Abelmoschus esculentus* (L.) Moench to locations prone to drought is the fact that these observations were achieved. They supply important data for exploring drought tolerance in this species and creating water management approaches for its production.

Keywords: Drought stress, *Abelmoschus esculentus*, Resilience, Water, Adaptation
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Introduction

The development of plants is vulnerable to the management of several environmental stressors, which may lead to losses in agricultural production (Yu, et. al., 2016, Ahanger *et al*., 2019). The influence of changing climates, and the possibility of catastrophic occurrences lead to losses in agriculture (Pachauri *et al*., 2014; Raja *et al*., 2020). Water deprivation is acknowledged to be one key abiotic stress factor affecting plant production because of its harmful consequences on plant development (Khan *et al*., 2017; Dąbrowski, 2019, Oraee and Tehranifar, 2020). Various physiological and biochemical systems, including photosynthesis, respiration, translocation, ion absorption, carbohydrates, nutrition metabolism, and growth stimulants reduce

plant development in times of drought stress (Li *et al*., 2020).

The main attribute to fluctuations in abiotic and biotic factors such as soil water content, temperature, and nutrient availability are following the stress-gradient hypothesis. In the 21st century, global warming has adversely impacted the style and quantity of rainfall in major areas of the planet which has led to lengthy periods of drought (Ma *et al*., 2022). The impact of drought on the interactions of plant species influences the carbon cycle and nutrient components in the ecosystem.

Response to stress circumstances by plants varies from either molecular, biochemical, or physiological responses. These include the expression of certain genes, increasing osmolyte accumulation, and activation of the antioxidant system whether enzymatic or non-enzymatic (Reddy *et al*., 2002). Physiological responses occur when plants regulate photosynthetic rates via the manipulation of photosystem II, low electron transport rate, and increase in stomata closure (Khan *et al*., 2023). However, creative solutions for the protection of crop production under adverse drought, salt, and heat stress conditions are undoubtedly the major difficulty being tackled by current agriculture (Albdaiwi *et al*., 2019).

Okra (*Abelmoschus esculentus* L.) is a traditional vegetable crop that belongs to the Malvaceae family and which is largely grown in East Africa. Countries such as Nigeria, India, and Sudan are classified as the greatest okra producers in the world, with 2.06 million tons, six million metric tons, and 0.297 million tonnes accordingly as stated by FAO (2019). It bears edible fruits and it is especially rich in carbohydrates, protein, fat, fibres, minerals, and vitamins. Okra is a key product in the pharmaceutical field owing to its high polysaccharides and bioactive compounds contents. Drought stress, according to Razi aand Muneer, (2023) and Adejumo *et al*. (2023) reduces the yield of Okra, thus the need for more studies that will quantify the degree of tolerance to drought so as to encourage such breeding efforts. This research was conducted to find out the impact of drought-related stress on plant development, physiology, Enzyme activity, and water consumption efficiency of *Abelmoschus esculentus*.

Materials and Methods

This research was done at the Department of Plant Science and Biotechnology's screen house, Federal University, Oye Ekiti, situated at latitude 7.80^{o}N and longitude 5.21^{o}E while the Laboratory studies were also carried out in the Departmental Laboratory.

Seeds of *Abelmoschus esculentus* employed in the study were acquired from a National Centre for Genetic Resources and Biotechnology (NAGRAB). They were planted in pots in the screen house while the sandy-loamy soil employed was purchased from the University community. The pH of the soil was tested in water using a pH meter. Twenty-five (25) planting pots were set up in five replicates per treatment and were structured in a Completely Randomized Block Design pattern along with the control experiment. Stress intervals of five (5), ten (10), fifteen (15), and twenty (20) days were employed as the treatments while a control experiment was not deprived of water at any stage. Drought was introduced a day after blossoming.

Soil Test

Pre-soil test was carried out before planting, data were collected and reported accordingly.

Morphological Parameters

The height of the plant was obtained by a metre rule, the meter rule was placed on the surface of the soil to the topmost part of the stem for each seedling while stem girth was measured using a Vernier Caliper.

Leaf area was calculated using this method (Length x Width x 0.75), number of leaves present in each plant was gathered by counting. Weights of shoot consisting of all the component plants above the soil level are computed using an automated weighing scale. Relative Growth Rate was computed using this formula (Hoffmann and Poorter, 2002)

Relative Growth Rate = $(\ln W_2 - \ln W_1)/(t_2 - t_1)$

Where:

In–natural logarithm
t_1 – initial time in days
t_2 – final time in days
W_1 – initial size at time
W_2 – final size at time
Assimilation Re was calculated according to Vrno and Allion (1963)

$$\frac{(InL_2 - In)(L_1W_2 - W_1)}{(t_2 - t_1)(L_2 - L_1)}$$

where W_1 and L_1 are the total dry matter and leaf area at time t_1 and W_2 and L_2 at time t_2, respectively.
Leaf Area Ratio was calculated using this formula

$$F = \frac{(L_1/W_1) + (L_2/W_2)}{2}$$

Stress tolerance index (STI)
STI determines high yield and tolerance stress potential, these were calculated as follows (Wilkins 1957)
RLSTI (Length of Root Stress Tolerance Index) = (stressed plant root length/control plant root length × 100 (Gardea-Torresdey *et al*., 2004)
SLSTI (Length of shoot Stress Tolerance Index) = (stressed plant shoot length/control plant shoot length) × 100 (Salunkhe *et. al*., 1998)
RFSTI (Weight of Fresh Root Stress Tolerance Index) = (fresh weight of plant root stressed plant/control plant root fresh weight) × 100 (Barnhart, 1997)
SFSTI (Weight of Fresh Shoot Stress Tolerance Index) = (stressed plant shoot fresh weight/control plant shoot fresh weight) × 100 (Pillay and Wang, 2003)
RDSTI (Root Dry Weight Stress Tolerance Index) = (stressed plant root dry weight/control plant root dry weight) × 100 (Kotaś and Stasicka, 2000)
SDSTI (Shoot Dry weight Stress Tolerance Index) = (stressed plant Shoot dry weight/ control plant Shoot dry weight) × 100 (Zayed *et. al*., 1998).

Relative Water Contents
Relative water contents were determined using a technique outlined by (Turner, 1981). Extracted leaves were immersed in double-distilled H2O in the dark. The foliage leaves were withdrawn from the double distilled water after 24 hours, cleaned with a blotting paper that was sanitized, and put on a digital weighing scale to acquire Turgid Weight (TW). Label bags were utilized, Leaves, and were kept at 65oC in an automated oven for 72hrs, afterward, data for the dry weight (DW) was obtained.
Relative Water Content was calculated using the (Turner, 1981) formula, i.e.

$$RWC(\%) = \frac{(Fresh\ Weight - Dry\ Weight)}{(Turgid\ weight - Dry\ weight)} \times 10$$

Chlorophyll Pigment Extraction and Measurement of Chlorophyll (a and b) Fluorescence
Leaves collected from the control and stressed groups were cut and processed for the measurement of chlorophyll. In general, 0.05 g of leaves were homogenized in aqueous buffered acetone using a mortar and pestle that was pre-cooled. This homogenate was placed in a centrifuge for 2 minutes at a 10000 rate per minute and refrigerated at 4ºC. The supernatant's volume was adjusted back to 5 ml using buffered acetone after the pellet was discarded. The ideal absorbance of the supernatants was obtained at 480, 645, and 663 nm wavelengths using a double-beam UV-VIS spectrophotometer against 80% buffered acetone which was used as blank. Chlorophyll and carotenoid pigments content were obtained using (Arnon, 1949) and (Lichtentaler and Buschman, 2001) procedures.
Enzyme assays
1.0 g of fresh young leaf material was homogenized with a mortar and pestle in 3 ml of ice-cold 100 mM K-phosphate buffer pH 6.8 containing 0.1 mM EDTA for 5 minutes. After filtration through cheesecloth, the homogenate was centrifuged at 16,000 g for

15 minutes, and the supernatant was used as the source of enzymes. All the steps were carried out at 0–4°C.

The activity of guaiacol peroxidase (POX) was determined by adding 25 µl of the crude enzyme preparation to 2 ml of a solution containing 50 mM potassium phosphate buffer pH 6.8, 20 mM guaiacol, and 20 mM H_2O_2. After incubation at 30°C for 10 minutes, the reaction was stopped by adding 0.5 ml 5% (v/v) H2SO4 and the absorbance was read at 480 nm (Urbanek *et al*., 1991). One POX unit was defined as the change of 1.0 absorbance unit per ml enzymatic extract and expressed as units of enzyme activity per g fresh matter per minute (UA g^{-1} FW min^{-1}).

Catalase (CAT) activity was determined by adding 50 µl enzymatic extract to 3 ml of a solution containing 50 mM potassium phosphate buffer pH 7.0 and 20 mM H2O2 and measuring the decrease in absorbance at 240 nm and 30°C (Havir & McHale, 1987). Enzyme activity was calculated using the molar extinction coefficient 36 × 103 mM^{-1} m^{-1} and expressed as µmol H_2O_2 oxidized g^{-1} FW min^{-1}.

The activity of SOD was determined by adding 50 µl of the enzymatic extract to a solution containing 13 mM methionine, 75 µM p-nitroblue tetrazolium chloride (NBT), 100 µM EDTA, and 2 µM riboflavin in a 50 mM potassium phosphate buffer pH 7.8.

It is important to note that the results of enzyme activities measured in both control and salt-treated leaves were not affected by the addition of serine and cysteine proteinase inhibitors (1 mM phenylmethylsulfonyl fluoride + 1 µg ml^{-1} aproptinin) in the extracting buffer when soluble proteins were extracted in the absence of these proteinase inhibitors. Therefore, the inhibitors were not included in the extracting buffer. The reaction took place in an illuminated chamber, under the light of a 30 W fluorescent lamp at a temperature of 25°C. The reaction was initiated by turning on the fluorescent lamp and stopped 5 minutes later by turning it off (Van Rossun *et al*., 1997).

The blue formazane produced by NBT photo-reduction was measured as an increase in absorbance at 560 nm. The control reaction mixture did not have enzyme extract. The blank solution had the same complete reaction mixture but was kept in the dark.

One SOD unit was defined as the amount of enzyme needed to inhibit 50% of the NBT photo-reduction in comparison with tubes that lacked the plant extract. The enzyme activity was expressed as units of enzyme activity (AU) g−1 FW min−1. Data obtained were analyzed using IBM SPSS Statistics 22.

Results

The findings demonstrated that there was no significant difference in the plant height between the control and the groups receiving treatment plants (Table 1), the same was observed in stem girth, however, there were significant differences in the number of leaves between the treated plants and the control, leaf numbers reducing drastically as the days of drought increases (Table 1).

In Table 2, it was observed that the highest values for all the measured parameters were seen in the control experiment. As the drought period interval increased, the values were reduced. There was no significant difference observed in the root length and shoot length. However, on day 10, there was a statistically significant difference observed in the shoot weight when compared to other treatments (Table 2).

In Table 3, it was observed that there was a significant difference in the Relative Water Content of the control experiment when compared to other treatment groups except for the day 20 drought period. Here, it was significantly different from other treatment groups and the control experiment. Significant differences were observed in the Leaf Area measurements as well. There was a significant difference between the control experiment and day 5. Additionally, the day 5 drought period was significantly different from the other treatment groups.

Table 4 shows a significant decrease in the values observed in all the stress tolerance indices measured in the experiment as the treatment period increases. Generally, not many significant differences were observed across the board. However, there were significant differences in RFWSTI and SDWSTI.

The chlorophylls a and b contents decrease with increasing drought stress when compared with the control. The effects are more pronounced on day 15 with a drop from 3.52 to 1.48 in chlorophyll a, and from 12.35 to 0.65 in chlorophyll b (Table 5).

Table 1: Effects of drought stress on the plant height, number of leaves and stem girth of Okra

Treatments	Plant height (cm)	Number of leaves	Stem Girth (cm)
Control	41.95±3.40a	4.33±0.33b	2.67±0.12a
Day 5	33.78±1.50a	4.33±0.67b	1.92±0.13a
Day 10	36.87±2.80a	3.00±0.58ab	2.15±0.08a
Day 15	35.23±12.09a	1.67±0.33a	1.73±0.48a
Day 20	36.33±3.79a	1.00±0.58a	2.00±0.06a

Values in the same column with different alphabets are significantly different at P≤0.05 according to Duncan Multiple Range Test (DMRT)

Table 2: Effects of drought stress on the root length, shoot length and shoot weight of Okra

Treatments	Root length (cm)	Shoot length (cm)	Shoot weight (g)
Control	9.00±1.53^{a}	37.00±4.04^{a}	6.38±0.77^{a}
Day 5	8.70±0.76^{a}	42.00±1.15^{a}	4.36±0.23^{a}
Day 10	4.17±1.47^{a}	32.33±6.36^{a}	3.89±0.60^{ab}
Day 15	4.77±1.62^{a}	35.33±5.24^{a}	2.62±0.49^{a}
Day 20	4.50±1.76^{a}	34.00±1.53^{a}	2.37±0.35^{a}

Values in the same column with different alphabets are significantly different at P≤0.05 according to Duncan Multiple Range Test (DMRT)

Table 3: Effects of drought stress on the Relative Water Content, Leaf Area Ratio and Water Use Efficiency of Okra

Treatments	Relative Water Content (%)	Leaf Area (cm^2)	Leaf Area Ratio (cm^2g^{-1})	Water Use Efficiency ($gha^{-1}mm^{-1}$)
Control	68.00±0.58^{a}	111.00±8.73^{a}	36.25±4.57^{a}	0.06±0.01^{a}
Day 5	22.00±0.58^{b}	79.33±16.17^{ab}	30.50±0.31^{ab}	0.02±0.01^{b}
Day 10	22.00±0.58^{b}	55.00±13.23^{bc}	29.89±0.34^{ab}	0.02±0.00^{b}
Day 15	24.00±0.58^{b}	6.50±3.76^{c}	26.25±1.37^{ab}	0.02±0.00^{b}
Day 20	11.00±0.58^{c}	18.00±2.00^{c}	20.79±1.33^{b}	0.01±0.00^{b}

Values in the same column with different alphabets are significantly different at P≤0.05 according to Duncan Multiple Range Test (DMRT)

Table 4: Effect of drought on the Stress Tolerance Index of Okra

Treatments	RLSTI (%)	SLSTI (%)	RFWSTI (%)	SFWSTI (%)	RDWSTI (%)	SDWSTI (%)
Day 5	93.67±22.70[a]	97.00±5.69[a]	57.00±12.34[a]	89.00±11.15[a]	59.00±16.00[a]	134.00±15.27[a]
Day 10	50.67±20.38[a]	89.33±17.63[a]	30.33±8.69[ab]	86.33±30.49[a]	28.67±8.67[a]	63.33±12.01[b]
Day 15	52.00±11.85[a]	98.67±21.07[a]	15.33±7.00[b]	58.33±8.35[a]	35.33±15.02[a]	39.00±3.05[b]
Day 20	50.00±5.77[a]	94.33±10.17[a]	14.00±4.26[b]	51.33±5.69[a]	16.33±4.41[a]	38.67±11.97[b]

Values in the same column with different alphabets are significantly different at P≤0.05 according to Duncan Multiple Range Test (DMRT)

Table 5: Chlorophyll contents (mg/g) in Okra plants exposed to drought treatments

Treatments	Chlorophyll a	Chlorophyll b	Total Chlorophyll
Control	14.45±0.03[a]	23.53±0.17[a]	41.06±0.12a
Day 5	5.35±0.24[b]	15.25±0.12[b]	14.36±6.14b
Day 10	3.52±0.06[c]	12.35±0.06[c]	15.87±0.12b
Day 15	1.48±0.16[d]	0.65±0.06[d]	1.01±0.02c
Day 20	0.82±1.32[e]	0.47±0.12[d]	0.13±0.01[c]

Values in the same column with different alphabets are significantly different at P≤0.05 according to Duncan Multiple Range Test (DMRT)

The SOD enzyme expression showed a drastic rise as the drought treatment increased, being highest on day 20 (Figure 1). The CAT enzyme expression was also increasing with the increase in drought exposure; however, it was much slower, gradually peaking at day 20 (Figure 2). The expression of Peroxidase also peaked on day 20 of drought treatment but its expression was also gradual (Figure 3).

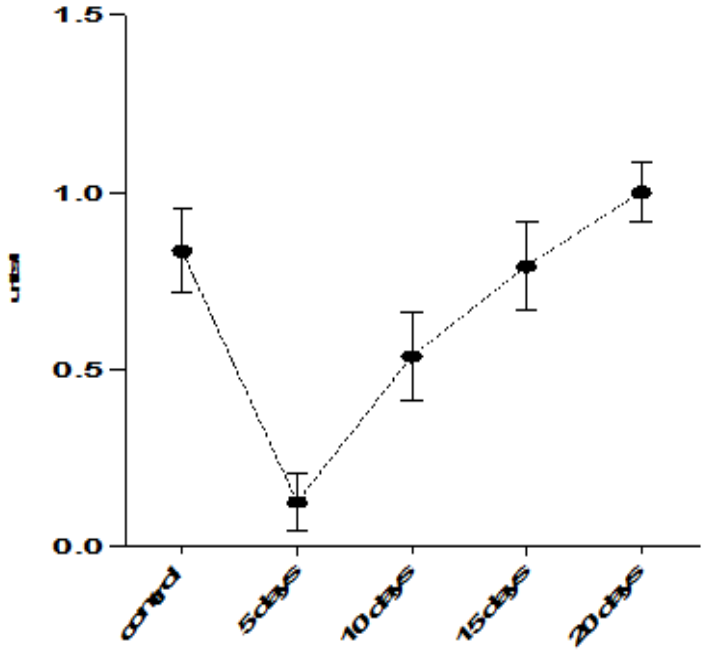

Fig 1: SOD enzyme expression in okra exposed to drought treatments

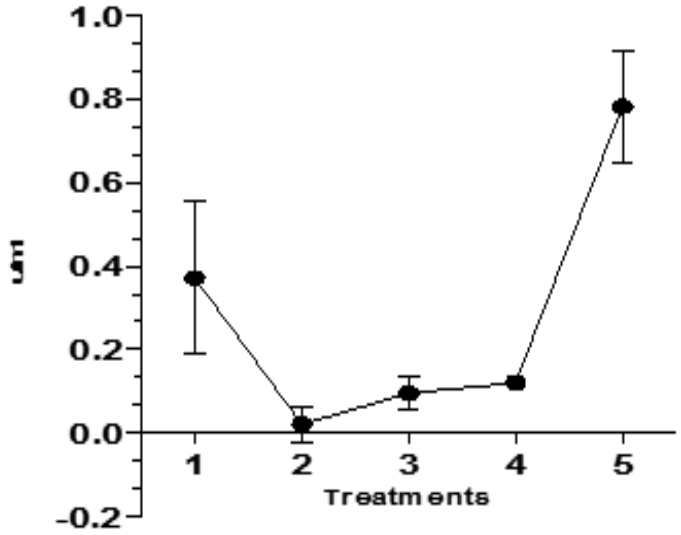

Fig 2: CAT enzyme expression in okra exposed to drought treatments

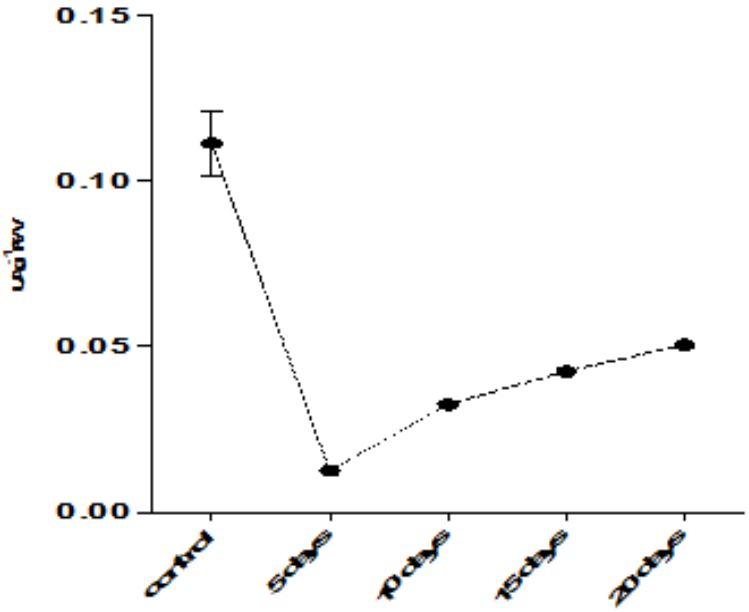

Fig 3: Peroxidase enzyme expression in okra exposed to drought

Discussion

Reduction in morphological parameter of *Abelmoschus esculentus* is a powerful indication of drought stress which displays sensitivity towards water deprivation. The investigation of plant growth and development under different drought stresses conducted in this study showed that the plant height, number of leaves, stem girth, leaf area, leaf area ratio, root weight, shoot weight, and plant weight in drought treatment were significantly decreased than those in control, indicating that drought stress affects the growth rate and development of *Abelmoschus esculentus*, which corroborates with the findings of (Tang, 2019) in the expression and regulation basis of drought resistant physiological molecules in commonly seen trees in the north. It was also observed that RLSTI, SLSTI, RDWTI,

RFWSTI, SFWSTI, and SDWTI in the treatment groups consistently decreased as the duration of stress treatment increased, which implies that *Abelmoschus esculentus* could not strive under long-term drought stress which correspond with the study of (Guoliang, 2009) in study on drought resistance and adaptability of different provenances of pinus softwood.

The ability of the crop to create biomass per unit of water transpired is referred to as water utilization efficiency. Our experimental results regarding water use efficiency showed a decrease in treatment with an increase in drought stress; this could be consistent with the finding that under drought conditions reduction in polysomal complexes was noted in plant tissues because of lower tissue water content which is corroborated by a study conducted by (Yamada *et al*., 2005) in effects of free proline accumulation in petunias under drought stress.

Relative water content is a critical indicator reflecting the water status of plants, a decrease in the hydraulic conductivity is usually experienced under plant stress conditions which in turn affects the relative water contents which is in line with the submission of (Sun *et al*., 2023) in physiological effects of drought stress on spinach seedlings. This study result showed that the relative water content of *Abelmoschus esculentus* decreased significantly under drought stress, indicating that the crop is significantly affected by drought stress; this finding is in line with previous studies (Suzuki *et al*, 2014) in abiotic and biotic stress combinations.

Effects of drought on chlorophyll content of *Abelmoschus esculentus* was found that an increase in dryness considerably affected the chlorophyll pigment concentration and at highest drought, the lowest values were reported. Collection and conversion of sunlight into food and energy are mostly influenced by Chlorophyll (a.b and total) pigments (Ahmad, 2019) in the effects of plant growth regulators on seed filling, endogenous hormone contents, and maize production in semiarid settings. Drought stress leads to a fast decline or expansion in the root length. In this study, the root growth is more influenced under drought stress as compared with its shoot growth in *Abelmoschus esculentus*.

The reduction in shoot length is due to the mechanisms of drought stress. Antioxidant enzymes such as CAT, SOD, and POD play significant roles in these systems (Guo *et al*., 2023). CAT activity of this study revealed an increasing trend throughout the treatment period, suggesting that it is a significant enzyme of the *Abelmoschus esculentus* antioxidant defense system. POD activity exhibits dynamic changes in various plant tissues, and is closely related to plant growth and development and the degree of oxidation (Alonso-Ramirez *et al*., 2009) in evidence for a role of gibberellins in salicylic acid-modulated early plant responses to abiotic stress in Arabidopsis seeds. This result revealed that *Abelmoschus esculentus*, POD activity was substantially lower in the drought stress treatment than in the control consistent with a recent study [Song *et al*., 2023) on the roles of salicylic acid in plant tolerance to abiotic and biotic stresses. SOD is a critical ROS-scavenging enzyme in plant cells. A constant rise was identified in SOD activity as treatment duration increased, indicating a breakdown in the balance between the production and clearance of free radicals within stamen and pistil cells, culminating in ROS accumulation and damage to membrane selective permeability.

In conclusion, physiological traits and yield in agricultural plants are the most critical factors influenced by drought on plants. This present experiment indicated that drought stress had a large impact on the overall development and created a notable drop in biochemical features of *Abelmoschus esculentus* and at bigger drought levels peak decreases in all studied parameters were documented. On the other hand, *Abelmoschus esculentus* subjected to varying drought degrees of RLSTI, SLSTI, RDWSTI, and SDWSTI indicates rising as the treatment increases. Antioxidant enzymes revealed positive results by growing as the treatment increased and decreasing chlorophyll content was found due to the drought stress.

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References

Ahanger, M. A., Aziz, U., Alsahli, A. A., Alyemeni, M. N. and Ahmad, P. (2019). Influence of exogenous salicylic acid and nitric oxide on growth, photosynthesis, and ascorbate-glutathione cycle in salt stressed *Vigna angularis*. *Biomolecules* 10(1): 42. https://doi.org/10.3390/biom10010042

Adejumo, S. A., Ezeh, O. S. and Mur, L. A. J (2023). Okra growth and drought tolerance when exposed to water regimes at different growth stages. *International Journal of Vegetable Science*. 25(3): 226-258. https://doi.org/10.1080/19315260.2018.1501788

Ahmad, I., Kamran, M., Meng, X., Ali, S., Bilegjargal, B., Cai, T., Liu, T. and Han, Q. (2019). Effects of plant growth regulators on seed filling, endogenous hormone contents and maize production in semiarid regions. *Journal of. Plant Growth Regulation*, 38 (4): 1467-1480.

Albdaiwi, R. N., Khyami-Horani, H. and Ayad, J. Y. (2019). Plant Growth-Promoting Rhizobacteria: An Emerging Method for the Enhancement of Wheat Tolerance against Salinity Stress-(Review). *Jordan Journal of Biological Sciences*, 12 (5): 525-534.

Alonso-Ramirez, A.; Rodriguez, D.; Reyes, D.; Jimenez, J.A.; Nicolas, G.; Lopez-Climent, M.; Gomez-Cadenas, A.; Nicolas, C. (2009). Evidence for a role of gibberellins in salicylic acid-modulated early plant responses to abiotic stress in *Arabidopsis* seeds. *Plant Physiology*, *150*, 1335–1344. https://doi.org/10.1104/pp.109.139352

Arnon, D. I.(1949). Copper enzymes in isolated chloroplasts. polyphenol-oxidase in *Beta vulgaris*. *Plant Physiology*, 24(1):1-15. doi: 10.1104/pp.24.1.1.

Barnhart, J. (1997). Occurrences, uses, and properties of chromium. *Regulatory Toxicology and pharmacology*. 26(1): S3-S7. http://dx.doi.org/10.1006/rtph.1997.1132

Dabrowski, P., Baczewska-Dabrowska, A. H., Kalaji, H. M., Goltsey, V., Paunoy, M., Rapacz, M., Wójcik-Jagła, M., Pawluśkiewicz, B., Baba, W. and Brestic, M. (2019). Exploration of chlorophyll a fluorescence and plant gas exchange parameters as indicators of drought tolerance in perennial Ryegrass. *Sensors* (Basel), 19(12): 2736. Doi:10.3390/s19122736.

Elings, A. (2000). Estimation of leaf area in tropical maize. *Agronomy Journal* 92, 436–444.

FAO (2019). Crop production statistics. Retrieved August 23, 2019, from, www.fao.org

Gardea-Torresdey J, Peralta-Videa J, Montes M, De la Rosa G, Corral-Diaz B. (2004) Bioaccumulation of cadmium, chromium and copper by *Convolvulus arvensis* L.: impact on plant growth and uptake of nutritional elements. *Bioresource Technology*, 92(3): 229-235.

Guo, P. R., Wu, L. L., Wang, Y., Liu, D. and Li, J. (2023) A. Effects of Drought Stress on the Morphological Structure and Flower Organ Physiological Characteristics of *Camellia oleifera* Flower Buds. *Plants (Basel)*, 12(13): 2585 - 2594. doi: 10.3390/plants12132585

Guoliang, S. (2009). Study on Drought Resistance and Adaptability of Different Provenances of Pinus Softwood. Master's Thesis, Beijing Forestry University, Beijing, China, 2009.

Hoffmann W. A. and Poorter, H. (2002). Avoiding bias in calculations of relative growth rate in soil microcosms. *Annals of Botany*, 80: 37-42, doi:10.1093/aob/mcf140

Khan, R.; Ma, X.; Hussain, Q.; Chen, K.; Farooq, S.; Asim, M.; Ren, X.; Shah, S.; Shi, Y. (2023) Transcriptome and anatomical studies reveal alterations in leaf thickness under long-term drought stress in tobacco. *Journal of Plant Physiology*, 281, 153-920. 281 doi: 10.1016/j.jplph.2023.153920

Kotas, J. and Stasicka, Z. (2000) Chromium Occurrence in the Environment and Methods of Its Speciation. *Environmental Pollution*, 107: 263-283. https://doi.org/10.1016/S0269-7491(99)00168-2

Li, D., Li, C., Sun, C., Sun, H., Liu, L., Zhang, Y. and Xiao, K. (2010). The Effects of Drought on Endogenous Hormone Contents and Balance in Main Stem Leaves of Cotton. *Cotton Science*, 22(3): 231 - 235. https://doi.org/10.11963/cs100306

Li, H., Guo, Y., Cui, Q., Zhang, Z., Yan, X., Ahammed, G. J. and Zhang, X. (2020) Alkanes (C29 and C31)-mediated intracuticular wax accumulation contributes to melatonin-and ABA-induced drought tolerance in watermelon. *Journal of Plant Growth Regulation.* 39(4):1441–1450. https://doi.org/10.1007/s00344-020-10099-z

Lichtentaler, H.K. and Buschman, C. (2001). Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. *Current Protocols in Food and Analytical Chemistry*, F4, 3.1–F4,3.8.

Ma, X.; Zeng, X.; Li, E.; Mei, H.; Luo, L.; Liu, Y. (2022). Drought tolerance of rice and its research progress. *Journal of Shanghai Agriculture*, 38: 36–45.

Oraee, A. and Tehranifar, A. (2020). Evaluating the potential drought tolerance of pansy through its physiological and biochemical responses to drought and recovery periods. *Scientia Horticulturae*, 265:109225. https://doi.org/10.1016/j.scienta.2020.109225

Pachauri, R. K., Allen, M. R., Barros, V. R., Broome, J., Cramer, W., Christ, R. and Dubash, N. K. (2014). Climate change 2014: Synthesis report. Contributions of working groups I, II, and III to the Fifth Assessment report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, 1-151

Pillay, A. and Wang, J. (2003). Modified Failure Mode and Effects Analysis using approximate Reasoning. *Reliability Engineering and System Safety*, 9: 69-85

Raja, V., Qadir, S. U., Alyemeni, M. N. and Ahmad, P. (2020). Impact of drought and heat stress individually and in combination on physio-biochemical parameters, antioxidant responses, and gene expression in *Solanum lycopersicum*, 3 *Biotech*, 10(5): 208-213. Doi: 10.1007/s13205-020-02206-4

Razi, K. and Muneer S. (2023). Grafting enhances drought tolerance by regulating and mobilizing proteome, transcriptome and molecular physiology in Okra genotypes. *Frontiers in Plant Science*, 14:1178935. https://doi.org/10.3389/fpls.2023.1178935

Reddy, A. R., Chaitanya, K. V. and Vivekanandan, M. (2004). Drought induced responses of photosynthesis and antioxidant metabolism in higher plants. *Journal of Plant Physiology*, 161(11): 1189-202. doi: 10.1016/j.jplph.2004.01.013

Salunkhe, P., Dhakephalkar, P. and Paknikar, K. (1998) Bioremediation of hexavalent chromium in soil microcosms. *Biotechnology letters.* 20(8): 749-751. https://doi.org/10.1023/A:1005338820430

Song, W., Shao, H., Zheng, A., Zhao, L. and Xu, Y. (2023). Advances in Roles of Salicylic Acid in Plant Tolerance Responses to Biotic and Abiotic Stresses. *Plants*, 12(19):3475-3484. https://doi.org/10.3390/plants12193475

Sun, Y., Wang, J., Wang, Q. and Wang, C. (2023). Responses of the Growth Characteristics of Spinach to Different

Moisture Contents in Soil under Irrigation with Magnetoelectric Water. *Agronomy*, 13(3):657. https://doi.org/10.3390/agronomy13030657

Suzuki, N.; Rivero, R.M.; Shulaev, V., Blumwald, E. and Mittler, R. (2014). Abiotic and biotic stress combinations. *New Phytologist*, 203 (1): 32–43. https://doi.org/10.1111/nph.12797

Turner, N.C. (1981). Techniques and experimental approaches for the measurement of plant water status.*Plant Soil*, 58: 339–366. https://doi.org/10.1007/BF02180062

Vernon, A.and Allison, J. (1963). A Method of calculating Net Assimilation Rate. *Natur*e, 200: 814-825. https://doi.org/10.1038/200814a0

Wilkins D. (1957). A technique for the measurement of lead tolerance in plants. *Nature*, 180,37–38 https://doi.org/10.1038/180037b0

Yamada, M., Morishita, H., Urano, K., Shiozaki, N., Yamaguchi-Shinozaki, K., Shinozaki, K., & Yoshiba, Y. (2005). Effects of free proline accumulation in petunias under drought stress. *Journal of Experimental Botany*, 56(417): 1975–1981. http://www.jstor.org/stable/24031455

Yu, X., Liang, C., Chen, J., Qi, X., Liu, Y. and Li, W. (2015). The effects of salinity stress on morphological characteristics, mineral nutrient accumulation and essential oil yield and composition in *Mentha canadensis* L. *Scientia Horticulturae*, 197: 579–583, doi:10.1016/j.scienta.2015.10.023

Zayed, A., Gowthaman, S. and Terry, N. (1998). Phytoaccumulation of trace elements by wetland plants: I. Duckweed. *Journal of Environmental Quality*, 27(3): 715-721. https://doi.org/10.2134/jeq1998.00472425002700030032xs