ASSESSMENT OF MORPHO-PHYSIOLOGICAL FEATURES OF TWO OKRA (*ABELMOSCHUS ESCULENTUS* L.) CULTIVARS AND THEIR HYBRIDS UNDER INDUCED SALT STRESS CONDITIONS

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Abstract. Salinization is an enormous problem affecting the horticultural crops. Salinity alters plant metabolism, reduces the endogenous water potential, and inhibits plant growth. Okra (*Abelmoschus esculentus* L.) is the most important annual vegetable, with high nutritional and medicinal values. It exhibits large genetic variation amongst cultivars, which shows better breeding material for improved crop production. Four concentrations of NaCl (0, 25, 50, and 75 mM) were applied to Japanese and Egyptian parents of okra and their F_1 hybrids (Egyptian × Japanese) and (Japanese × Egyptian). Salt stress significantly enhanced the reduction of shoot length, root length, dry weight, and fresh weight in Egyptian cultivars and both hybrids. The efficiency of photo system two [PSII (Fv/Fm)] in the Egyptian mothers showed a significant decrease. Increasing NaCl concentrations significantly reduced protein content in the Japanese (J) and Egyptian (E) cultivars. While both hybrids (J×E and E×J) showed a significant increase (P value < 0.05) in protein content from 249 to 310.9 and 240 to 312.5 mg/g DW (dry weight). Also, there was an increase in total phenolics, proline, and steroids in hybrids compared to their parents under high salt concentrations (75 mM), reaching (15, 14.75, and 82.44 mg/g DW, respectively) for the J×E hybrid and (22.5, 8.98, and 64.45 mg/g DW, respectively) for the E×J hybrid. These results clearly indicated the successfully higher salt-tolerant nature of hybrids, which correlated with a higher level of protein, proline, steroid, and total phenolics at high salt concentrations. Cross-breeding between Japanese and Egyptian okra cultivars improved the okra salt tolerance.
Keywords: *plant breeding, salinity, Antioxidants, DPPH, growth traits*

Introduction

Salinity is one of the most severe problems responsible for limiting the growth and productivity of agricultural crops. It is common in arid and semiarid regions (Kaashyap et al., 2018). Proximity to coastal regions, high temperatures, floods, and wind erosion are the natural reasons that lead to increasing salinity (Shrivastava and Kumar, 2015). Poor irrigation and drainage, the use of imbalanced fertilizers, and soil degradation are also thought to contribute to salinity (Sandhu et al., 2017).

About 20% of the cultivated and irrigated land in the world is salt-affected (Machado and Serralheiro, 2017). Na+ and Cl- ions build up in a variety of plant tissues because of salt stress. Salinity stress affects all aspects of growth, changing water status,

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transpiration, and respiration rate, causing cellular damage and degradation of chlorophylls, resulting in growth reduction (Allel et al., 2018).

Plants possess different mechanisms, such as producing osmolytes like proline, regulating salt accumulation, and producing different enzymatic or non-enzymatic antioxidants, which eliminate the harmful effects of reactive oxygen species (ROS) (Sharma et al.,2019). In addition, changes in plant physiology and metabolites play a vital role in plants surviving salinity stress and maintaining osmotic balance (Borrelli et al., 2018). Plants accumulate compatible metabolites, e.g., soluble sugars, amino acids, organic acids, polyamines, and lipids, in order to lower the osmotic potential and protect against the damaging effects of osmotic stress (Ghosh et al., 2021). Under environmental stresses, chlorophyll fluorescence analysis is considered an efficient, rapid, and sensitive method to characterize and inspect plant performance (Bąba et al., 2019).

Breeding salt-tolerant crop varieties is the most effective and utilized method to reduce the drastic effects of salinity. The development of resistant varieties to salt stress through selection and breeding depends on the presence of genetic variability within the crop species in response to salt stress (Razzaq et al., 2021). Physiological and biochemical responses such as ion exclusion, ion accumulation, compatible solute synthesis, antioxidant activity, polyamines, and osmotic adjustment have been attributed to genetic variation and are necessary to design an effective breeding program and choose the best salt-tolerant parents for hybridization (Singh et al., 2021).

Okra (*Abelmoschus esculentus* L.), which belongs to the family of Malvaceae, is ranked as one of the most important annual vegetables grown in tropical and subtropical areas (Azooz et al., 2015). Okra is cultivated and consumed as a fresh food in Egypt due to its high nutritional and medicinal values. Soil salinization affects okra quality and productivity, and it is classified as a moderately salt-tolerant vegetable (El-Shaieny et al., 2022; Davis, 2022). Okra genotypes have a wide genetic variation to tolerate NaCl salinity; therefore, breeding and genetic improvement are possible (Priyanka et al., 2018).

The present study was conducted to evaluate the physiological and metabolic mechanisms of adaptation to salt stress of two genetically different okra cultivars (Japanese and Egyptian) and their hybrids. Japanese and Egyptian cultivars were chosen for the study of okra due to their unique characteristics and adaptability to different environmental conditions as well as seeds of Japanese cultivar were available. The information gained would be useful in the breeding programs, which may further be used to improve the productivity of the crops.

Materials and methods

Plant material and growth conditions

Two Okra (*Abelmoschus esculentus* L.) cultivars (Japanese and Egyptian) were crossbred in both directions, according to Ahmed and El-Sayed (2019). Seeds of all okra cultivars and their F1 hybrids were set to germinate in plastic pots filled with fine sand and vermiculite (1:1 w/w) as a growth medium. Five seeds per pot were sown. After 15 days of germination, the seedlings were thinned out to three. The experiment was carried out with three replications. The pots were kept in the greenhouse of the Research Unit for Studying Plants of Arid Lands (RUSPAL), Aswan University (23°59’56”N, 32°51’36”E) under normal climatic conditions for 60 days. The salt

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treatment was initiated using NaCl at final concentrations of 0 (control), 25, 50, and 75 mM. To minimize the osmotic shock, the NaCl concentrations were imposed in 25 mM increments every day until the final concentrations were reached after a three-day interval. Plants were grown for 10 days under salt-stressed conditions. At the end of the experiment, roots and shoots were separated and washed properly with distilled water. The mean root length, shoot length, fresh mass, and dry mass were recorded for all treated seedlings.

Chlorophyll fluorescence

Chlorophyll fluorescence was measured from non-detached young and fully expanded leaves using an infrared gas analyzer (IRGA, CI 340) photosynthesis system (CID Bio-Science, Inc.). Following the manufacturer's instructions using the (CI-510 CF) chlorophyll fluorescence module, the data recorded was between 11:00 a.m. and 1:00 p.m. Chlorophyll fluorescence parameters, including initial fluorescence (*Fo*), maximum fluorescence (*Fm*), variable fluorescence (*Fv*), and maximum quantum efficiency of PSII (*Fv/Fm*), were monitored on the 4th leaf under salt stress conditions.

Total chlorophyll content and metabolic changes measurements

Determination of chlorophyll content

Fresh leaves (0.1 g) were extracted in 80% acetone to determine the total chlorophyll contents (Ni et al., 2009), using 100 mg FW of leaf material ground in a pre-chilled mortar in 3 ml of acetone 80% (v/v). After complete extraction, the mixture was filtered and the volume adjusted to 10 ml with cold acetone. The absorbance of the extract was read at 663 and 645 nm, and pigment concentrations were calculated according to Ni et al. (2009).

Determination of total proteins

Total proteins were determined according to the method adopted by Lowry et al. (1951). Fifty mg of dried leaf sample was hydrolyzed in 2 ml of (1N) NaOH, then centrifuged for 20 min, then the supernatant was transferred into a test tube and 1 ml of alkaline reagent (2% Na2CO3 prepared in 0.1 N NaOH (reagent A) and 0.5% CuSO4.5H2O prepared in 1% sodium-potassium tartrate (reagent B). Alkaline reagent was prepared by mixing 50 mL of reagent A and 1 ml of reagent B. The mixture was incubated at room temperature for at least 10 min, and 0.5 ml of diluted Folin-Ciocalteu reagent was added to the mixture. After 30 min, the absorbance against the proper blank was measured at 700 nm. Egg albumin was prepared in a diluted series and treated as the samples to construct the calibration curve. The content of protein was expressed as mg/g dry weight.

Determination of total carbohydrates

The water-soluble carbohydrates and insoluble carbohydrates were determined by the anthrone-sulfuric acid method according to Fales (1951). 50 mg of leaf sample was hydrolyzed with 4N HCl for 2 h in a boiling water bath, and after cooling, 9 ml of anthron reagent was added to the hydrolyzed sample. The developed blue-green color was read at the wavelength of 620 nm.

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Determination of proline

Proline content of leaves was determined according to a modification of the method of Bates et al. (1973). Dry leaf (250 mg) was homogenized in 10 ml of 3% sulfo-salicylic acid. The homogenate was centrifuged, and 2.0 ml of the supernatant were mixed with 2.0 ml of acid ninhydrin (2.5 g ninhydrin in 40 ml of 6 ml orthophosphoric acid and 60 ml glacial acetic acid) and 2.0 ml of glacial acetic acid in a test tube. All test tubes were incubated at 100°C for 1 h. The mixture was cooled in an ice bath. After cooling, to each test tube, 4 ml of toluene were added and vortexed for 1 min. The absorbance of the supernatant was read at 520 nm using a UV-visible spectrophotometer. The proline concentration was determined from a standard curve and calculated on a dry weight basis.

Determination of steroids

The steroid content was spectrophotometrically measured according to Bischoff and Turner (1958). A dried leaf sample (20 mg) was placed in a test tube with 1.0 ml of glacial acetic acid. Then, 4.0 ml of a cool mixture consisting of 0.5 ml. of a solution of 10% FeCls. 6H20 (prepared in glacial acetic acid), 50 ml of 96% sulfuric acid, and 50 ml of glacial acetic acid was added. Two hours after mixing, the solution was measured with a spectrophotometer against a blank.

Determination of total phenolics

Total phenolic compounds were determined spectrophotometrically according to the Folin-Ciocateu method (Ough et al., 1988). Okra dry leaf (50 mg) was soaked in 2.0 ml of 80% methanol and was homogenized and centrifuged (13,000 rpm, 20 min). One ml of the supernatant was transferred to a test tube, and 1 ml of 80% methanol and 0.5 ml of Folin-Ciocalteu phenol reagent were added. After an incubation period of 5 min, 1 ml of 5% Na2CO3 was added, mixed well, and kept for 1 h. The samples were vortexed, and absorbance was measured at 700 nm. Phenolic content was reported as mg galic acid equivalent per g dry weight (DW).

Determination of total flavonoids

The total flavonoid content of the plant extract was determined by the aluminum chloride colorimetric method (Chang et al., 2002). In brief, dry leaf (50 mg) was soaked in 2.0 ml of 80% methanol. The sample was homogenized and centrifuged at 13,000 rpm for 20 min. One ml of the supernatant was transferred to a test tube, then 0.3 ml of 5% NaNO2 solution and 0.3 ml of 10% AlCl3 solution were added after 5 min of incubation, and the mixture was allowed to stand for 6 min. Then, 2 mL of 1 M NaOH solution were added. The mixture was allowed to stand for 15 min. The absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg quercetin equivalent per g dry weight (DW).

Determination of total saponins

Total saponin content was determined spectrophotometrically at 473 nm (Ebrahimzadeh and Niknam, 1998) with a modification as follows: 20 mg of dried leaf sample was dissolved in 2 ml of 80% methanol, and then 5 ml of 0.7% vanillin in 65%

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H2SO4 was added. After vortexing, test tubes were maintained at 60°C in a water bath for 1 h. Then, test tubes were transferred into crushed ice, and absorbance was determined after 10 min. Saponin content was calculated based on the average value of absorbance at each concentration of the diosgenin standard.

Determination of total antioxidant capacity

The assay was performed according to Prieto et al. (1999). Tubes containing 1 ml of plant extract and 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) were incubated at 90°C for 90 min. The antioxidant capacity was expressed as ascorbic acid equivalent (AAE).

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The reaction mixture (total volume, 3 ml), containing 1 ml of 0.2 mM DPPH in ethanol and 0.5 ml of methanol plant extract, was vigorously shaken. After incubation at room temperature for 30 min, the amount of DPPH remaining was determined by measuring absorbance at 517 nm (Blois, 1958).

Statistical analysis

Significant variances among different treatments of salinity were calculated using one-way analysis of variance (ANOVA) in Minitab version 18.1. Significant variance is obtained when the P-value is < 0.05. Multi-correlation analyses were performed using principal component analysis (PCA) by using SPSS to categorize the different growth patterns and to designate the response of metabolic and growth traits to a different NaCl concentration between cultivars. Three biological replicates ($n = 3$) were performed.

Results

The data in *Table 1* shows that salinity stress significantly decreased the plant fresh weight (FW) and dry weight (DW) in both okra cultivars and their hybrids. The fresh weight (FW) of E×J was highly affected, with a 61.5% reduction at high salt concentrations (75 mM) compared to the control, followed by the Japanese cultivar with 54% and J×E with 41.3%. However, the Egyptian cultivar was the least affected by the 29.8% reduction in biomass. Salinity significantly decreased the dried biomass of the Egyptian cultivar and both F_1 hybrids (J×E and E×J). The Japanese cultivar showed different responses among the different salinity levels. The highest values of DW were observed at 25 mM with a mean value of 2.6 g, then decreased at 75 mM with a mean value of 1.4 g. The magnitude of shoot and root reduction was highly dependent on NaCl concentration.

Root and shoot morphology showed no perceptible changes in both okra cultivars and their hybrids under NaCl treatment. There was no severe damage or apparent necrosis, even in high concentrations. *Figure 1* showed the mean shoot length of the cultivars under different levels of salt stress. The shoot length of the Egyptian cultivar and the hybrid (E×J) was not significantly affected by salinity stress. The Japanese cultivar showed a significant increase in shoot length at high salt concentrations, while a significant decrease was observed in the J×E cultivar at the same salinity stress level.

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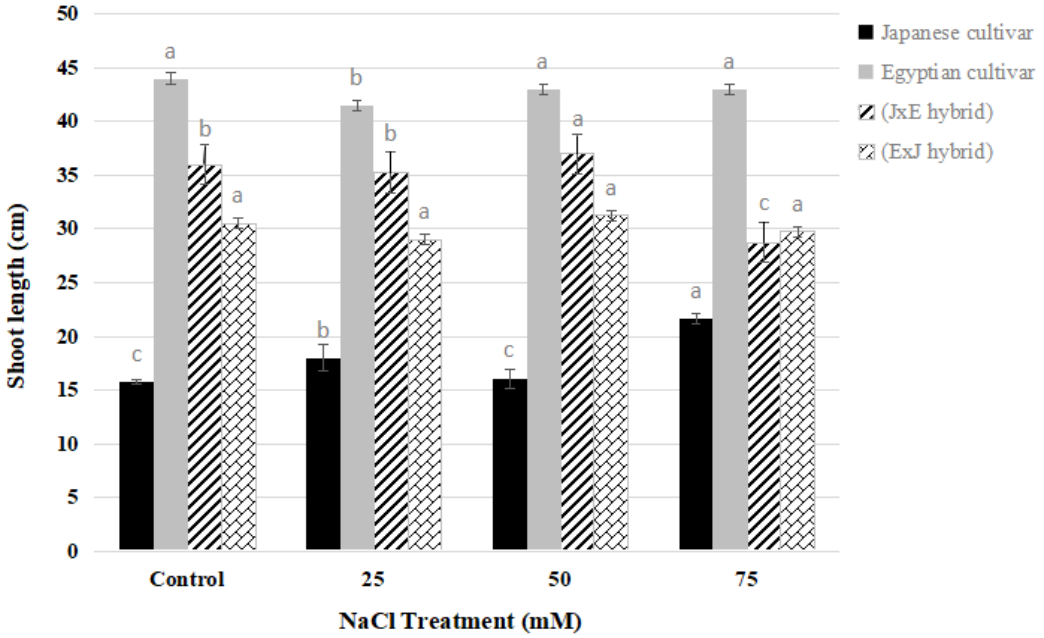

Figure 1. *Mean shoot length under different levels of salinity*

Table 1. *Fresh weight, dry weight, total chlorophyll and Fv/Fm of two hybrid of okra in comparison with their parent cultivars under varying NaCl level*

Variety	Treatment	FW (g)	DW (g)	Fv/Fm	Total chlorophyll (mg/g FW)
Japanese cultivar	Control	13.13 ± 0.5 ^{a}	0.82 ± 0.00 ^{c}	0.54 ± 0.05^{a}	2.52 ± 0.058^{ab}
	25	12.46 ± 4.9 ^{a}	2.64 ± 0.56 ^{a}	0.139 ± 0.01^{c}	2.56 ± 0.043^{a}
	50	7.25 ± 1.42 ^{b}	1.77 ± 0.04 ^{b}	0.334 ± 0.0^{b}	2.35 ± 0.06^{ac}
	75	6.20 ± 0.24 ^{b}	1.40 ± 0.0 ^{b}	0.55 ± 0.03^{a}	2.41 ± 0.03^{cb}
Egyptian cultivar	Control	17.9 ± 0.73^{a}	4.06 ± 0.58 ^{a}	0.54 ± 0.02^{a}	2.49 ± 0.030^{a}
	25	13.7 ± 0.57^{b}	2.33 ± 0.56 ^{b}	0.61 ± 0.03^{a}	1.79 ± 0.029^{c}
	50	11.5 ± 1.22^{b}	2.26 ± 0.29^{b}	0.42 ± 0.04^{b}	1.99 ± 0.058^{b}
	75	12. 6 ± 0.47^{b}	2.5 ± 0.12^{b}	0.39 ± 0.04^{b}	2.10 ± 0.027^{b}
Japanese cultivar × Egyptian cultivar (JxE hybrid)	Control	13.37 ± 1.18 ^{a}	2.7 ± 0.30^{a}	0.59 ± 0.06^{a}	2.23 ± 0.08^{ab}
	25	10.85 ± 0.49^{ab}	2.2 ± 0.58^{ab}	0.49 ± 0.02^{a}	2.46 ± 0.090^{a}
	50	10.1 ± 2.95^{ab}	2.8 ± 0.26^{a}	0.51 ± 0.03^{a}	2.36 ± 0.10^{ab}
	75	7.83 ± 2.95^{b}	1.7 ± 0.57^{b}	0.56 ± 0.11^{a}	2.12 ± 0.075^{b}
Egyptian cultivar × Japanese cultivar (ExJ hybrid)	Control	14.30 ± 1.88^{a}	3.09 ± 1.20^{a}	0.51 ± 0.1^{a}	2.32 ± 0.09^{a}
	25	16.49 ± 2.38^{a}	2.23 ± 1.51^{ab}	0.49 ± 0.05^{a}	2.31 ± 0.08^{a}
	50	5.25 ± 0.61^{b}	1.51 ± 0.16^{b}	0.42 ± 0.004^{a}	2.32 ± 0.02^{a}
	75	5.55 ± 0.44^{b}	1.20 ± 0.25^{b}	0.411 ± 0.02^{a}	2.30 ± 0.06^{a}

All values are mean ± SD, Values in the same group with different superscript letters are significantly Different, P < 0.05 (ANOVA after Tukey's test analysis)

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Figure 2 showed the mean root length of the studied cultivars under different levels of salt stress. A significant decrease in the root length was observed in Egyptian and J×E cultivars with increasing salt stress. E×J cultivars show no significant differences in the root length.

The *Fv/Fm* values were observed to be significantly decreased only in Egyptian cultivars. The two hybrids J×E and E×J remained constant in terms of *Fv/Fm* values even under high saline conditions. The Japanese cultivars exhibited a sharp reduction at 25 mM with a mean value of 0.139 (*Table 1*).

Total chlorophyll content showed a significant decrease in both okra parents and J×E hybrid, while no significant change in chlorophyll content was recorded in E×J hybrid under high salt concentration (*Table 1*).

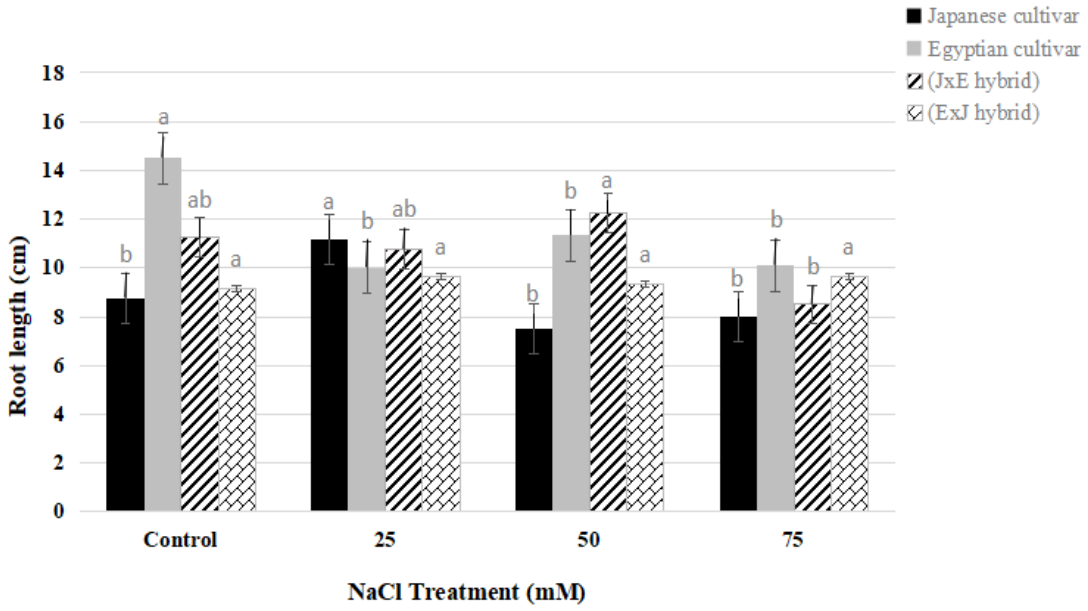

Figure 2. *Mean root length under different levels of salinity*

Table 2 shows the effect of salinity levels on different metabolite contents. Under high salt concentration, total protein content insignificantly increased in Egyptian cultivars and increased significantly in both F_1 hybrids (J×E and E×J) with mean values of 352.5, 310.9, and 296.3 mg/g DW, respectively. In contrast, the Japanese cultivar showed a significant decrease in total protein content (205.5 mg/g DW).

Compared with the control, results showed a significant increase in total carbohydrate content under high salt concentrations (75 mM) for the Japanese cultivar. However, the Egyptian cultivar and E×J hybrid experienced a decrease in total carbohydrates. Meanwhile, the J×E hybrid exhibited no significant changes.

Salinity stress significantly affected proline and steroid accumulation. Both parents showed a decrease in proline and steroid contents under high saline conditions, corresponding to their control; in contrast, J×E showed a highly significant increase in both proline and steroid content, while in the other hybrid, E×J, proline and steroid content were not significantly affected. The mean highest values for proline and steroid accumulation were recorded at 75 mM of salt concentration in J×E with values of 14.7 and 82.4 mg/g DW, respectively.

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Table 2. *Different growth traits, total chlorophyll and Fv/Fm of two hybrid of okra in comparison with their parent cultivars under different NaCl levels*

Variety	Treatment	Total protein	Total carbohydrate	Proline	Steroids	Phenolics	Flavonoids	Saponin
Japanese cultivar	Control	348.00±1.52 ^{a}	169.80±6.78 ^{c}	33.94±1.96 ^{a}	79.87±2.09^{a}	12.94±1.51 ^{a}	61.50±3.94^{a}	31.62±2.89 ^{a}
	25	341.13±2.90 ^{b}	182.67±4.24^{bc}	22.59±1.78 ^{b}	72.07±0.30^{b}	11.37±0.61^{ab}	56.00±1.63^{ab}	36.54±2.11 ^{a}
	50	213.63±2.75 ^{c}	202.56±1.39 ^{b}	18.17±0.59 ^{bc}	63.41±1.39^{c}	8.97±0.76^{b}	52.33±1.91^{b}	34.02±0.74 ^{a}
	75	205.50±7.55 ^{c}	208.71±1.39 ^{a}	13.52±3.44 ^{c}	63.84±1.64 ^{c}	10.57±0.82 ^{ab}	36.60±1.63^{c}	34.92±2.90 ^{a}
Egyptian cultivar	Control	321.83±31.88 ^{a}	246.67±1.98^{a}	9.63±1.22 ^{ab}	94.02±2.09 ^{a}	16.62±0.23 ^{b}	69.17±0.14^{a}	50.1±0.05 ^{a}
	25	348.00±1.63 ^{a}	245.98±3.29^{a}	11.26±1.48 ^{a}	90.54±0.34 ^{a}	18.22±0.21^{a}	55.83±3.67^{b}	52.68±0.49 ^{a}
	50	325.25±0.81 ^{a}	236.57±2.35^{b}	6.97±2.14 ^{ab}	87.80±4.99 ^{a}	14.24±0.13 ^{c}	59.33±3.81^{b}	54.66±3.38 ^{a}
	75	325.50±12.25 ^{a}	225.72±1.47^{c}	4.94±0.59 ^{b}	62.86±2.74 ^{b}	14.24±0.15 ^{c}	46.56±0.87^{c}	34.32±0.49 ^{b}
Japanese cultivar × Egyptian cultivar	Control	249.50±12.60^{b}	219.97±4.53^{a}	11.49±0.32^{b}	64.57±1.05^{c}	11.27±1.75 ^{c}	50.67±3.54^{a}	35.80±1.38 ^{a}
	25	273.25±4.69 ^{ab}	233.25±4.76 ^{a}	5.85±0.03^{c}	60.79±0.15^{c}	12.33±0.74 ^{bc}	48.00±1.36 ^{a}	35.27±0.77 ^{a}
	50	266.63±14.39^{ab}	225.90±2.77 ^{a}	15.26±0.59^{a}	72.68±0.40^{b}	13.27±0.53 ^{ab}	52.17±3.40 ^{a}	34.56±2.06^{a}
	75	310.91±27.02 ^{b}	234.19±7.07 ^{a}	14.75±1.00^{a}	82.44±2.40^{a}	15.24±1.74 ^{a}	48.50±1.77 ^{a}	34.24±1.82 ^{a}
Egyptian cultivar × Japanese cultivar	Control	240.00±0.40^{c}	244.67±2.07 ^{a}	5.93±0.02^{c}	60.54±2.74 ^{a}	17.01±0.17 ^{b}	62.33±0.27 ^{a}	52.88±4.42 ^{a}
	25	280.38±5.80^{b}	222.51±2.93 ^{b}	7.79±0.04^{b}	64.45±1.54 ^{a}	16.89±0.87 ^{b}	63.17±2.04 ^{a}	50.04±2.16^{a}
	50	297.50±2.04^{b}	205.20±2.66 ^{c}	8.98±0.22^{a}	63.17±2.19 ^{a}	21.39±1.06 ^{a}	48.67±1.63 ^{b}	35.88±1.08 ^{b}
	75	312.50±10.2^{a}	200.17±4.69 ^{c}	9.16±0.36^{a}	67.07±3.49^{a}	22.59±0.41 ^{a}	48.83±2.25^{b}	49.40±0.76 ^{a}

All values are mean ± SD. Values in the same group with different superscript letters are significantly different, $P < 0.05$ (ANOVA after Tukey's test analysis)

Total phenolic content differed significantly between okra parents and their hybrids. A significant decrease was observed in Japanese and Egyptian parents (10.5 and 14.27 mg/g DW, respectively). In contrast, both F1 hybrids (J×E and E×J) showed a significant increase in total phenolic content (15.2 and 22.5 mg/g DW, respectively). Total flavonoid contents showed a significant decrease in all okra parents and their hybrids E×J, while J×E showed no significant change. Saponin content showed no significant change in all Okra parents and their hybrids.

Figure 3 shows that the total antioxidant activity exhibited a significant decrease in all okra cultivars and their hybrids (J×E and E×J). Okra cultivars showed a much weaker antioxidant capacity with increasing NaCl concentrations to 75 mM.

Figure 3 illustrates a significant decrease in TAC exhibited by all okra cultivars and their hybrids (J×E and E×J). Okra cultivars showed a much weaker antioxidant capacity with increasing NaCl concentrations to 75 mM.

Figure 4 shows that the DPPH radical scavenging activity of Egyptian cultivars and E×J was significantly decreased with an increased concentration of NaCl as compared to the control. The Egyptian cultivar showed a highly antioxidant activity at low NaCl concentrations (25 and 50 mM) with 92% activity.

In Japanese cultivars, the DPPH activity provides a slight increase at high salt concentrations (66%), while there are no significant changes in both hybrids E×J and J×E.

According to PCA multivariate data analysis (*Fig. 5*), the response of metabolites and growth parameters varied with different salt treatments and cultivars. In each cultivar, the control and different salt-treated plants separated very distinctly from each other. The findings suggested that the plants responded differently to different treatment concentrations of NaCl, and the efficiency of separation in the case of the main factors was checked. The sharp angle means a positive correlation between the measured

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parameters; the obtuse angle means a negative correlation; and the right angle means no correlation between the parameters. Most of the metabolites and growth traits such as fresh weight, dry weight, shoot length, and root length have positive loadings in PC1 except proline and chlorophyll contents. Furthermore, PCA analysis allowed us to group okra cultivars and their F_1 hybrids according to their performance against salt stress. The trend of increase or decrease of metabolites and growth traits in response to NaCl, as analyzed by ANOVA.

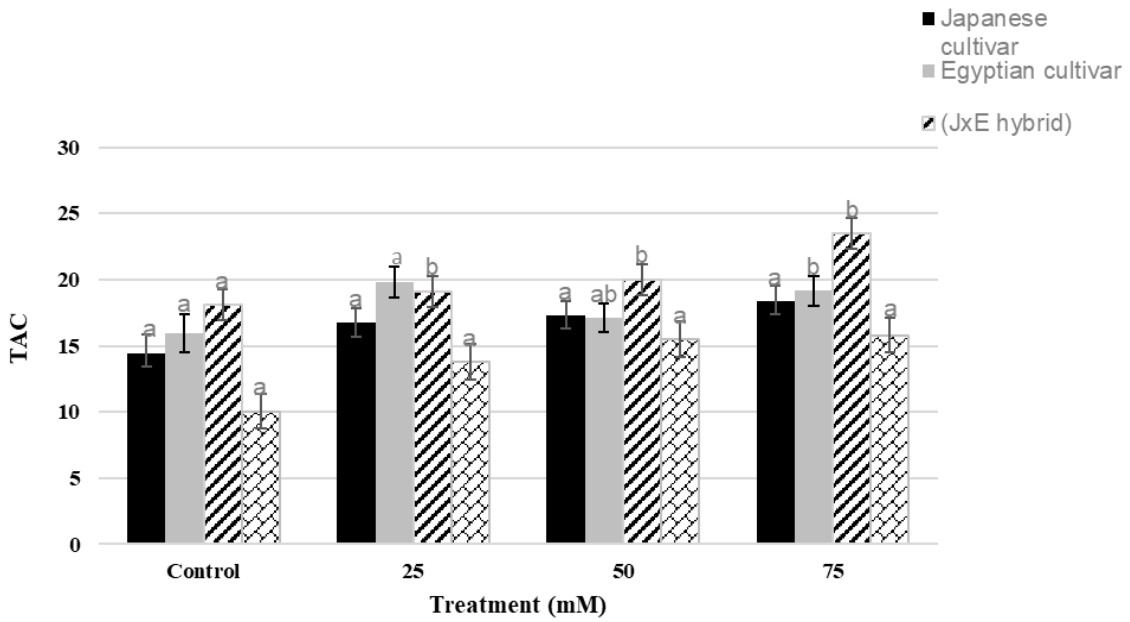

Figure 3. *Total antioxidant capacity under different levels of salt stress*

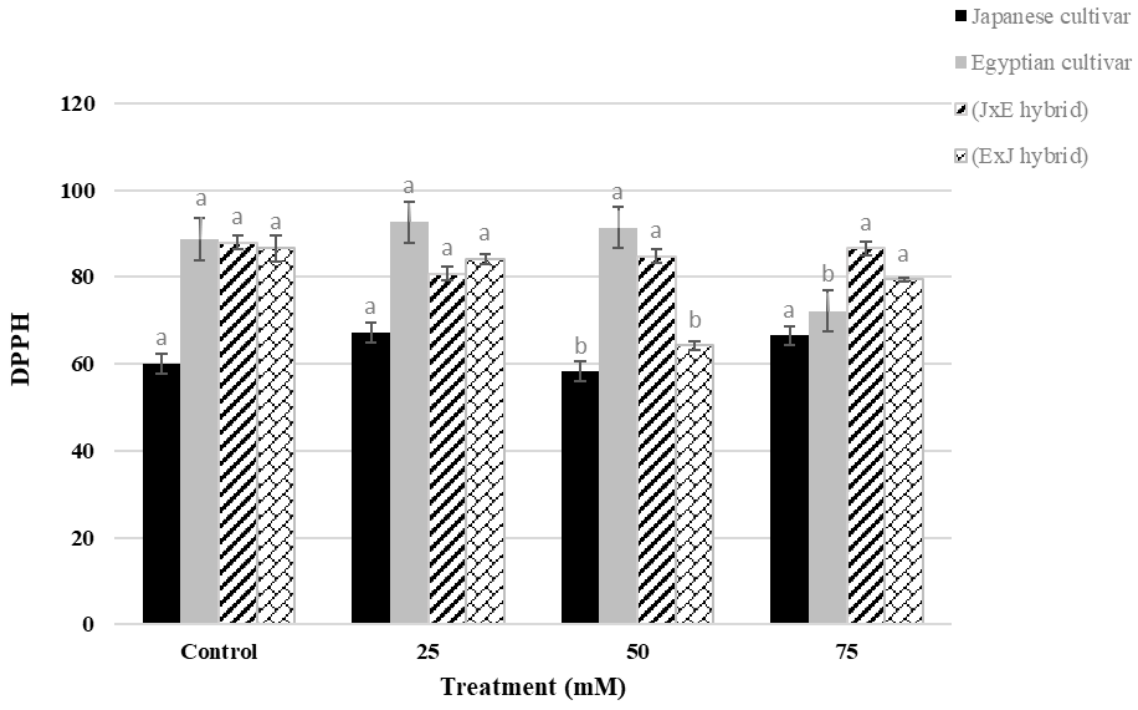

Figure 4. *DPPH radical scavenging activity under different levels of salt stress*

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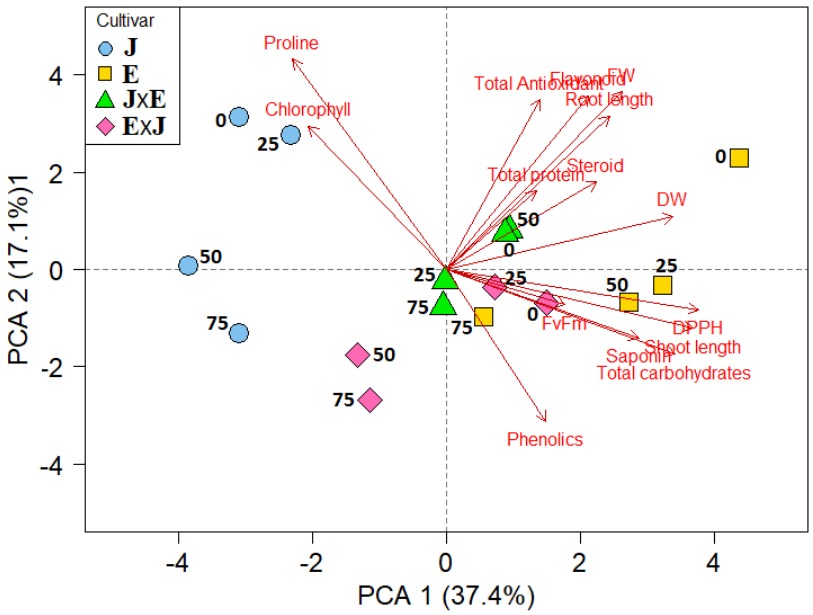

Figure 5. *Principal component analysis for identification of metabolic and growth traits in two okra varieties (Egyptian and Japanese) and their hybrids (Egyptian cultivar ×Japanese cultivar and Japanese cultivar ×Egyptian cultivar) grown under different NaCl concentrations (0 (control), 25, 50, 75 mM). The factor loading values for variables are indicated by red arrows radiating from the center, showing the direction (angle) and magnitude (length), showing how each metabolite contributes to the individual correlations represented by PCA1 and PCA2*

Discussion

Salinity is one of the most serious abiotic stresses that results in deterioration of growth and crop production (Munns et al., 2006). Global okra production is controlled by a complex genetic background and is frequently associated with negative agronomic characters. The successful breeding technique delivers new opportunities to fulfill current consumer needs and even anticipate consumer needs in the near future. Growth performance of okra cultivars showed a reduction in plants' fresh and dry weights of both okra cultivars and their hybrids (E×J and J×E). This reduction might be due to the toxic effect of NaCl and the low water potential of saline soil (Greenway and Munns, 1980). In addition, Gouveitcha et al. (2021) stated that the retardation in growth of Okra under salt stress is due to cell dehydration at low water potential, nutritional imbalance caused by interference of saline ions with essential nutrients in both uptake and translocation processes, and toxicity of Na^+ and Cl^- in the cytoplasm. Our results are also consistent with the results of Pungin et al. (2023) and Kaur et al. (2014) in two halophytes and chickpea seedlings, respectively. Moreover, Datta et al. (2009), working on wheat (*Triticum aestivum* L.), demonstrated that an increase in salt concentration significantly affected the FW and DW. Growth attributes like shoot and root length of okra cultivars and their F1 were severely affected by salinity stress. The reduction in shoot and root length was observed in the J×E under salt stress, and it might be due to failing to develop efficient osmotic adjustment (Kapoor and Pande, 2015). Salinity inhibited root and shoot elongation in wheat (*Triticum aestivum)* due to slowing down the water uptake for overall osmotic adjustments of the plant body under high salt stress conditions (Datta et al., 2009). Also, no significant change in shoot and root length of

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Egyptian cultivars or E×J was recorded. It might be due to the inhibition of the initiation of new roots and radical growth at high salt concertation (Neumann, 1995). Japanese cultivars showed an increase in shoot length and a decrease in root length. These different responses between cultivars in shoot and root length may be correlated to the genetic potential of the cultivars (Puvanitha and Mahendran, 2017).

Estimations of chlorophyll content and fluorescence parameters such as *Fv*/*Fm* were performed to prove the results of growth parameters and the integrity of the photosystem II reaction center. The sensitivity of a crop to salinity depends on the *Fv/Fm* value. Our results revealed that there is no major change in the maximum quantum yield across Japanese cultivars and both hybrids. Only the Egyptian cultivar showed a decrease in the *Fv/Fm* ratio. These results were consistent with the results of Dkhil and Denden (2012), who found no changes in chlorophyll fluorescence parameters (Fo and Fv/Fm) in okra seedlings. In addition, Saleem et al. (2011) discovered that salt stress induced by NaCl did not affect the chlorophyll fluorescence-related characteristics such as photochemical quenching, non-photochemical quenching, and efficiency of photosystem II (Fv/Fm). Furthermore, Allel et al. (2018) stated that the effective quantum yield of light and dark-adapted tests (Fvs/Fms and Fv/Fm, respectively) were probably not useful tools to screen barley for salinity tolerance.

Chlorophyll and carotenoid are the main photosynthetic pigments of higher plants. The photosynthetic pigment concentration was reduced by increasing the salt concentration. Yang et al. (2011) reported that sodium ions cause chlorophyll degradation. The reduction of chlorophyll contents indicated that there was a photoprotection mechanism through reducing light absorbance by decreasing chlorophyll contents (Taïbi et al., 2016). Leaf chlorophyll contents of parent cultivars are generally decreased under salinity stress, unlike the two hybrids, which showed no significant change in chlorophyll content. Decreased chlorophyll contents under conditions of increased salinity have also been reported in sugarcane (Gomathi and Rakkiyapan, 2011) and *Oryza sativa* (Teh et al., 2015).

Plant responses to salt stress also include changes in plant metabolism. Our observations in this study indicated that Egyptian and Japanese parents differ greatly in their response to salinity when compared with their F_1 hybrid (E×J and J×E). Increasing protein content in both hybrids rather than mother cultivars indicates salt tolerance, which is suggested to be an adaptive mechanism in order to maintain ion homeostasis and protect the cellular structure and components (Sairam and Tyagi, 2004).

The accumulation of carbohydrates plays an important role in enhancing plant tolerance to stresses and osmotic regulation (Sami et al., 2016). Total carbohydrates declined in Egyptian mothers and E×J, while J×E showed no significant change. A greater accumulation of sugar was observed in Japanese mothers, and this led to a lower osmotic potential (Singh, 2004).

Proline accumulates in plants exposed to severe salinity-stress conditions. They are involved in scavenging reactive radicals and osmotic regulation of plants (Lee et al., 2016; Kordrostami et al., 2017). Our results showed that proline and steroid content were significantly reduced by increasing NaCl concentration for both Japanese and Egyptian cultivars, while a slight increase in proline content was recorded in J×E and E×J cultivars. The accumulation of proline in the J×E as well as E×J hybrids rather than Egyptian and Japanese mothers may be due to the expression of genes encoding key enzymes of proline synthesis, and this could contribute to the protection against salt stress (Ashraf and Harris, 2004; Liang et al., 2013). Ghanem et al. (2021) recorded

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different behaviors in two halophytes species toward proline accumulation and confirmed that the tolerance mechanism in halophytes is species-specific, which provides a new strategy for saline areas.

Total phenolic compounds and steroids increased in stressed okra hybrids (E×J and J×E). Le et al. (2019) recorded that *Launea sarmentosa* leaves contain a high amount of polyphenol compounds and a lower amount of flavonoids. Singh (2004) recorded the same results on chickpea (Cicer arietinum). Phenolic compounds are induced under osmotic stress to minimize subcellular damage caused by ROS (reactive oxygen species) production (Hichem et al., 2009; Latha et al., 1989). In contrast, a significant reduction in phenolic compounds and flavonoids was recorded in Egyptian and Japanese mothers. Pungin et al. (2023) recorded a slight decrease in the content of phenolic compounds when NaCl was added to *Spergularia marina*. The difference in the behavior of different varieties towards the content of phenolic compounds under salt stress may be due to the genetic variations within the cultivars (Misra and Dwivedi, 2004).

The antioxidant defense system plays an important role in plants responses to adverse environmental conditions. DPPH showed the capacity of plants to fight stress. They play crucial roles in protecting plants from oxidative damage (Miranda et al., 2014). DPPH radical-scavenging activity is a measure of non-enzymatic antioxidant activity. DPPH activity and TAC increased insignificantly in okra cultivars and hybrids with the increase in NaCl concentrations. Kaur et al. (2014) stated that DPPH increased significantly in the roots of different Chickpea (*Cicer artetinum* L.) genotypes with increasing salt stress. Our results showed that all cultivars and hybrids at higher NaCl concentrations had a high antioxidant capacity and could still cope with salt stress, and these results are in agreement with Miljuš-Djukić et al. (2013). Zhang et al. (2024) recoded that two rice hybrids exhibited better salt tolerance than their parents, with increased fresh weight, higher survival rate, improved ionic homeostasis, and higher expression of salt-stress-responsive genes. It was reported by Nilov et al. (2023), that two hybrids of *Vitis vinifera* (L.) showed varied responses to salt stress compared to parent cultivars in growth characteristics, sugar concentrations, leaf water potential and yield due to genotype differences. Two rice cultivars (salt-tolerant and salt-sensitive) exhibited differential responses to salt stress compared to parent cultivars due to variations in antioxidant and osmotic activities, highlighting potential genes for salt tolerance (Fang et al., 2023). In addition, two hybrid of *Solanum spp.* showed higher tolerance than their parents. Better growth parameters such as plant height, root fresh weight and shoot fresh weight at intermediate levels of salt stress were recorded (Ortega-Albero et al. 2023).

Conclusion

In the current study, okra cultivars and their hybrids responded to salt stress in different ways. Increased salt stress significantly reduced biomass production and chlorophyll content in all Okra cultivars and their brids. There was no major change in the maximum quantum yield across Japanese cultivars and both hybrids. DPPH activity and TAC increased insignificantly in all okra cultivars and hybrids with the increase in NaCl concentrations. A significant reduction in total protein, proline, and phenolic compounds was recorded in Egyptian and Japanese parents. In contrast, Okra hybrids (E×J and J×E) tolerate salinity by enhancing total proteins, proline, steroids, and

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phenolic compounds. We concluded and recommended that both okra varieties will be useful in breeding programs where there is a dire need to devise methodologies to enhance crop production, particularly in the stressed regions of the world. Producing products that were desired within the local markets and meeting export standards by selecting varieties and hybrids that were suitable for local growing should be a prime target for plant breeders.

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Conflicts of interest. The authors declare that they have no competing interests.

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