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Naphthalene Acetic Acid Mediated Morphological and Biochemical Changes in Squash [Lagenaria siceraria (Molina) Standl.] Under Alkaline Stress

Uswa Ashiq Nadeem
The University of Lahore
Naila Ali
The University of Lahore
Zaib-un-Nisa .
The University of Lahore
Anis Ali Shah
anisalibot@gmail.com

University of Education
Muhammad Iftikhar
University of Education
Hosam O. Elansary
King Saud University
Shankarapp Sridhara
University of Agricultural and Horticultural Sciences

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Abstract

Alkaline stress promotes ionic toxicity and ultimately reduce plant growth and yield. *Cucurbita pepo* is an annual plant of the family cucurbiteacea and moderately sensitive to alkalinity but limited literature is available on the use of plant growth regulators to alleviate alkali stress in C. pepo. In this study, a pot experiment was carried out to study the effect of foliar application of NAA (0, 50, 75, 100 ppm) on C. pepo growth, yield and biochemical parameters under control and alkaline stress conditions (0,40 mM), respectively. The results showed that, alkali stress prominently reduced the plant growth, biomass, leaves and fruit count but NAA application enhanced all growth and yield attributes both under stress and non-stress conditions. Further, alkaline stress significantly reduced the photosynthetic pigments but 75% NAA application increased Chl a by 43%, Chl b by 53% and carotenoids by 66% compared to only salt treated plants. In addition, a significant improvement in plant primary and secondary metabolites such as total soluble proteins by (27%), total free amino acids by (38%), total phenolics and flavonoid contents by 28% and 27%, respectively as compared to only stress treated plants. To further investigate the effects of NAA on antioxidant enzymes, catalase (CAT) and peroxidase enzyme activities (POD) were calculated and results showed that alkali stress increased the enzyme activities while under the foliar applied NAA in stress conditions further enhanced these enzyme activities compared to only stress treated plants. These findings suggests the positive role of NAA exogenous application under alkali stress conditions to mitigate harmful effects of stress on *C.pepo.*

Introduction

Saline-alkaline stress is a major abiotic stress factor, that limits plant growth and agricultural productivity worldwide[1]. Specifically, plant under saline-alkaline stress face metabolic disorders by elevating levels of osmotic pressure and accumulation of reactive oxygen species[2,3]. In turn, plants use upregulation of a series of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and peroxidase (POD) as well as enhance the activities of enzymes which scavenge ROS and stabilize the structural integrity of membrane area by reduction in lipid peroxidation rates[4,5]. Higher levels of alkaline salts in plant growth medium reduce K^+ content and enhance Na^+ uptake and accumulation, leads towards an efflux of K^+ ions and promote K^+ leakage from plant cells. Moreover, under alkaline stress conditions, Na^+ content rises above that of K+, resulting in poor nutrient uptake and lack of Na^+/K^+ homeostasis. Higher pH also causes abnormality in root morphology, causing leaf chlorosis and necrotic lesions in leaves, all of which hamper plant growth and development, and ultimately lead to reduced crop yield[6]. Even though saline-alkaline stress occurs simultaneously, however their mechanisms intertwined. Saline stress is mostly caused due to accumulation of neutral salts, which is further intensified by high pH values greater than 8.5, which renders root vitality, photosynthesis, and cell membrane integrity[7,8]. Alkaline stress also affects plant metabolism at physiological and biochemical levels, integrating changes in levels of phytohormones as well as altering the gene expression networks[5,9].

There are significant spatial and temporal variations have been reported by many researchers in phytohormones under responses of different abiotic stress conditions. Among phytohormones cytokinins (CK), salicylic acid (SA), auxins, ethylene (ET), gibberellins (GA), abscisic acid (ABA), jasmonates (JA), and brassinosteroids (BR) play important roles in signaling pathways under abiotic stresses. In general, ABA regulates osmotic stress, SA enhances the important enzymes of antioxidant system, IAA and CK are involved in sustaining plant development under high pH growth medium[1,10].

Napthalene acetic acid (NAA) application could increase the root development of plants in different plant species such as in maize, citrus, rice and malus under abiotic conditions[11–14]. It has been reported that, the stress mediated auxin gradient regulates various physiological and biochemical mechanisms such as production of aquaporins, stomatal opening, lateral roots positioning, and the accumulation of important osmolytes such as soluble sugars and proline. In addition, auxin has been reported to increase the stress tolerance of plants via ROS efficient scavenging, lowering the rates of Na^+ accumulation and via protecting photosystem II from stress damages[15,16]. Recently[17], concluded in his research that auxin in higher amounts in rice roots regulated the development of root systems which results in salt resistant genotypes of rice.

Summer squash (*Cucurbita pepo*), which belongs to the cucurbitaceae family is available throughout Indo-pak and consumed as vegetable in various parts of the world also. Extensive investigations have led to isolation of several bioactive compounds from *C. pepo* which includes phenolic compounds such as chlorgenic acid, benzoic acid, quercetin, luteolin and kaempferol[18,19], p-coumaric acid, sinapic acid. Several biochemical and physiological changes have been associated with abiotic stresses like alkaline stress, but little is identified about the alkaline tolerance in squash. Moreover, less evidence exists regarding the protective roles of growth hormone to enhance crop yield under stress conditions. As far as literature is concerned, there are limited findings available regarding the effects of exogenous application of NAA on squash grown under alkaline stress. In the current study it is hypothesized that "exogenous applications of Naphthalene acetic acid may enhance the growth and yield of squash under alkaline stress.

Material and Methods

The experiment was conducted in the wire house of IMBB, the University of Lahore, Lahore, Pakistan. It was designed to study the effect of foliar application of NAA on *C. pepo* under alkaline stress. The laboratory work was performed in General Botany Research Lab, Institute of Molecular Biology and Biotechnology (IMBB), University of Lahore, Lahore, Pakistan.

Plant materials and Stress application

The seeds of *C. pepo* were collected from local seed market and sown in clay pots with 7 kg soil in each pot. Five seeds were sown in each pot and allowed to germinate under natural conditions up to the two

leaf stage. After one week of seed germination, the pots were divided into two sets, one was considered as a control while the other set of pots was irrigated with 40 mM $NaHCO_3$.

Foliar application of NAA

After two weeks of stress applications, the plants were treated with (0, 50, 75 and 100 ppm) NAA foliarly, details described in Table 1. A surfactant (tween- 20) was added in the alkaline solution of NAA at the ratio of 5% per liter. The plants were sprayed with respective concentrations of NAA three times at an interval of 5 days while control plants were sprayed with distilled water. Plants were harvested after a month at the onset of fruits, ensuring undamaged roots and shoots to measure the growth parameters.

Table 1
Treatment details of Pot Experiment.

Treatments	Conditions	NAA application
T^0	0 mM $NaHCO_3$	0 mg/L
T1	40 mM $NaHCO_3$	0 mg/L
T2	0 mM $NaHCO_3$	50 mg/L
T3	40 mM $NaHCO_3$	50 mg/L
T4	0 mM $NaHCO_3$	75 mg/L
T5	40 mM $NaHCO_3$	75 mg/L
T6	0 mM $NaHCO_3$	100 mg/L
T7	40 mM $NaHCO_3$	100 mg/L

Determination of growth and yield parameters

Plants were harvested at fruiting stage and the roots and shoots fresh weigh were taken on a weigh balance. Root and shoot samples of each treatment were taken and dried in laboratory oven at 65°C for two weeks at the temperature of 70° and then their dry biomass was recorded.

Root and shoot lengths of harvested plants were recorded with measuring tape. Further, the fresh weight of fruits was measured on weight balance. Fruits were separated from petiole and number was recorded. Leaves of each plant of respective treatment were collected and their number per pot was recorded. The volume of fruit was calculated with the volume displacement method[20]. The volume has calculated by using following formula:

Fruit volume (ml) = Initial water level - Final water level

Determination of Physiological parameters

Plant extract was exuded out by taking 0.5 g of fresh leaf sample from four replications of each treatment in 10ml phosphate buffer solution and stored at 21° C in freezer. The falcon tubes were kept in freezer for further analysis.

Photosynthetic pigments

Photosynthetic pigments including chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids were determined by following the methods of [21,22]. Fresh leaves (0.5g) of all pot plants of each were chopped and immersed in 80% acetone in the falcon tubes, separately. The tubes were kept at 4°C overnight and absorbance was taken at spectrophotometer at different wavelengths 480, 645 and 663 nm. Acetone (80%) without plant extract was taken as blank.

Determination of primary and secondary metabolites

Total free amino acids were determined by the protocol proposed by[23] and absorbance was noted at 570 nm. Bradford method [23](Bradford, 1976) was used to determine total soluble proteins in plant leaves. The absorbance of BSA solution was measured at 595 nm on spectrophotometer (UV/V spectrophotometer HALO SB-10). Whereas, total phenols contents determined by the protocol proposed by Julkunen-Titto (1985)[24]. The absorbance was noted at 760 nm on Spectrophotometer.

Antioxidant enzymes assays

Fresh leaves (500 mg) were homogenized in 5 mL of phosphate buffer (50 mM, pH 7.8) at 4°C. For 15 minutes, this extract was centrifuged at 12,000 rpm Supernatant was collected in autoclaved eppendrof tubes and used for the determination of peroxidase (POD) and catalase (CAT). CAT and POD activity was determined by using the protocol of[25] Chance and Maehly (1955).

Statistical Analysis

The experiment data was analyzed by computer based software (STATISTIX 8.1) by analysis of Variance (ANOVA). Pairwise comparison between the groups were taken by Tukey HSD test[26].

Results

NAA foliar application promoted growth indices under alkaline stress

To investigate the effects of NAA on squash plant growth and biomass, various parameters were studied shown in Table 2. It was observed that alkalinity in the soil reduced the root and shoot lengths by 42.1% and 33.8%, respectively, as compared to control plants. Biomass of a plant comprises of root and shoot fresh weights decreased by 53.4% and 29.3%, respectively, in saline stressed plants as compared to control plants. The foliar application of NAA solution in return accelerated the growth characteristics under stressed and non-stressed plants. Foliar application of 75mg/L NAA solution increased the shoot

length by 53% and 46%, root length by 51% and 4/%, plant fresh biomass by 68% and 62% under stressed and non-stressed plants, respectively. The foliar application of NAA solution also accelerated number of leaves in squash plant under both conditions (Table 2). It is noted that alkali treated plants exhibited 43% reduction in leaf count as compared to control plants. While 75mg/L NAA application increases the leaf count by 65% under stressed condition and by 47% under control plants. The results showed the significant effect of NAA on plant growth attributes.

Table 2
Interactive effect of different concentrations of naphthalene acetic acid (NAA) under alkaline stress on growth indices of squash plant

Conditions	Treatments (mg/L)	Root Length (cm)	Shoot length (cm)	Root fresh weight (g)	Shoot fresh weight (g)	Number of leaves (n)
Control (0mM)	0	7.33^{d}	19.06^{cd}	5.00^{cd}	10.24^{de}	3.66^{cd}
	25	9.33^{c}	$24.73a^{bc}$	7.36^{bc}	20.25^{c}	5.00^{e}
	50	10.66^{ab}	29.06^{ab}	8.65^{b}	30.27^{b}	6.33^{b}
	75	12.22^{a}	35.66^{a}	10.33^{a}	38.32^{a}	7.01^{a}
Alkaline Stress (40mM)	0	4.24^{f}	12.60^{d}	2.33^{d}	7.23^{e}	2.06^{d}
	25	6.01^{ef}	18.70^{cd}	4.00^{cd}	13.34^{d}	3.38^{cd}
	50	7.66^{e}	22.06^{bcd}	4.66^{c}	20.28^{c}	4.68^{c}
	75	8.66^{d}	26.02^{abc}	6.33^{b}	28.25^{b}	6.01^{ab}
ANOVA (F Value)						
Treatments		***	*	***	***	*
Conditions		***	***	***	***	***
Treatments*Conditions		ns	ns	ns	ns	ns

Each value is a mean of four replicates; values sharing different alphabetic letters indicate significant differences ($P \leq 0.05$) among treatments; *, ** and *** indicate significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively; ns indicates non-significant differences.

Foliar application of NAA improved photosynthetic pigments in squash plants under Alkaline stress

Data in Fig. 1 showed the response of foliar application of NAA solution on plant photosynthetic pigments under control and alkaline stressed plants. In stressed plants, chlorophyll *a* content was reduced by 2%, chlorophyll *b* by 78%, total chlorophyll by 43%, and carotenoids content by 54% as

compared to control plants (Fig. 1a, b, c, d). But exogenously applied NAA solution enhanced the plant pigments, although more concentrated solution exhibited more increase in the pigment as 75 mg/L NAA solution showed an increase of chlorophyll *a* by 54% and 43%, chlorophyll *b* by 53% and 87%, total chlorophyll by 64% and 55%, and carotenoids by 45% and 66% under non-stressed and stressed plants, respectively.

NAA application altered the concentrations of plant metabolites in squash plants under Alkaline stress

Data in Fig. 2a, b showed the effect of NBS foliar application on plant primary metabolites such as total free amino acids(TFA) and total soluble proteins(TSP). It is noted that the total amount of soluble proteins and free amino acids decreased (8%) in stressed squash plants, but significant improvement was noticed with increasing concentration of NAA (25, 50 and 75mg/L) solution foliarly applied. The foliar application of 75 mg/L NAA exhibited an increase of 27% in total soluble proteins under both stressed and non-stressed conditions. At the same concentration of NAA, an increase of 38% and 46% was noted under stressed and unstressed conditions.

NAA application also altered the contents of plant secondary metabolites (Fig. 2c, d) although, alkaline stress increases the total phenolics and flavonoids contents by 12% and 45% as compared to control plants, respectively. But 75 mg/L NAA application modulates the phenolics contents by 28% and 24% as well as flavonoids contents by 27% and 20% under non-stressed and stressed plants, respectively.

NAA application improved the antioxidant defense system in squash plants under Alkaline stress

Antioxidant activities of the squash plant were also investigated in squash plant under the effect of foliar application of NAA grown in alkaline soil (Fig. 3a, b). Data showed that alkaline stress increased the catalase (CAT) and peroxidase (POD) enzyme activities by 26% and 40% as compared to control plants, respectively. While, the application of 75 mg/L NAA regulate CAT activity by 42% and 32%, while POD activity was normalized by 51% and 34% under non-stressed and stressed plants, respectively. Results depicts positive role of NAA in plant defense against stress conditions.

Discussion

Plant growth regulators (PGRs) play a pivotal role in plant's life as they coordinate and regulate many physiological processes governing crop growth and yield. Lower concentrations of PGRs may be effective in enhancing the physiological aspects of crops, which ultimately aid in increasing their yield[27].

Alkaline stress is one of the abiotic stresses, alkaline, usually caused by Na_2CO_3 and $NaHCO_3$. High-pH stress affects the growth of the roots and damages their physiological function and cell structure. At the same time, alkaline stress inhibits the ion uptake and destroys the ion homeostasis of the root, causing Ca^{2+}, Mg^{2+}, $H_2PO_4^-$ precipitation. Consequently, the production of ROS accelerates, which disrupts the

metabolic pathways[28]. Alkaline salts, such as Na_2CO_3 and $NaHCO_3$, are particularly damaging to plant growth and development, as they can increase the pH of the soil and disrupt nutrient absorption[29,30]. Effect of NAA (0, 50, 75, 100ppm) applied foliar on squash growth parameters were studied (Table 2). It was observed that salinity decreased plant length, fresh biomass and leaf count while the application of NAA improved these parameters. Reduction in growth of wheat, periwinkle and tomato plants have also been studied under alkaline stress[31–33].

Photosynthetic pigments including chlorophyll *a, b*, total and carotenoids decreased under alkaline stress as compared to the control plants in this study (Fig. 1). Photosynthetic pigments are the key components of light reactions in the mechanism of photosynthesis. It is widely reported that almost all types of stresses including alkaline stress damage the thylakoid membrane, the site where all different type of photosynthetic pigments accumulated[34]. However, amino acids under alkaline stress enhance the biosynthesis of total chlorophyll and carotenoid contents in order to preserve more functioning of the photosynthetic system. During abiotic stress conditions, chlorophyll initiated the synthesis of important signaling molecule to support plant growth and development [35]Enzyme that involve in chlorophyll concentration are suppressed by high alkaline stress so chlorophyll concentration is ultimately reduced. On the other hand, the findings of this experiment revealed that the foliar application of NAA improved both chlorophyll *a* and *b* in squash plant (Fig. 1). Similarly, [36]Jeber and his coworker (2019) worked on wheat plant and observed the positive effect of NAA on plant growth and yield.

Total free amino acid and Total soluble protein content are the primary products of inorganic assimilation which decreased in squash plant under alkaline condition, while, exogenously applied NAA positively regulate amino acid under stressed condition (Fig. 2a, b). The current experimental trial agrees with the work of[37] who reported the effect of NAA in cantaloupe (*Cucumis melo* L.). In the current study, it is observed that alkalinity enhanced the synthesis of secondary metabolites (Phenolics and Flavanoids) (Fig. 2c, 2d), these results are in accordance with[38] who worked on cotton plant and observed the effect of alkaline stress on cotton plant and found that alkaline stress increase secondary metabolites of cotton plant.

Scavenging of Reactive oxygen species is carried out in plant via antioxidant enzyme system[39]. Antioxidant production in response to abiotic stress such as salinity and water stress in an effective strategy for combating oxidative stress [40]. Plants generate antioxidants in response to stress, which operate as a protective mechanism against the detrimental effect of ROS. In response to stress-triggered ROS accumulation, the plants activated antioxidant defense mechanisms to eliminate excess ROS from their cells [27].

In present investigation, it is revealed that the antioxidant enzymes catalase (CAT) and peroxidase (POD) activities conspicuously enhance by alkaline stress which agrees with the result of zhang[27] who suggested that root cell damage, and consequently growth inhibition, of rice seedlings under alkaline stress is closely associated with ROS accumulation. The antioxidant activity of catalase, peroxidase,

increased under alkaline stress. Overall, all the concentrations of NAA (0, 50, 75, 100ppm) applied exogenously affects the morphological, physiological and biochemical attributes positively in squash plants grown under both control and alkaline environment.

Conclusion

Alkaline stress significantly reduced all growth and yield parameters in C. pepo especially at higher concentrations. However, NAA application under alkali stress minimized these harmful effects via strengthening photosynthetic pigment contents, efficient increase of primary and secondary metabolites as well as regulation of CAT and POD enzyme activities compared to only alkali treated plants. 75 mg/L concentration of NAA proved to be more effective to alleviate stress responses. In future, the cellular pathways of NAA can be explored to further clarify its roles in alkali stress responses.

Abbreviations

ROS, Reactive oxygen species; CAT, catalase; SOD superoxide dismutase; Peroxidase (POD); Cytokinins (CK); Salicylic Acid (SA); Ethylene (ET); Gibberellins (GA); Abscisic Acid (ABA); Jasmonates (JA); and Brassinosteroids (BR); Napthalene acetic acid (NAA); Plant growth regulators (PGRs). (HM) Heavy metals.

Declarations

Competing interests

The authors declare that they have no competing interests.

Authors contributions

[UAN] conceived the idea and did the experiment. [NA] collected all the materials and draft it. [ZB] revised the manuscript. [AAS] supervised and gave some suggestions and advice. [MI] revised manuscript critically for important intellectual content arranged the figures and tables. [HOE] and [SS] involved in funding, revision and gave final approval to publish. All authors approved the research work to be published.

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Ethics approval and consent to participate

Not applicable

Consent for publication

All authors give permission to the publisher to publish this research work.

Availability of data and materials

All data is presented in this manuscript. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

The authors have no conflict of interest.

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References

1. Yu, Z. *et al.* How Plant Hormones Mediate Salt Stress Responses. *Trends Plant Sci* **25**, 1117–1130 (2020).
2. Ahanger, M. A., Aziz, U., Alsahli, A. A., Alyemeni, M. N. & Ahmad, P. Influence of Exogenous Salicylic Acid and Nitric Oxide on Growth, Photosynthesis, and Ascorbate-Glutathione Cycle in Salt Stressed Vigna angularis. *Biomolecules* **10**, 42 (2019).
3. Luo, X. *et al.* The ABI4-RbohD/VTC2 regulatory module promotes reactive oxygen species (ROS) accumulation to decrease seed germination under salinity stress. *New Phytologist* **229**, 950–962 (2021).
4. Sun, J., He, L. & Li, T. Response of seedling growth and physiology of Sorghum bicolor (L.) Moench to saline-alkali stress. *PLoS One* **14**, e0220340 (2019).
5. Nisa, Z. *et al.* Strigolactone signaling gene from soybean GmMAX2a enhances the drought and salt-alkaline resistance in Arabidopsis via regulating transcriptional profiles of stress-related genes. *Funct Integr Genomics* **23**, 216 (2023).
6. Mir, M. A., John, R., Alyemeni, M. N., Alam, P. & Ahmad, P. Jasmonic acid ameliorates alkaline stress by improving growth performance, ascorbate glutathione cycle and glyoxylase system in maize seedlings. *Sci Rep* **8**, 2831 (2018).
7. An, M. *et al.* Application of compound material alleviates saline and alkaline stress in cotton leaves through regulation of the transcriptome. *BMC Plant Biol* **20**, 462 (2020).
8. Fang, S., Hou, X. & Liang, X. Response Mechanisms of Plants Under Saline-Alkali Stress. *Front Plant Sci* **12**, (2021).
9. Guo, Q., Li, X., Niu, L., Jameson, P. E. & Zhou, W. Transcription-associated metabolomic adjustments in maize occur during combined drought and cold stress. *Plant Physiol* **186**, 677–695 (2021).
10. Hyoung, S. *et al.* Cytokinin oxidase PpCKX1 plays regulatory roles in development and enhances dehydration and salt tolerance in Physcomitrella patens. *Plant Cell Rep* **39**, 419–430 (2020).

11. Wu, J. *et al.* Genome-wide comprehensive analysis of transcriptomes and small RNAs offers insights into the molecular mechanism of alkaline stress tolerance in a citrus rootstock. *Hortic Res* **6**, 33 (2019).
12. Wen, T. *et al.* Changes in root architecture and endogenous hormone levels in two Malus rootstocks under alkali stress. *Sci Hortic* **235**, 198–204 (2018).
13. He, Y. *et al.* Exogenous IAA Alleviates Arsenic Toxicity to Rice and Reduces Arsenic Accumulation in Rice Grains. *J Plant Growth Regul* **41**, 734–741 (2022).
14. Mehmood, A. *et al.* IAA and flavonoids modulates the association between maize roots and phytostimulant endophytic *Aspergillus fumigatus* greenish. *J Plant Interact* **13**, 532–542 (2018).
15. Shiraz, M., Sami, F., Siddiqui, H., Yusuf, M. & Hayat, S. Interaction of Auxin and Nitric Oxide Improved Photosynthetic Efficiency and Antioxidant System of Brassica juncea Plants Under Salt Stress. *J Plant Growth Regul* **40**, 2379–2389 (2021).
16. Mir, A. R., Siddiqui, H., Alam, P. & Hayat, S. Foliar spray of Auxin/IAA modulates photosynthesis, elemental composition, ROS localization and antioxidant machinery to promote growth of Brassica juncea. *Physiology and Molecular Biology of Plants* **26**, 2503–2520 (2020).
17. Chaudhry, U. K. *et al.* Salinity Tolerance in Rice. in *Modern Techniques of Rice Crop Production* 275–293 (Springer Singapore, Singapore, 2022). doi:10.1007/978-981-16-4955-4_16.
18. Iswaldi, I. *et al.* Profiling of phenolic and other polar compounds in zucchini (Cucurbita pepo L.) by reverse-phase high-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. *Food Research International* **50**, 77–84 (2013).
19. Peričin, D., Krimer, V., Trivić, S. & Radulović, L. The distribution of phenolic acids in pumpkin's hull-less seed, skin, oil cake meal, dehulled kernel and hull. *Food Chem* **113**, 450–456 (2009).
20. Costa, A. G., Ribeiro, E., Braga, R. A. & Pinto, F. A. C. Measurement of volume of macaw palm fruit using traditional and the digital Moiré techniques. *Revista Brasileira de Engenharia Agrícola e Ambiental* **20**, 152–157 (2016).
21. Arnon, D. I. Copper Enzymes in Isolated Chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol* **24**, 1–15 (1949).
22. Davies, B. H. Carotenoids. Chemistry and biochemistry of plant pigments. *Ac. Press. London.* **II**, (1976).
23. Bradford, M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal Biochem* **72**, 248–254 (1976).
24. Julkunen-Tiitto, R. Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. *J Agric Food Chem* **33**, 213–217 (1985).
25. Chance, B. & Maehly, A. C. [136] Assay of catalases and peroxidases. in 764–775 (1955). doi:10.1016/S0076-6879(55)02300-8.
26. Kenneth M. Steele, Tamera N. Ball & Rebecca Runk. Listening to Mozart Does Not Enhance Backwards Digit Span Performance. *Percept Mot Skills*.

27. Zhang, H., Sun, X. & Dai, M. Improving crop drought resistance with plant growth regulators and rhizobacteria: Mechanisms, applications, and perspectives. *Plant Commun* **3**, 100228 (2022).
28. Fan, Y. *et al.* Cotton transcriptome analysis reveals novel biological pathways that eliminate reactive oxygen species (ROS) under sodium bicarbonate (NaHCO3) alkaline stress. *Genomics* **113**, 1157–1169 (2021).
29. Zhang, H. *et al.* Effects of four types of sodium salt stress on plant growth and photosynthetic apparatus in sorghum leaves. *J Plant Interact* **13**, 506–513 (2018).
30. Wang, W. *et al.* Alkaline Salt Inhibits Seed Germination and Seedling Growth of Canola More Than Neutral Salt. *Front Plant Sci* **13**, (2022).
31. Farghaly, F. A., Nafady, N. A. & Abdel-Wahab, D. A. The efficiency of arbuscular mycorrhiza in increasing tolerance of Triticum aestivum L. to alkaline stress. *BMC Plant Biol* **22**, 490 (2022).
32. Gong, B. *et al.* Sodic alkaline stress mitigation by interaction of nitric oxide and polyamines involves antioxidants and physiological strategies in Solanum lycopersicum. *Free Radic Biol Med* **71**, 36–48 (2014).
33. Pahare, P. and D. J. N. Effect of Alpha-Naphthalene Acetic Acid [NAA] on Growth, Flowering and Yield of Vinca rosea cv Catharanthus caramel. *Int.J.Curr.Microbiol.App.Sci* **9**, 1961–1967 (2020).
34. Billah, M. *et al.* Exploring Regulatory Roles of Plant Thylakoid-Bound Proteins Involved in Abiotic Stress Responses. *J Plant Growth Regul* **43**, 1570–1591 (2024).
35. Shaheen, S., Naseer, S., Ashraf, M. & Akram, N. A. Salt stress affects water relations, photosynthesis, and oxidative defense mechanisms in *Solanum melongena* L. *J Plant Interact* **8**, 85–96 (2013).
36. Jeber, B. A. & Khaeim, H. M. effect of foliar application of amino acids, organic acids, and naphthalene acetic acid on growth and yield traits of wheat. vol. 19 (2019).
37. Ali, M.). Detection of Xanthomonas campestris pv. cucurbitea from bacterial leaf spot disease of cucumber and evaluation of it biological control. . *Advances in Bioresearch,* **9**, 41–46 (2018).
38. Fan, Y. *et al.* Cotton transcriptome analysis reveals novel biological pathways that eliminate reactive oxygen species (ROS) under sodium bicarbonate (NaHCO3) alkaline stress. *Genomics* **113**, 1157–1169 (2021).
39. Huang, H., Ullah, F., Zhou, D.-X., Yi, M. & Zhao, Y. Mechanisms of ROS Regulation of Plant Development and Stress Responses. *Front Plant Sci* **10**, (2019).
40. Hasanuzzaman, M. *et al.* Regulation of Reactive Oxygen Species and Antioxidant Defense in Plants under Salinity. *Int J Mol Sci* **22**, 9326 (2021).

Figures

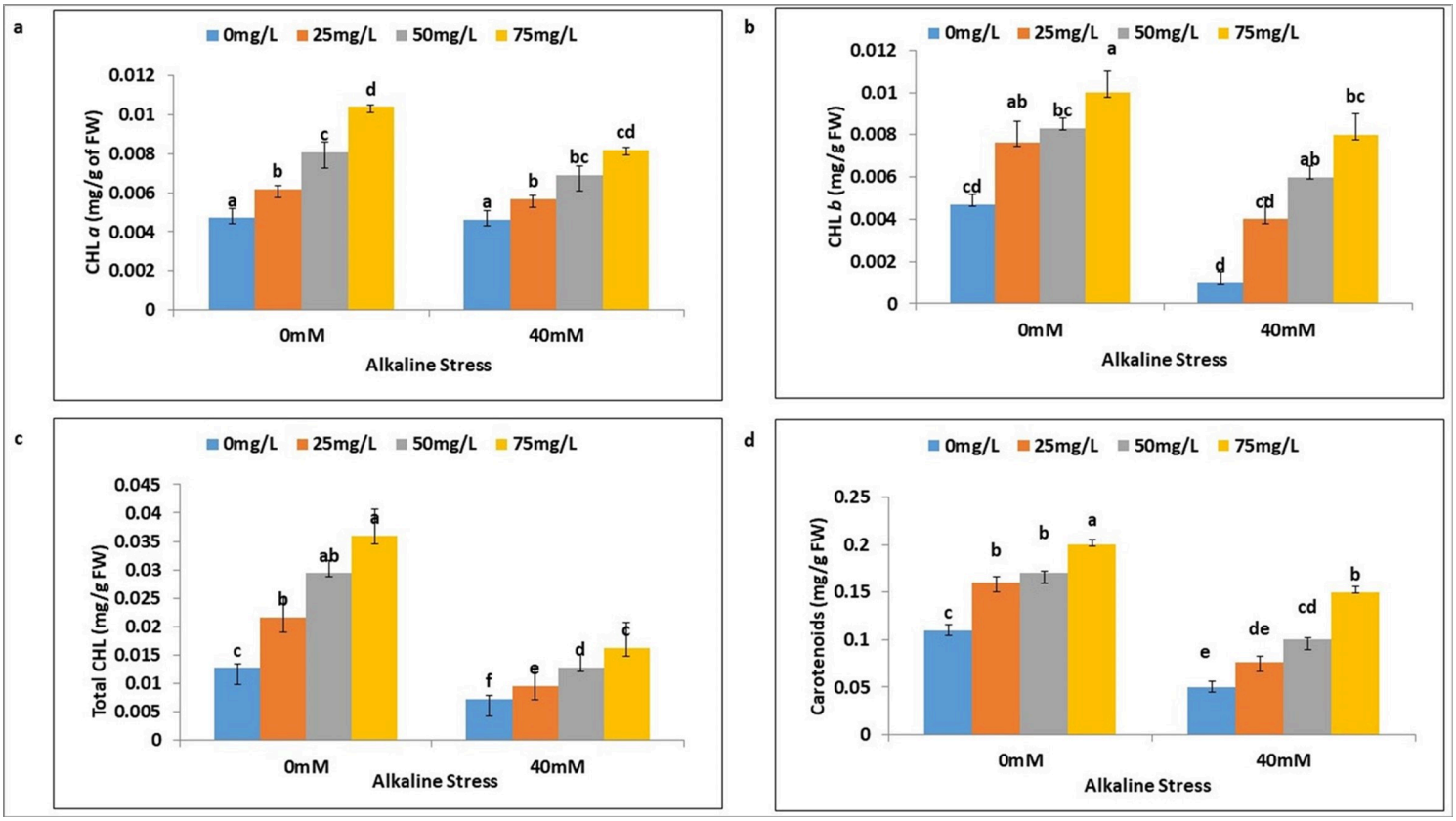


Figure 1

The effect of naphthalene acetic acid (NAA) under alkaline stress on photosynthetic pigments of squash plant. (a) Chlorophyll *a*, (b) Chlorophyll *b*, (c) Total chlorophyll, and (d) Caratenoids. Bars sharing similar alphabetic letters do not differ significantly ($P \leq 0.05$).

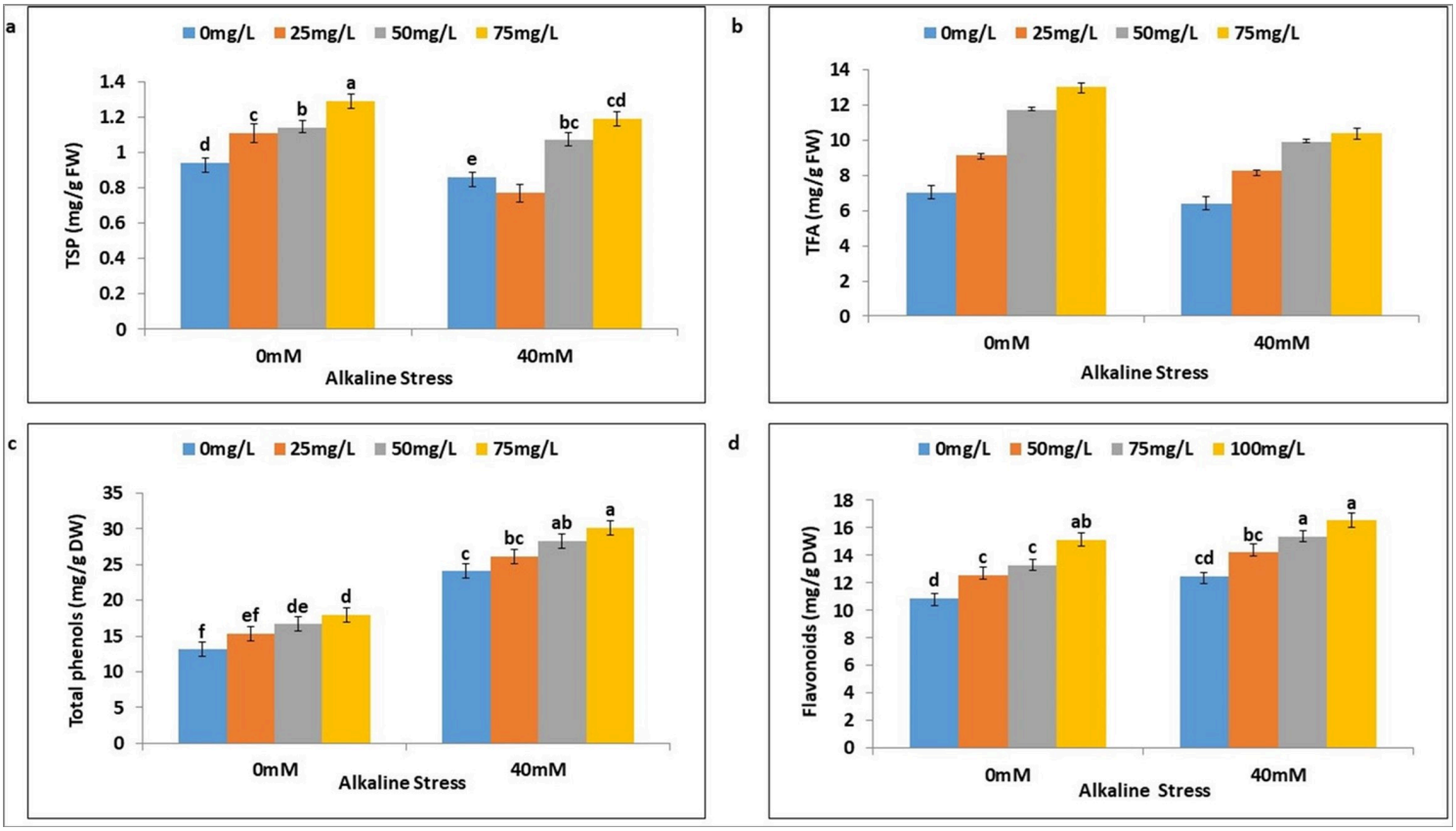


Figure 2

The effect of naphthalene acetic acid (NAA) under alkaline stress on metabolites of squash plant. (a) Total soluble proteins (TSP), (b) Total free amino acids (TFA), (c) Total phenols, (d) Falvanoids. Bars sharing similar alphabetic letters do not differ significantly ($P \leq 0.05$).

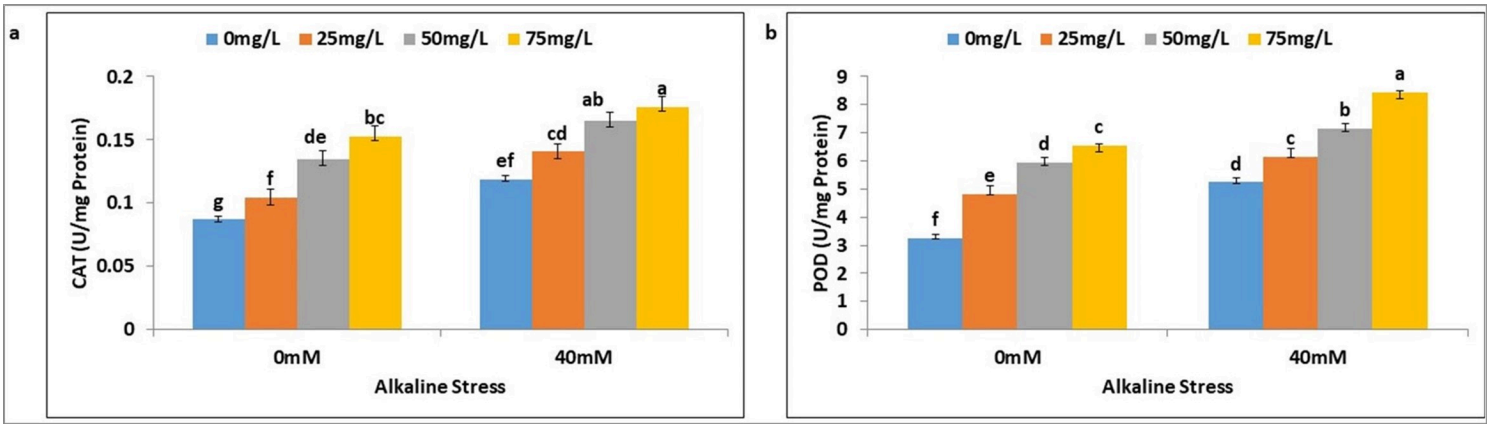


Figure 3

The effect of naphthalene acetic acid (NAA) under alkaline stress on antioxidant enzymes of squash plant. (a) Catalase; (b) Peroxidase. Bars sharing similar alphabetic letters do not differ significantly ($P \leq 0.05$).