IJABR Vol. 15(1): 7- 17 (2024)

Original article

EFFECTS OF DROUGHT-RELATED STRESS ON THE GROWTH AND DEVELOPMENTAL STAGES OF *AMARANTUS CRUENTUS* L.

***Oluwajobi A.O.* and Ajewole T.O.**

Department of Plant Science and Biotechnology, Federal University Oye Ekiti, Nigeria

Submitted: February 2024; Accepted: May 2024; Published: June 2024

ABSTRACT

Food insecurity and climate change directly affect the growing population and these effects can be overwhelming. The vegetable used in this study, *Amaranthus cruentus* L., which is a very common vegetable in Nigeria, has been confirmed to have some drought-tolerant characteristics. The study was carried out to relatively compare how the physiological properties of *Amaranthus cruentus* were affected by drought. Sandy-loamy soil was collected and drought treatments of (5, 10, 15, and 20 days) were set up while a control experiment that was not devoid of water at any point was set up. These levels of stress treatments were introduced at twelve (12) Weeks after Planting (WAP). From the study, both the Relative Water Content (RWC) and Net Assimilation Rate (NAR) reduce as treatment levels of drought increase. The stress tolerance index exhibits a little variation across all treatment regimes, for example, the Shoot Length Stress Tolerance Index (SLSTI) of *A. cruentus* swings gradually as the period of drought stress reduced; nevertheless, on day 10, the treatment showed considerably low (89.33 %) SLSTI. Superoxide Dismutase (SOD) exhibited its activities at the highest in the 15-day group while Catalase (CAT) and Peroxidase (POD) showed the highest expression in the 5-day group in comparison to other groups. The tolerance exhibited by this plant in the face of changing global climatic conditions is fascinating, thus *A. cruentus* presents itself as a succour for vegetal food availability in this time of global food shortage. Its production should therefore be encouraged.

Keywords: Food insecurity, climate change*, Amaranthus cruentus,* Relative water content, Stress Tolerance Index

Corresponding author's email: ayoola.oluwajobi@fuoye.edu.ng +2348035036877

INTRODUCTION

Several environmental stressors influence plant growth and this leads to a great reduction in crop production (1,2). Due to high levels of climate change, global warming, and long periods of droughts which will likely increase in years to come, local areas are said to be a major beneficiary of low water availability which will ultimately result in low agricultural yield. Water deficit is a major abiotic stress factor that affects plant productivity due to how it negatively affects plant growth (3,4) and photosynthesis (5). Stress as a result of drought causes a huge reduction in growth this occurs as a result of its effect on some physiological and biochemical processes like photosynthesis, translocation, carbohydrates, and nutrient metabolism (6).

Limitations such as drought and heat stress are threats that limit processes like photosynthetic rate and stomata function. An investigation (7) indicated that determined prolonged exposure to environmental pressures increases reactive oxygen species (ROS) generation and consequently leads to detrimental oxidative damage, disrupting normal cell functions including causing degradation of molecules such as proteins, lipids, and nucleic acids. Heat stress as well as water scarcity also influence the electron transport rate (ETR) and harm the photosynthetic machinery PSII (8). Changes in both biotic and abiotic conditions such as soil water content, temperature, and availability of nutrients are the major cause of competition switching and facilitation in several plant species this is according to the stress-gradient hypothesis. Alteration in the patterns and amount of rainfall in the 21st century has been caused by global warming; this alteration eventually led to long periods of droughts (9). There is alteration as a result of interactions in plant species which changes carbon cycling and nutrient elements in terrestrial ecosystems (10).

Restriction of plants under water promotes the synthesis and retention of numerous osmolytes in the cell to reduce their osmotic flux, thereby increasing the control and osmoprotection of the antioxidant defence system (10).

As a way of adaptation and coping strategy to stress, there is a molecular, physiological, and biochemical lingering like gene expression alteration and activation of the antioxidant pathway which may either be enzymatic or non-enzymatic (11). Photosynthetic rates in plants can be regulated by modifying photosystem II and stomata enhancement (12). The major challenge currently faced in agriculture is getting the latest coping strategies to maintain crop production under severe abiotic stress conditions (13). Several gene expression techniques that help in the study of drought resistance in addition to their economic importance very vital in so many developing countries, especially in Africa where drought has become massive and it is as a result of climate change challenges and the ability to access these gene expression techniques is very limited as a result of low investment in crop production (14).

Amaranthus cruentus is a very highly nutritious meal since all portions of the plant's shoots are edible, leaves from new *A. cruentus* stems serve a purpose in salad as well as soup preparation, whereas the grains may be utilized to manufacture cakes, biscuits, and breads (15). The vegetable also contains special metabolites that help in improving the human diet (16). It is also advantageous owing to its activity

as an antioxidant, enhanced pro-vitamin A, as well as anticancer genic chemicals, all of which may aid in a balanced diet. Although Amaranthus has previously been identified as drought-tolerant (17), knowledge regarding the effect of drought along with nitrogen availability on plant performance, when applied alone, has already been thoroughly stated, however, less information is available on their interacting effects. Sufficient nitrogen delivery is important for optimum plant function. The current research was done to investigate the influence of drought tolerance on plant development, physiology, enzymatic activity, and water usage efficiencies of *A. cruentus*

MATERIALS AND METHODS

Research Location

This study was undertaken in the screen house of the Department of Plant Science and Biotechnology, Federal University, Oye Ekiti (latitude 7.80°N and longitude 5.21°E) Oye Ekiti and the field experiment was set up behind the Faculty of Education, Federal University, Oye Ekiti while the Laboratory analyses were carried out in the Laboratory.

Source of Materials and Description of Species

Seeds of *Amaranthus cruentus* used in the study were collected from a well-established vegetable farm around Ado Ekiti and identified at the Department of Plant Science and Biotechnology's herbarium, Federal University Oye Ekiti with FUOH 0110 and FUOH 0111 as voucher numbers respectively. Sandy loamy soil for planting was collected within the University community; pH of the soil was measured in water using a pH meter to be 6.5. Fifty (50) planting pots were set up with 25 pots these pots were set up to have four replicates per treatment. Seeds of *Amaranthus cruentus* were planted and drought treatments of 5, 10, 15 and 20 days were arranged using a Completely Randomized Block Design pattern along with the control experiment. Drought was introduced at 12 weeks after planting. Data obtained were analysed using IBM SPSS Statistics 22.

Soil Test

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

Morphological Parameters

To determine the height of each plant, a meter rule was used to measure the length of the shoot from the ground surface to the apex. In addition, the diameter of the stem was measured using a Vernier Caliper.

Leaf area was obtained with this formula (Length x Width x 0.75), number of leaves per seedling was observed and obtained by counting. Weights of shoot which includes all part plants above the soil level determined using a digital weighing balance. Relative Growth Rate was obtained with this formula (18)

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

The Net Assimilation Rate was calculated according to this formula:

$$\frac{(InL_2 - In)(L_1W_2 - W_1)}{(t_2 - t_1)(L_2 - L_1)} \qquad (19)$$

where L_1 and W_1 represent the leaf area and the total dry matter at time t_1 and W_2 and L_2 at time t_2, respectively.

Leaf Area Ratio was calculated using this formula

$$F = \frac{(L_1/W_1) + (L_2/W_2)}{2} \quad (20)$$

Stress tolerance index (STI)

STI which is calculated to specifically determine high yield/tolerance stress potential.

RLSTI (Length of Root Stress Tolerance Index) = (stressed plant root length/control plant root length × 100 [21]

SLSTI (Length of shoot Stress Tolerance Index) = (stressed plant shoot length/control plant shoot length) × 100 [22]

RFSTI (Weight of Fresh Root Stress Tolerance Index) = (fresh weight of plant root stressed plant/control plant root fresh weight) × 100 [22]

SFSTI (Weight of Fresh Shoot Stress Tolerance Index) = (stressed plant shoot fresh weight/control plant shoot fresh weight) × 100 [23].

RDSTI (Root Dry Weight Stress Tolerance Index) = (stressed plant root dry weight/control plant root dry weight) × 100 [24]

SDSTI (Shoot Dry weight Stress Tolerance Index) = (stressed plant Shoot dry weight/ control plant Shoot dry weight) × 100 [25]

Physiological Parameters

Relative Water Contents

Relative water contents were obtained using a method described by [26]. Excised leaves were dipped in double-distilled H_2O in the dark. The leaves were removed from the double distilled water after 24 hrs, wiped with a blotting paper that was sterilized, and placed on a digital weighing balance to obtain Turgid Weight (TW). The leaves were subjected to 72 hours of automated oven heating at 65^{o}C, after which the dry weight (DW) was accurately recorded..

RWC (%) was determined employing the formula:

$$\frac{(Fresh\ Weight - Dry\ Weight)}{(Turgid\ weight - Dry\ weight)} \times 100 \quad [27]$$

Chlorophyll Pigment Extraction and Measurement of Chlorophyll (a and b) Fluorescence

Leaves obtained from the control and stressed group were cut and processed for determination of chlorophyll. Approximately, 0.05 g of leaves was homogenized in aqueous buffered acetone on a mortar and pestle that was pre-chilled. This homogenate was placed in a centrifuge for 2 minutes at 10000 rates per minute and cooled at 4^{0}C. The supernatant's volume was adjusted back to 5 ml using buffered acetone after the pellet was discarded. The optimum absorbance of the supernatants was taken at 480, 645, and 663 nm wavelengths with a double-beam UV-VIS spectrophotometer against 80% buffered acetone which was used as blank [28]. Chlorophyll and carotenoid pigments content were obtained using the procedure.

Enzyme Extraction and Assay (SOD, CAT and POD)

One (1 g) gram fresh leaf was homogenized in 3 ml ice-cold 100 mM K-phosphate buffer of pH 6.8 which contains 0.1 mM EDTA, this was carried out for 5 min. cheese cloth was used to filter the mixture, and the homogenate obtained was then centrifuged at 16000 rpm for 15 min, after which the supernatant was used as the enzyme source. Peroxidase (POX) was evaluated by the addition of 25 µl of the crude enzyme extract to 2 ml of a solution prepared by the mixing of 50 mM a solution of potassium phosphate buffer, 20 mM guaiacol, and 20 mM Hydrogen peroxide. Incubation is carried out at an ambient temperature of 30°C for 10 min, following which 0.5 ml 5%

(v/v) H_2SO_4 was added, and absorbance was measured at 480nm. One POX unit was defined as the change of 1.0 absorbance unit per ml enzymatic extract; this is also represented as units of enzyme activity per g fresh matter per min (UA g−1 FW min−1).

The activity of Catalase (CAT) was obtained by the addition of 50 µl enzymatic extract to 3 ml of a solution that comprises 50 mM potassium phosphate buffer with a of pH 7.0 and 20 mM of Hydrogen peroxide absorbance was determined at 240 nm at a temperature of 30°C.
SOD enzyme activity was measured by the addition of 50 µl of the enzymatic extract to a solution obtained by the addition of 13 mM-methionine, 75 µM p-nitroblue tetrazolium chloride, 100 µM EDTA and 2 µM riboflavin in a 50 mM potassium phosphate buffer with a pH 7.8, this procedure was carried out under illumination of a 30 W fluorescent lamp at an operating temperature of 25°C. The fluorescent light was put on for 5 min, and the blue formazane generated by NBT photo-reduction was assessed as an increase in absorbance at 560 nm. A unit of SOD was defined as the amount of enzyme sufficient to block 50% of the NBT photo reduction when compared with tubes that are without the plant extract and expressed as units of enzyme activity (AU) g−1 FW min−1. (29).

RESULTS

The experiment revealed that all the parameters taken (root length, shoot length and plant weight) showed a significant reduction after 5, 10, 15 and 20 days of drought stress, however, there was a significant difference in the weight of the plants on day 10 when compared to other treatment groups (Table 1).

Table 1: Effects of drought stress on the Root length, shoot length and plant weight of *A. cruentus* exposed to drought stress

Treatments	Root length (cm)	Shoot length (cm)	Plant 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).

The results of the Relative Water Content analysis for the *A. cruentus* plant showed a significant decrease after 5, 10, 15, and 20 days of drought stress. This trend was also observed in the leaf area, leaf area ratio, and water use efficiency (Table 2). However, there was a significant difference between the day 10 treatment and the control and day 5 treatments. Additionally, a significant difference was observed between the control group and other treatment groups.
During the drought stress, the RLSTI of *A. cruentus* decreased gradually with time, with no significant variation among treatments on day 5, 10, and 20. The SLSTI

of *A. cruentus* showed a fluctuating pattern with lesser drought stress duration; however, on day 10, the treatment showed a significant decrease (89.33%) in SLSTI as compared to other treatments (Table 3). On day 15, the treatment had a significantly higher (98.67%) SLSTI. The RFWSTI rate decreased significantly with the prolongation of drought stress. Similarly, SFWSTI in *A. cruentus* also decreased significantly on 5, 10, 15, and 20 days of drought stress.

Table 2: Effects of Drought Stress on the Relative Water Content, Leaf Area, Leaf Area Ratio and Water Use Efficiency in A. *cruentus*

Treatments	Relative Water Content(cm)	Leaf Area	Leaf Area Ratio	Water use efficiency
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 3: Effects of Drought Stress on the Stress Tolerance Index of *A. cruentus*

Treatments	RLSTI (%)	SLSTI (%)	RFWSTI (%)	SFWSTI (%)
Day 5	93.67±22.70^{a}	97.00±5.69^{a}	57.00±12.34^{a}	89.00±11.15
Day 10	50.67±20.38^{a}	89.33±17.63^{a}	30.33±8.69^{ab}	86.33±30.49
Day 15	52.00±11.85^{a}	98.67±21.07^{a}	15.33±7.00^{b}	58.33±8.35
Day 20	50.00±5.77^{a}	94.33±10.17^{a}	14.00±4.26^{b}	51.33±5.69

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

The analysis indicated that drought stress on *A. cruentus* significantly affected total chlorophyll and chlorophylls-*a* and *b* as shown in Table 4. The study showed that after 5, 10, 15, and 20 days of drought stress, the treatment of the total Chlorophyll significantly decreased as the lowest values were observed in the day 20 treated groups.

Table 4: Effect of drought stress on chlorophyll a, chlorophyll b and carotenoids in A. *cruentus*.

Treatments	Chlorophyll a	Chlorophyll b	Carotenoid
Control	14.45±0.03^{a}	23.53±0.17^{a}	41.06±0.12^{a}
Day 5	5.35±0.24^{b}	15.25±0.12^{b}	14.36±6.14^{b}
Day 10	3.52±0.06^{c}	12.35±0.06^{c}	15.87±0.12^{b}
Day 15	1.48±0.16^{d}	0.65±0.06^{d}	1.01±0.02^{c}
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 CAT activity of *A. cruentus* dramatically increased compared to the control group following 15 days of drought stress treatment (Figure 1). CAT activity steadily rose in both the treatment and control groups over time. The SOD expression was highest on day 15 of the therapy, whereas the lowest value was found on day 10 (Figure 2).
POD activity in *A. cruentus* under drought stress considerably increased on days 5 and 10, however, for plants on days 15 and 20, the POD activity dropped dramatically compared to the control (Figure 3). After days 5, 10, 15, and 20, the POD activity in *A. cruentus* was considerably enhanced under drought stress with the duration of therapy. However, after 5 days, the POD activity was considerably lower in *A. cruentus* in the treatment group than in the control.

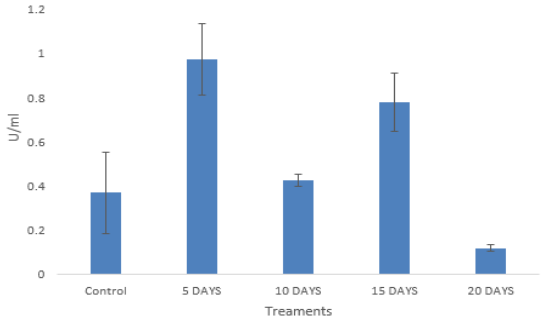

Figure 1: Expression of Catalase enzyme in *A. cruentus* exposed to drought stress

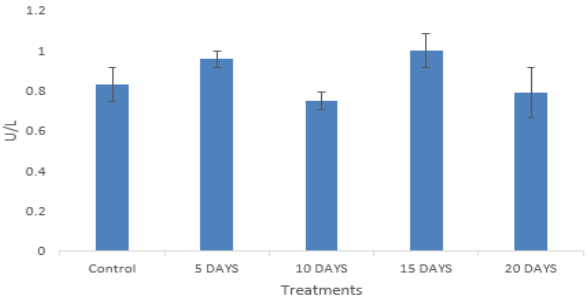

Figure 2: Expression of SOD enzyme in *A. cruentus* exposed to drought stress

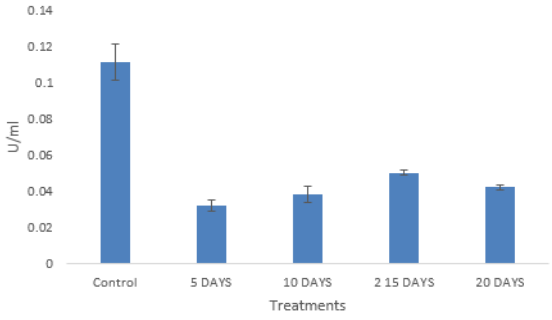

Figure 3: Expression of Peroxidase enzyme in *A. cruentus* exposed to drought stress

DISCUSSION

A decrease in morphological characteristics is a clear indicator of drought stress on *A. cruentus*, which demonstrates vulnerability toward water deficit. This research demonstrates that with increasing drought stress, there is a substantial drop in all growth indices evaluated in the study. Stomata closure to decrease transpiration and cell reduction, which are some of the main responses of plants to water stress, were also detected in the study, corroborating earlier report (30). Reduced availability of water due to drought may also have negative impacts on photosynthesis.

There was a decline in relative water content during drought as seen in this study. Significant alterations in plant growth and development under varied stress periods were detected, notably in the morphology of the plants. This is consistent with earlier findings (31). This research demonstrated that the relative water content of *A. cruentus* reduced considerably under varied drought stress circumstances, which shows that the crop is damaged by drought stress, in keeping with earlier research results (32). In plants, drought stress leads to a fast decline or growth in the root length. In this research, the root growth gets more impacted under drought stress as opposed to its shoot growth. This may be attributed to a reduction in cell elongation.

This research demonstrated that CAT activity in *A. cruentus* under drought stress was greatest in the 15-day treatment as compared to other treatment groups and the control. This shows that the enzyme is an essential antioxidant defence mechanism, consistent with the studies of Li *et al.* (2020). The research also demonstrated that POD activity was lower in the drought stress-treated groups than in the control group but considerably reduced after 15 days of treatment than the control. This is on par with the previous outcomes on such study (33).

CONCLUSION

Drought is a crucial factor that influences the development and productivity of agricultural plants such as *A. cruentus*. A more recent study has demonstrated that drought stress has a substantial influence on the overall development of *A. cruentus*, leading to a severe loss in its biochemical properties. As the amount of drought rises, the largest drop in all the examined parameters is found, including RLSTI, SLSTI, RDWSTI, and SDWSTI. However, antioxidant enzymes have demonstrated promising outcomes by rising as the therapy progressed. A reduction in chlorophyll concentration was detected owing to the dry stress. Despite the reduction in growth and biochemical properties, *A. cruentus* displayed resilience to drought, which is promising in the face of changing climatic circumstances worldwide. Therefore, *A. cruentus* offers itself as an option for vegetal food supply in this time of global food crisis, and its cultivation should be promoted.

Declarations

Authors' contributions: OAO conceptualized and designed the study, ATO handled the fieldwork and data collection and performed the data analyses. OAO and ATO prepared the manuscript. Bothauthors contributed to the development of the manuscript and also approved its submission.

Conflict of Interest: None

Funding: This study did not receive any external funding

REFERENCES

1. 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

2. 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. Doi: 10.1007/s13205-020-02206-4

3. 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.

4. Fahad, S., Bajwa, A. A., Nazir, U., Anjum, S. A., Farooq, A., Zohaib, A., Sadia, S., Nasim, W., adkins, S., Saud, S., Ihsan, M. Z., Alharby, H., Wu, C., Wang, D. and Huang, J. (2017). Crop production under drought and heat stress: plant responses and management options. *Frontiers in Plant Science*, 8:1147. Doi:10.3389/fpls.2017.01147

5. 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.

6. 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.* 2020; 39(4):1441–1450. https://doi.org/10.1007/s00344-020-10099-z

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

8. He, Z., Liu, C., Zhang, Z., Wang, R., and Chen, Y. (2022). Integration of mRNA and miRNA analysis reveals the differentially regulatory network in two different *Camellia oleifera* cultivars under drought stress. Frontiers in Plant Science, Volume 13 https://doi.org/10.3389/fpls.2022.1001357

9. Kumar,A., Basu, S., Ramegowda, H. V. and Pereira, A. (2017). Mechanisms of drought tolerance in rice. In: Achieving sustainable cultivation of rice Volume 1. Takuji Sasaki ed. doi:10.19103/AS.2106.0003.08

10. Li, Y. (2021). Physiological Response and Transcriptome Analysis of *C. oleifera* to Drought
Stress. Master's Thesis, Central South University of Forestry and Technology, Changsha, China, 2021.

11. 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

12. 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 ofPlant Physiology,* 281, 153-920. 281:153920. doi: 10.1016/j.jplph.2023.153920.

13. 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.
.

14. Ajewole, T.O.,Okpeku, M., Chukwuma, D. M., Iwuala, E. N., Popoola, K. M.,Agbolade, J. O., Adeoye, A. O. and David, O. A. (2023). Comparative Analysis of Gene Expression in selected drought Resistant vegetables. *Asian Journal of Plant Sciences*, 22:215-226

15. Rastogi, A. and Shukla, S. (2013). Amaranth: a new millennium crop of nutraceutical values. *Critical Reviews in Food Science and Nutrition.* 53(2):109-25. doi: 10.1080/10408398.2010.517876.

16. Neugart, S., Baldermann, S., Ngwene, B., Wesonga, J. and Schreiner, M. (2017). Indigenous leafy vegetables of Eastern Africa—A source of extraordinary secondary plant metabolites. *Food Research International.*100:411–422. doi: 10.1016/j.foodres.2017.02.014

17. Liu, F. and Stu¨tzel, H.(2007). Leaf water relations of vegetable amaranth (Amaranthus spp.) in response to soil drying. *European Journal of Agronomy*, 16(2):137–150.

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

19. Guo, Y.Y., Yu, H.Y., Yang, M.M., Kong, D.S. and Zhang, Y.J. (2018). Effect of drought stress
on lipid peroxidation, osmotic adjustment and antioxidant enzyme activity of leaves and roots of Lycium ruthenicum Murr. seedling. *Russian Journal of Plant Physiology,* 65,244-250.https://doi.org/10.1134/S1021443718020127

20. Tanveer, M., Shahzad, B., Sharma, A. and Khan, E.A. (2019). 24-Epibrassinolide application
in plants: An implication for improving drought stress tolerance in plants. *Plant Physiology and Biochemistry*, 135:295-303. doi: 10.1016/j.plaphy.2018.12.013

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

22. Gardea-Torresdey, J., Peralta-Videa, J., Montes, M., De la Rosa, G. and 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. https://doi.org/10.1016/j.biortech.2003.10.002

23. 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

24. 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

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

26. 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

27. 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/jeq1998.00472425002700030032x

28. 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

29. Fabio Rossi Cavalcanti, José Tadeu Abreu Oliveira, Aparecida Simone Martins-Miranda, Ricardo Almeida Viégas and Joaquim Albenísio Gomes Silveira (2004). Superoxide dismutase, catalase and peroxidase activities do not confer protection against oxidative damage in salt-stressed cowpea leave. *New Phytologist* 163:563–571. https://doi.org/10.1111/j.1469-8137.2004.01139.x

30. 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.

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

32. 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 conents and maize production in semiarid regions. *Journal of. Plant Growth Regulation*, 38 (4): 1467-1480. https://doi.org/10.1007/s00344-019-09949-2

33. 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