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Research Article

Field-Based Evaluation of Salt Stress Tolerance in Okra (*Abelmoschus esculentus* L.) across Contrasting Soil Environments

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ABSTRACT

Salinity is a major abiotic stress limiting crop production worldwide, particularly in arid and semi-arid regions. Okra is considered moderately tolerant to salinity; however, its early growth and reproductive stages are highly sensitive. The present study was conducted to evaluate the response of different okra genotypes under naturally saline and normal soil conditions. The experiment was carried out at two locations: Location 1 comprised normal soil at the experimental field of the Department of Plant Breeding and Genetics, while Location 2 consisted of saline soil (electrical conductivity = 8.3 dS m^{-1}) at Proka-2 Farms, University of Agriculture, Faisalabad. Twenty genotypes were evaluated for morphological, yield-related, physiological, ionic, and antioxidant traits using analysis of variance, correlation, and biplot analysis. Salinity stress significantly affected all studied parameters, resulting in reductions in germination, reproductive performance, and yield components. Under salt stress, Na^+ accumulation increased, whereas K^+ accumulation decreased. Antioxidant enzyme activities, including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), were enhanced, indicating activation of plant defense mechanisms against stress. Among the tested genotypes, Nirali and Okra-3A exhibited superior performance for yield-related traits under saline conditions, which was attributed to better ionic homeostasis and enhanced antioxidant capacity. These genotypes may be recommended for cultivation in saline environments and can be utilized in breeding programs to develop salt-tolerant hybrids.

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Introduction

Okra (*Abelmoschus esculentus* L. Moench) is an important summer vegetable crop cultivated in tropical and subtropical regions worldwide. It is commonly grown in Southeast Asian countries (Gul et al., 2025). Okra production in Pakistan is relatively low, primarily due to several biotic and abiotic stresses that limit yield. Among biotic factors, major constraints include yellow mosaic virus, root-knot nematodes, and insect pests, which cause significant annual yield losses (Yasin et al., 2023, 2024; Ounis et al., 2024). Abiotic stresses, particularly drought and salinity, also substantially reduce okra productivity. Another critical factor contributing to low production is the unavailability of pure and healthy seeds (Meng et al., 2025). Moreover, there is a shortage of okra cultivars possessing resistance to diseases and tolerance to abiotic stresses (Mkhbela et al., 2022; Haq et al., 2023).

Among abiotic stresses, salinity and water scarcity are

major constraints that cause considerable yield reduction in vegetable crops. Soil salinity reduces productivity by inducing physiological stress, limiting water uptake, and altering water use efficiency within the plant. Salinity also causes ionic imbalance by disrupting sodium (Na^+) and potassium (K^+) ratios, leading to water deficiency and impaired physiological functions (Derbew et al., 2026). Salt stress can result in wilting, reduced photosynthesis and respiration, loss of cell integrity, leaf abscission, tissue necrosis, stunted growth, and ultimately plant death (Liu et al., 2024). Globally, approximately 10% of total land area (~950 million hectares) and 50% of irrigated land (~230 million hectares) are affected by salinity, resulting in estimated annual agricultural losses of around 12 billion USD in the United States alone. With increasing population pressure and the use of poor-quality soil and irrigation water, the severity of salinity stress is expected to intensify (Behera et al., 2022).

Abiotic stresses, including salinity, decrease photosynthetic activity and induce the accumulation of reactive oxygen species (ROS), such as superoxide radicals (O_2^-), hydroxyl radicals (OH·), and hydrogen peroxide (H_2O_2) (Hasanuzzaman and Fujita, 2022). Plants activate antioxidant defense mechanisms to mitigate ROS-induced damage. Key enzymatic antioxidants, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX), neutralize ROS (Rao et al., 2025). Salt stress also induces non-enzymatic antioxidants, such as tocopherols, flavonoids, carotenoids, phenolic compounds, and ascorbic acid (ASA), which contribute to oxidative defense (Ashraf et al., 2019).

Proline accumulation under salt stress is not merely an indicator of stress but also enhances plant tolerance by improving photosynthesis, regulating osmolytes, maintaining Na^+/K^+ homeostasis, and enhancing enzymatic and non-enzymatic antioxidant activity (Hosseinifard et al., 2022). Plants under salinity stress regulate cytosolic Na^+ and K^+ concentrations through transporter proteins and H^+ pumps, ensuring ionic balance (Almeida et al., 2017). Although the precise sensors for salinity stress are not fully elucidated, some signaling pathways have been identified. For instance, salt-induced calcium signals activate the SOS3-SOS2 protein kinase complex, which in turn phosphorylates and activates ion transporters, including the plasma membrane Na^+/H^+ antiporter SOS1, to maintain ionic homeostasis (Hao et al., 2021; Zhu, 2003).

Okra is moderately tolerant to salinity; however, salt stress adversely affects seedling growth, flowering, and pod formation (Devi and Arumugam, 2019). High salinity has been reported to reduce root and shoot length, fresh and dry biomass, leaf number, and chlorophyll content in okra seedlings (Misra et al., 2019). At maturity, salinity decreases plant height, branch length, flower number, pod length, pod girth, pod weight, and seed quality, while increasing intermodal distance (Kafi et al., 2025). Some tolerant genotypes may exhibit early flowering under salt stress conditions (Haq et al., 2012).

The success of breeding programs relies on genetic diversity and divergence, focusing on genotypes with high yield potential and other favorable traits (Chen, 2025). Traits such as the number of branches per plant and total fruit count, analyzed through genotypic coefficients of variation, correlation, and path coefficient analyses, provide reliable selection criteria (Yadav et al., 2024). Evaluating both indigenous and introduced okra varieties aids in identifying superior genotypes for developing high-yielding and quality-improved cultivars (Abhilash et al., 2024). For effective crop improvement, it is essential not only to develop high-yielding cultivars but also to target traits conferring stress tolerance (Behera et al., 2022). High heritability and genetic advance of key traits are crucial for selection efficiency. Therefore, available genetic variability combined with appropriate statistical analyses forms a strong foundation for breeding superior okra genotypes with enhanced yield and stress tolerance (Saleem et al., 2023).

The present study aimed to identify salt-tolerant okra genotypes and recommend promising candidates for further breeding programs.

Materials and Methods

The germplasm, consisting of 70 genotypes (Table 1), was obtained from the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, and cultivated at two different locations in Faisalabad. One location was the field area of the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. The soil at this site was sandy loam with low organic matter and a pH of 7.5. The soil had an electrical conductivity (EC) of 2.5 dS m^{-1} and was used as the control, with canal water as the irrigation source. The second location was the Proca-2 saline area of the University of Agriculture, Faisalabad, which had an EC of 8.3 dS m^{-1}, and irrigation was provided through tubewell water.

The experimental design was a randomized complete block design (RCBD) under a split-plot arrangement, with salinity levels as the main plots and genotypes as subplots, replicated three times. Five seeds were sown per hole, maintaining a plant-to-plant distance of 9 inches and a row-to-row distance of 2.5 feet. Data were collected from the germinated genotypes for various morphological, physiological, and biochemical traits, including: days to emergence, germination percentage (%), plant height (cm), number of branches per plant, internodal distance (cm), leaf area (cm^2), days to flowering, number of pods per plant, pod weight (g), pod length (cm) and girth (cm), number of seeds per pod, marketable fruit yield per plant (g), K^+/Na^+ ratio, superoxide dismutase (SOD, U/mg), catalase (CAT, U/mg), and peroxidase (POD, U/mg).

Table 1. Okra genotypes screened under varying salinity treatments.

Sr. No.	Name of genotype	Sr. No.	Name of genotype	Sr. No.	Name of genotype
1	P-21	25	G-25 (019221)	50	PBG-Okra 38
2	G-2 (Karanti)	26	Okra-3A	51	PBG-Okra 39
3	G-3 (Punjab Selection)	27	PBG-Okra 18-1	52	PBG-Okra 41
4	G-4 (Pusa Swani)	28	PBG-Okra 18-2	53	PBG-Okra 42
5	G-5	29	PBG-Okra 18-6	54	PBG-Okra 43
6	G-6 (OK-1307)	30	PBG-Okra 18-7	55	PBG-Okra 44
7	Anmol	31	PBG-Okra 18-8	56	PBG-Okra 45
8.	Nirali	32	PBG-Okra 18-9	57	PBG-Okra 46
9.	Sabz Pari	33	PBG-Okra 18-10	58	PBG-Okra 47
10	Krishna	34	PBG-Okra 18-15	46	PBG-Okra 34
11	G-11 (Okra Malka)	35	PBG-Okra 24-8	59	PBG-Okra 48
12	G-12	36	PBG-Okra 24-10	60	PBG-Okra 49
13	G-13 (Tulsi)	37	PBG-Okra 24-13	61.	PBG-Okra 50
14	G-14 (Green Gold)	38	PBG-Okra 25	62.	PBG-Okra 51
15.	G-15 (Durga)	39	PBG-Okra 26	63.	PBG-Okra 52
16.	G-16 (Line Broud)	40	PBG-Okra 28	64.	PBG-Okra 53
17.	G-17 (Sel Super Green)	41	PBG-Okra 29	65	PBG-Okra 54
18	G-18 (ever green)	42	PBG-Okra 30	66	PBG-Okra 55
19	G-19 (Anmol)	43	PBG-Okra 31	67	PBG-Okra 20-3
20	G-20 (Irka Anamika)	44	PBG-Okra 32	68	PBG-Okra 20-5
21	G-21 (Patel)	45	PBG-Okra 33	69	PBG-Okra 20-9
22	G-22 (Okra-RM)	47	PBG-Okra 35	70	PBG-Okra 20-10
23	G-23 (Green Star)	48	PBG-Okra 36	-	-
24	G-24 (Green Makhnali FH)	49	PBG-Okra 37	-	-

K^+ and Na^+ contents in leaves

Okra leaves from each genotype were collected, washed with distilled water, and stored in zipper bags at -80°C for one week. To extract cell sap, leaves were crushed using small iron balls, followed by centrifugation to separate the liquid fraction. The sap was diluted with deionized water, and Na^+ and K^+ ion concentrations were analyzed using a flame photometer (Jenway PFP7). Standard curves were generated using solutions of 5-25 mg L^{-1} for both ions, and these curves were used to calculate the total concentrations of Na^+ and K^+ (Gorham et al., 1990).

The potassium uptake relative to sodium was assessed by calculating the K^+/Na^+ ratio. This ratio provides insights into the ionic balance in the seedlings, which is critical for understanding their physiological responses under saline conditions.

Biochemical analyses

Leaves of each genotype were collected separately,

rinsed with distilled water, placed in zipper bags, and stored at -80°C for one week. Cell sap was extracted by crushing the leaves in microfuge tubes using small iron balls, followed by centrifugation according to standard procedures. The activities of SOD, CAT, and POD were quantified using a spectrophotometer.

Superoxide Dismutase (SOD) (Units/mg)

Superoxide dismutase (SOD) activity was determined by assessing its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). For the reaction mixture, 800 µl of distilled water was combined with 100 µl of enzyme extract, followed by 200 µl of Triton X, 200 µl of methionine, and 100 µl of NBT. The pH was adjusted to 5 using potassium phosphate buffer. The mixture was exposed to ultraviolet light for 15 min to initiate the reaction. Subsequently, 100 µl of riboflavin was added, and absorbance was measured at 560 nm using a spectrophotometer to determine the extent of NBT reduction, reflecting SOD activity and its role in mitigating oxidative stress (Zafar et al., 2022).

Peroxidase (POD) (Units/mg)

POD activity was measured by evaluating enzyme-mediated guaiacol oxidation. The same enzyme extract used for the SOD assay was employed. The reaction mixture consisted of 800 µl of potassium phosphate buffer (pH 5), 100 µl of hydrogen peroxide (40 mM), 100 µl of guaiacol (20 mM), and 100 µl of enzyme extract in Eppendorf tubes. Absorbance was recorded at 470 nm using a spectrophotometer, and POD activity was calculated based on the rate of guaiacol oxidation (Zafar et al., 2022).

Catalase (CAT) (Units/mg)

CAT activity was determined by measuring the decomposition of hydrogen peroxide (H_2O_2) into water and oxygen. A 100 µl aliquot of the enzyme extract, prepared for the SOD assay, was mixed with 100 µl of H_2O_2 in cuvettes. Absorbance was measured at 240 nm using a spectrophotometer to quantify catalase activity based on the rate of H_2O_2 consumption (Zafar et al., 2022).

Statistical analysis

Analysis of variance (ANOVA) was performed to determine the level of significance among genotypes at the maturity stage across two locations (Steel et al., 1997). Graphical representations were used to illustrate the mean differences among genotypes for various traits. Correlation analysis was conducted to examine the relationships among genotypes for different parameters (Kwon and Torrie, 1964). Multivariate analysis, including principal component analysis (PCA), was performed using RStudio with R version 4.5.1 and the ggplot2 package to identify patterns and genetic diversity among traits (Team, 2021; Settanni and Sarai, 2025).

Results

Germination under salinity

Out of seventy genotypes, only twenty genotypes germinated in saline soil. Therefore, data were collected only from the germinated genotypes, and statistical analysis was performed accordingly. The list of germinated genotypes is presented in Table 2.

Analysis of variance (ANOVA) was performed to assess the presence of variation among the 20 okra genotypes at the maturity stage. The mean squares for all characters are presented in Table 3. The ANOVA revealed significant ($p < 0.01$) genotypic variation for all characters except the number of locules per pod. Replication effects were found to be non-significant. Significant effects of the saline environment were observed for all characters except the number of locules per pod. The significant location × genotype interaction indicated that genotypes responded differently to increasing salinity levels for internodal distance, pod weight, pod length, pod girth, fruit yield per plant, K^+ content, Na^+ content, and K^+/Na^+ ratio, whereas non-significant interactions were observed for days to emergence, germination percentage, days to flowering, plant height, number of leaves per plant, number of nodes per plant, leaf area, number of pods per plant, and number of locules per pod.

Table 2. List of okra genotypes germinated under salt treatment.

Sr. No.	Name of genotype	Sr. No.	Name of genotype	Sr. No.	Name of genotype
1	P-21	8	G-25 (019221)	15	PBG-Okra 38
2	G-2 (Karanti)	9	Okra-3A	16	PBG-Okra 39
3	G-3 (Punjab Selection)	10	PBG-Okra 18-1	17	PBG-Okra 41
4	G-4 (Pusa Swani)	11	PBG-Okra 18-2	18	PBG-Okra 42
5.	G-5	12	PBG-Okra 18-6	19	PBG-Okra 43
6	G-6 (OK-1307)	13	PBG-Okra 18-7	20	PBG-Okra 44
7	Anmol	14	PBG-Okra 18-8	-	-

Table 3. Analysis of variance of okra genotypes for morphological and biochemical traits under normal and saline soils.

SoV	Replication	Location	Error 1	Genotype	Loc×Geno	Error 2
D.F.	1	1	1	19	19	38
DE	2.5	12.8ns	0.8	1.3**	0.1ns	0.5
G%	20	3920*	20	410.5**	77.9ns	167.4
DF	9.8	180**	8×10^{-30}	22.9**	6.8×10^{-29}ns	0.7
PH	0.01	18252.9**	0.1	178.3**	0.01ns	0.1
NOLP	0.1	500**	0.2	14.5**	2.3×10^{-30}ns	0.4
NONP	1.5	177.01**	0.01	2.7**	0.01ns	0.3
IND	0.7	392*	0.3	3.4**	0.9**	0.2
LA	0.09	18361.8**	1×10^{-28}	905.5**	1×10^{-28}ns	0.1
NOPP	4.5	177**	0.01	33.01**	0.01ns	0.6
PW	0.002	217.8**	1.7×10^{-32}	1.1**	1×10^{-30}ns	0.02
PL	0.006	2.2**	5×10^{-33}	1.2**	3.3×10^{-31}ns	0.01
PG	3.20×10^{-04}	1.8**	3.3×10^{-34}	0.1**	1.9×10^{-32}ns	0.001
FYP	164.5	40590**	18.2	1351.4**	101.8**	22.1
K^+	0.002	8854.1**	2×10^{-05}	111.7**	14.3**	9.5×10^{-06}
Na^+	0.002	405.3**	5×10^{-06}	8.8**	3.2**	5×10^{-06}
K^+/Na^+	0.002	228.9**	1.1×10^{-32}	2.9**	0.6**	8.4×10^{-32}
SOD	0.02	199.7**	5×10^{-06}	0.6**	6.6×10^{-04}ns	8.2×10^{-04}
POD	0.001	200.9**	0.003	0.6**	0.001ns	0.001
CAT	1.8×10^{-04}	198.6**	6.1×10^{-04}	0.7**	0.001ns	0.001

** = highly significant at 1%; * = significant 5%; ns = non-significant; D.F.: degree of freedom; DE: days to emergence; G%: germination percentage; DF: days to flowering; PH: plant height; NOLP: number of leaves per plant; NONP: number of nodes per plant; IND: internodal distance; LA: leaf area; NOPP: number of pods per plant; PW: pod weight; PL: pod length; PG: pod girth; NLP: number of locules per pod; FYP: fruit yield per plant; K^+: potassium ion; Na^+: sodium ion; SOD: superoxide dismutase; POD: peroxidase; CAT: catalase.

Morphological and biochemical variations in okra genotypes under saline stress

Morphological and biochemical traits exhibited significant variation among okra genotypes when evaluated under both normal and salt-affected soil conditions. These variations reflect the differential responses and adaptive potential of genotypes to saline stress. Under salinity, notable changes were observed in key morphological parameters such as germination, plant height, root and shoot development, and overall plant vigor. Similarly, biochemical attributes, including the accumulation of osmolytes, antioxidant enzyme activities, and stress-related metabolites, varied markedly among the genotypes. Such variability indicates the presence of inherent genetic diversity, which can be exploited for the selection and development of salt-tolerant okra cultivars.

Days to emergence

The genotypes exhibited higher values for days to emergence (DE) under salt-affected soils compared to normal soil conditions. Narali recorded the minimum DE (2) under salt stress (Figure 1).

Germination and flowering traits

For germination percentage (G%), the genotypes expressed a lower range under salt stress, whereas a greater number of plants germinated under normal conditions. The maximum G% was observed in Okra-3A (70%) (Figure 2). Days to flowering (DF) also showed an increased range under salt stress compared to normal conditions. Narali recorded the minimum DF (48) under salt stress and performed better than the other genotypes (Figure 3).

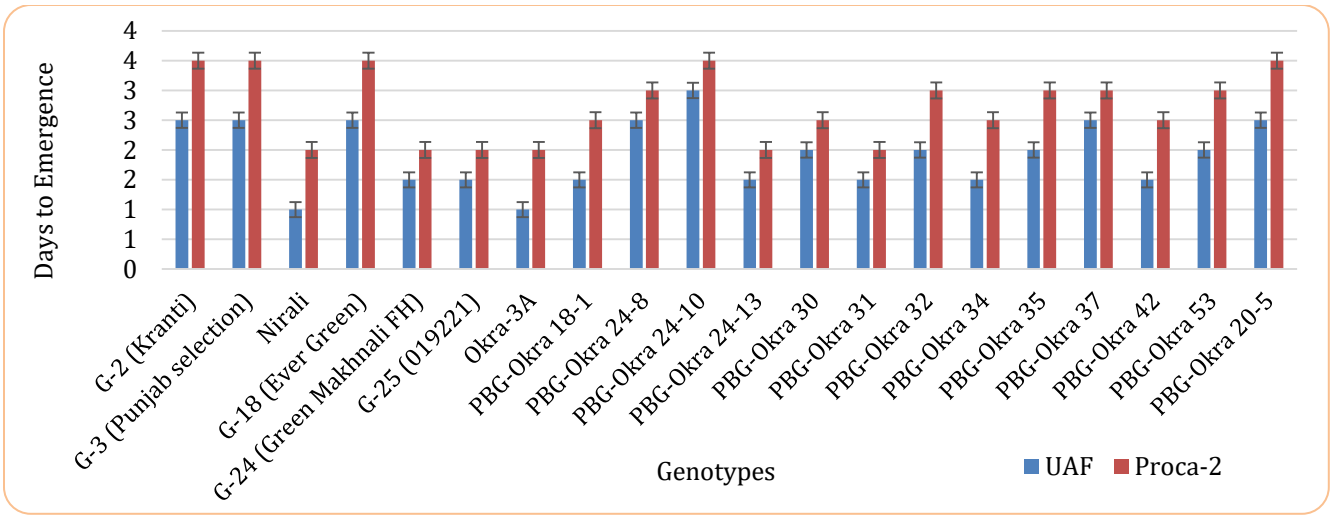


Figure 1. Effect of soil salinity on days to emergence in okra genotypes.

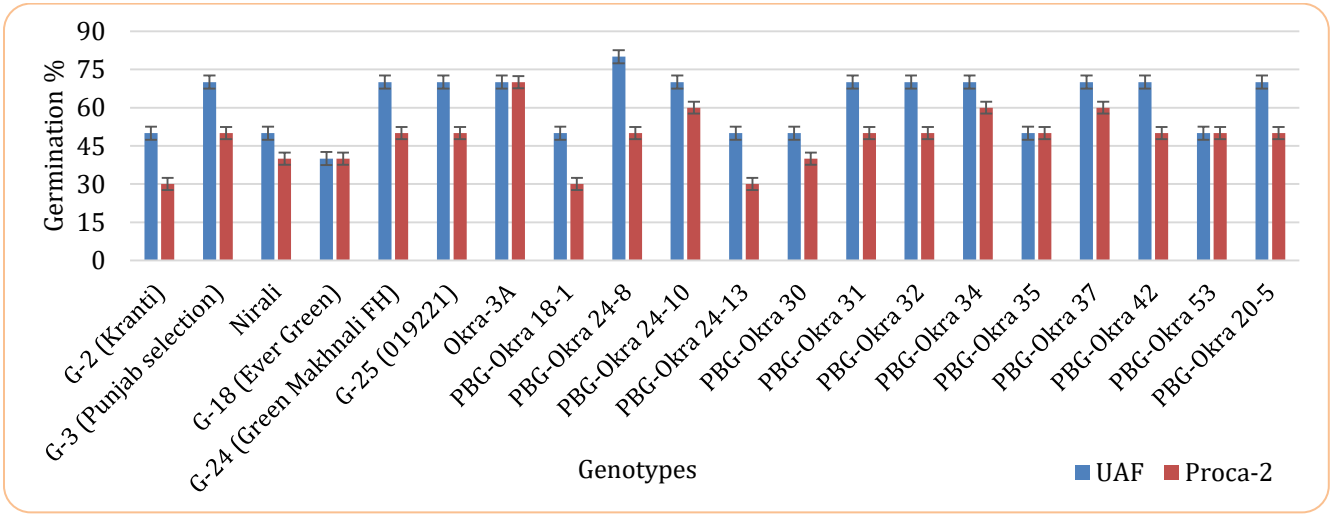


Figure 2. Effect of soil salinity on germination percentage in okra genotypes.

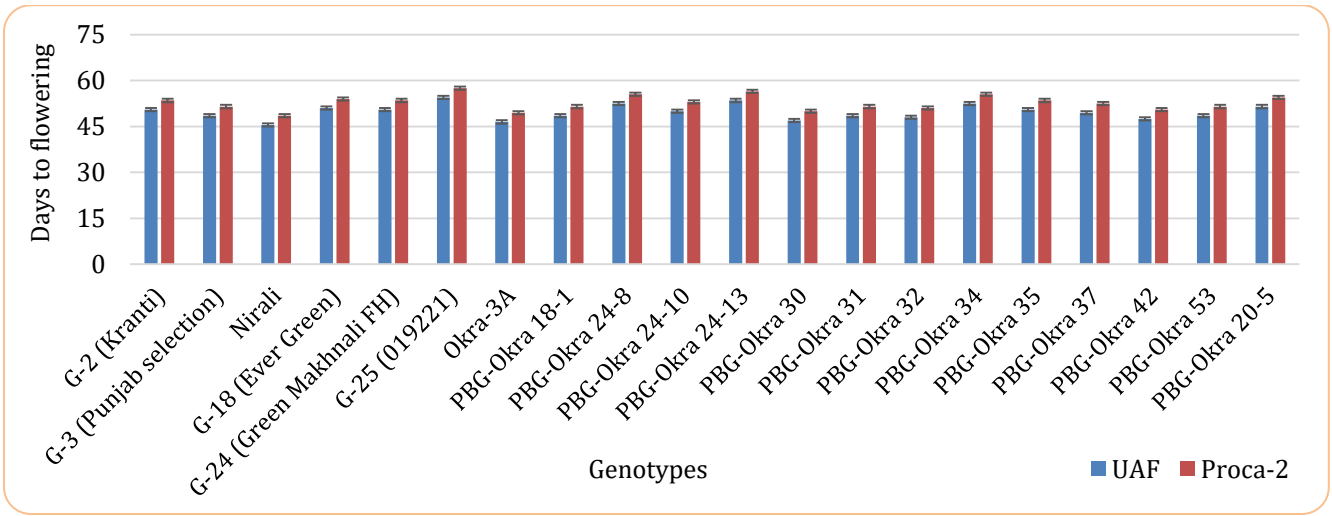


Figure 3. Effect of soil salinity on days toflowering in okra genotypes.

Growth parameters

In salt-affected soils, plant height (PH) decreased, and PBG-Okra 53 performed better with the minimum reduction in PH (40.05 cm) as shown in Figure 4. The number of leaves per plant (NLPP) decreased under stress conditions, whereas G-8 Ever Green showed the highest NLPP (10.50) under stress (Figure 5). The number of nodes per plant (NNP) also decreased under salt-affected soil and increased under normal soil. The maximum NNP (6.50) was observed in Okra-3A (Figure 6). Internodal distance (ID) decreased under stress and increased under normal conditions. The maximum ID (5.95 cm) under salt-affected soil was observed in PBG-Okra 20-5 (Figure 7). Leaf area (LA) also decreased under salt stress and increased under normal conditions. G-18 Ever Green showed the maximum LA (98.54 cm^2) as shown in Figure 8.

Pod and yield traits

Pod-related traits, including number of pods per plant (NPP), pod weight (PW), pod length (PL), and pod girth (PG), also decreased under salt stress among the twenty okra genotypes. Nirali showed the maximum values for NPP (13.50), PW (4.70 g), and PL (5.95 cm), whereas Okra-3A exhibited the maximum PG (1.26 cm) under salt-affected soil (Figure 9, 10, 11, 12). Fruit yield per plant (FYP) also decreased under salt-affected soil, with Nirali recording the maximum FYP of 63.47 g (Figure 13).

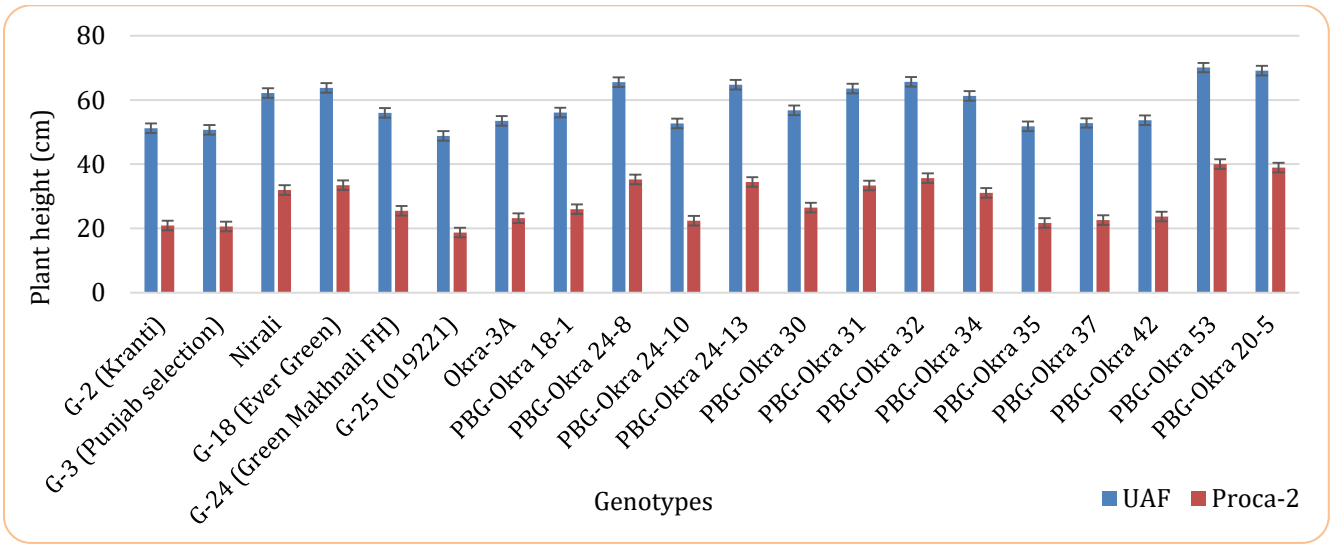


Figure 4. Effect of soil salinity on plant height in okra genotypes.

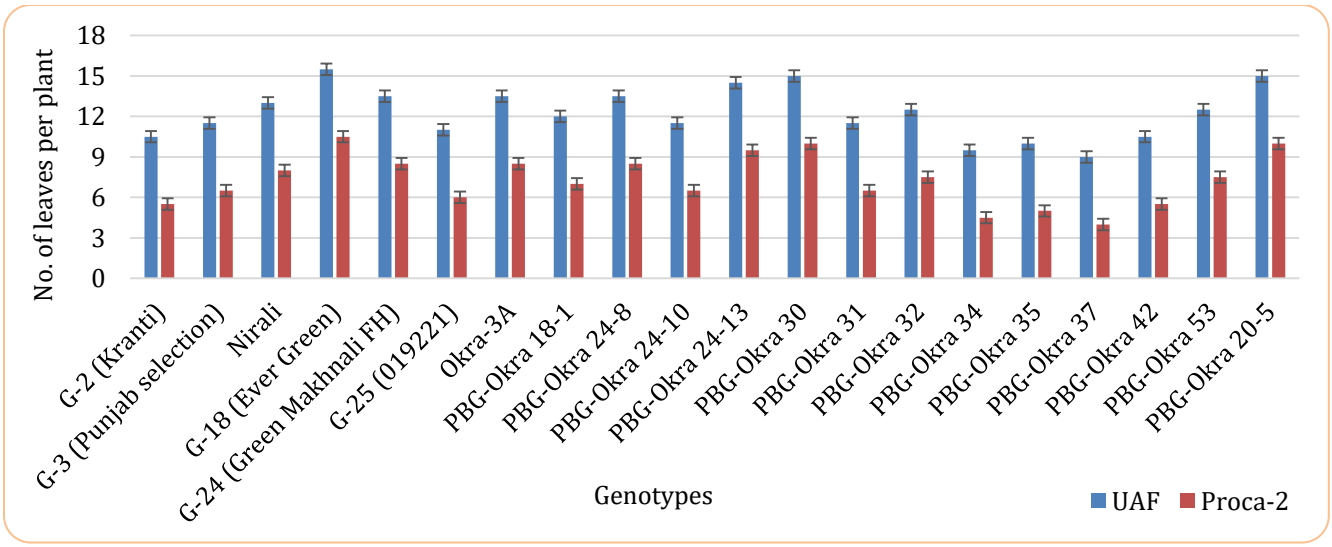


Figure 5. Effect of soil salinity on number of leaves per plant in okra genotypes.

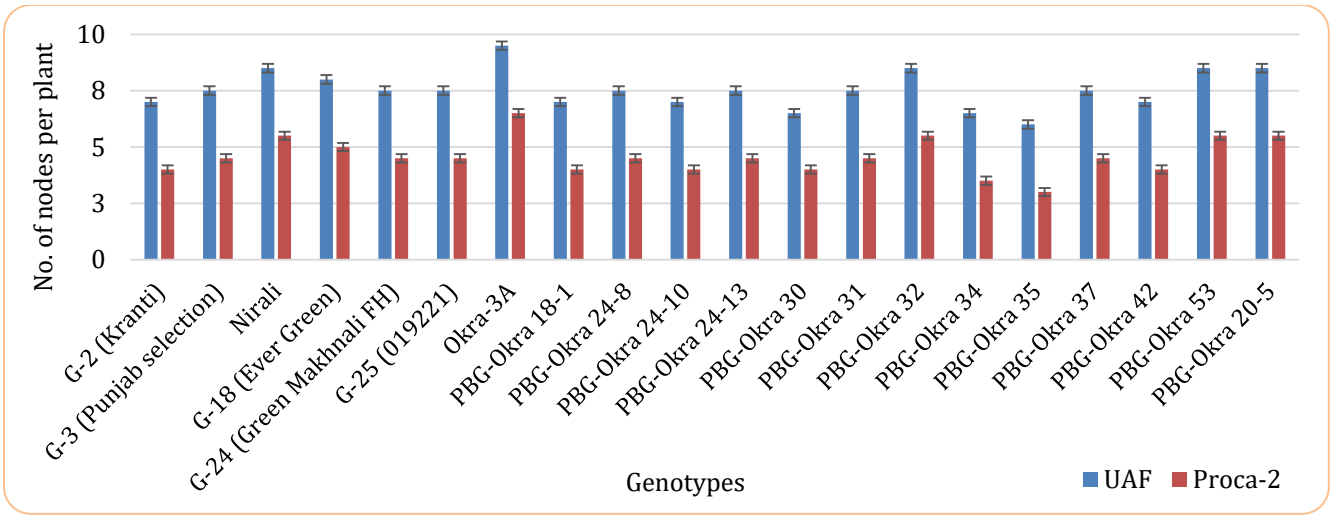


Figure 6. Effect of soil salinity on number of nodes per plant in okra genotypes.

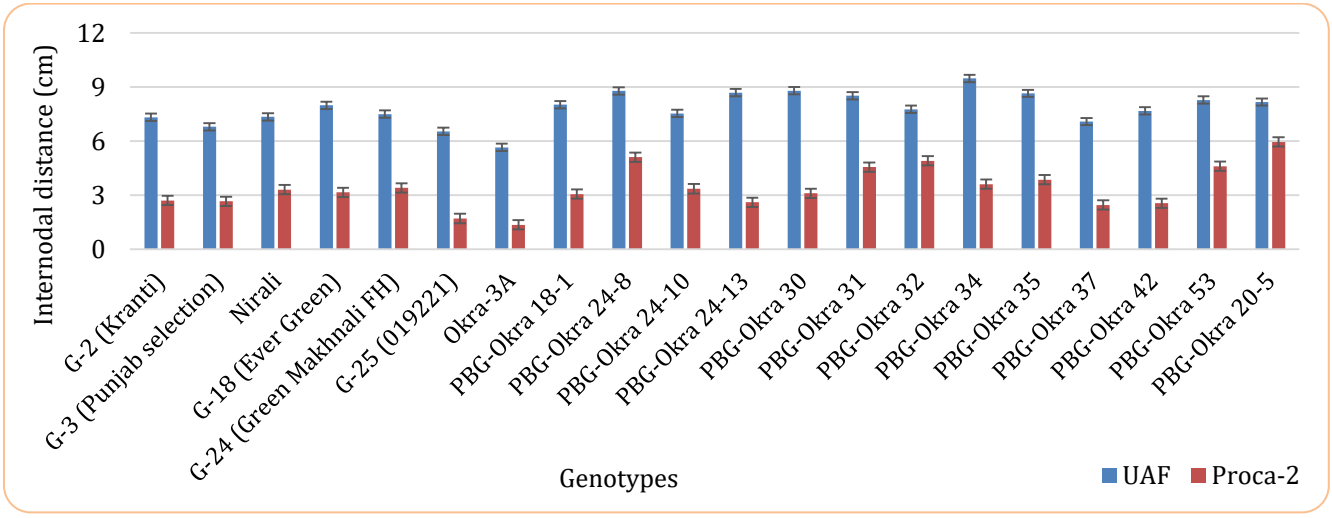


Figure 7. Effect of soil salinity on intermodal distance in okra genotypes.

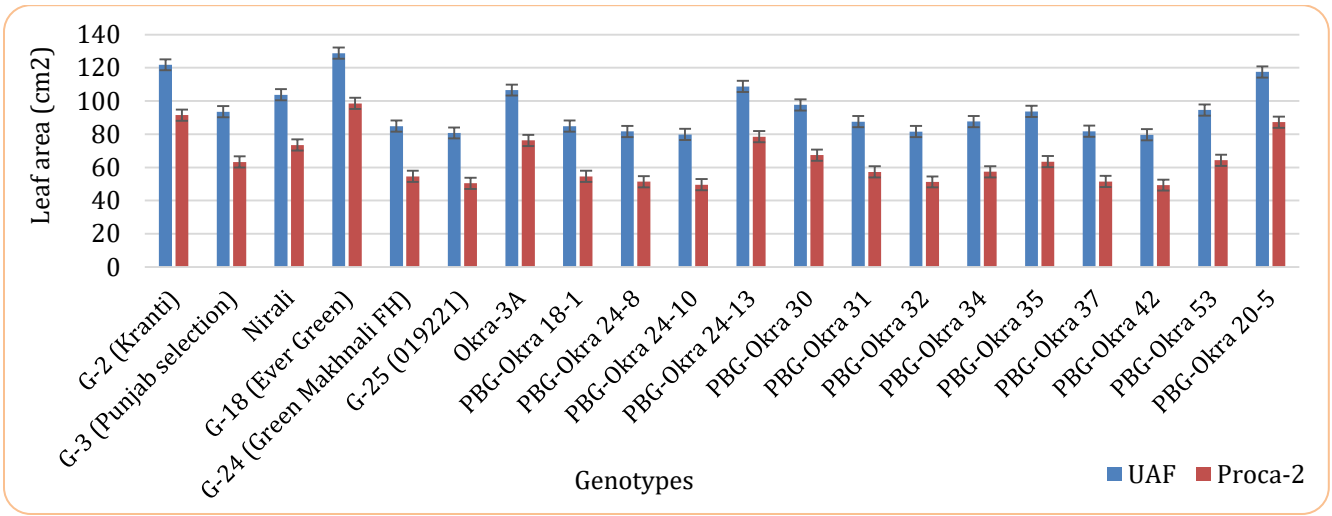


Figure 8. Effect of soil salinity on leaf area in okra genotypes.

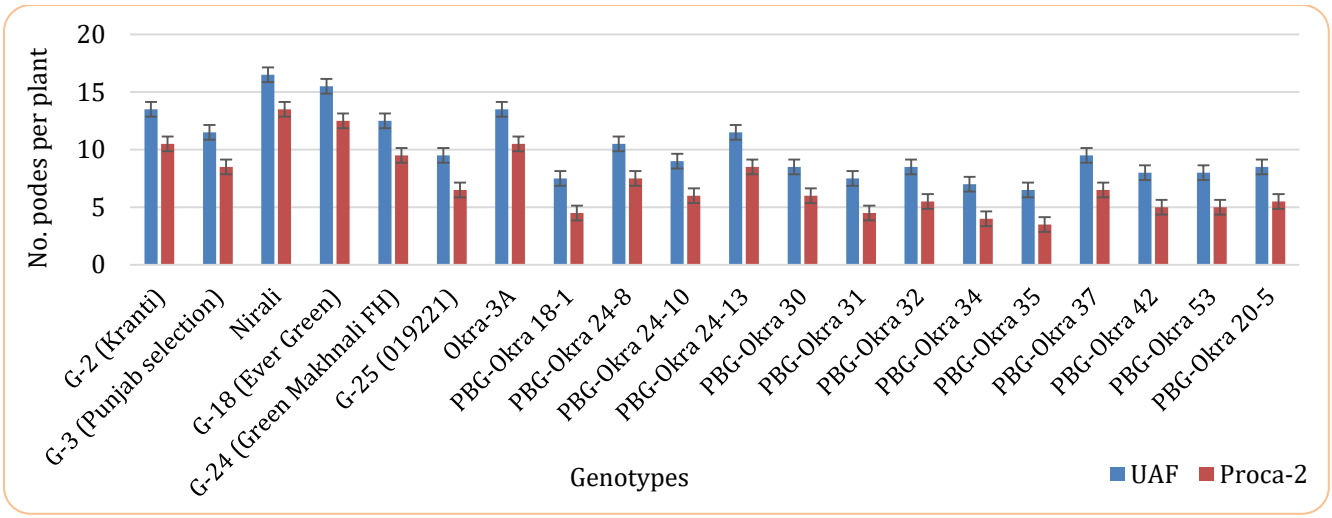


Figure 9. Effect of soil salinity on number of pods per plant in okra genotypes.

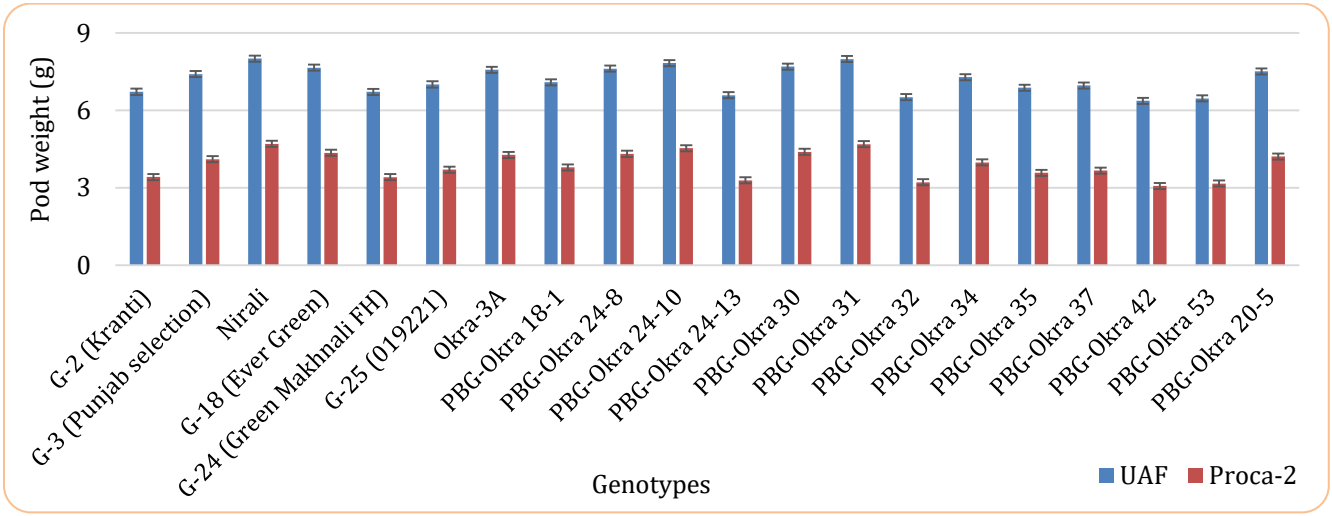


Figure 10. Effect of soil salinity on pod weight in okra genotypes.

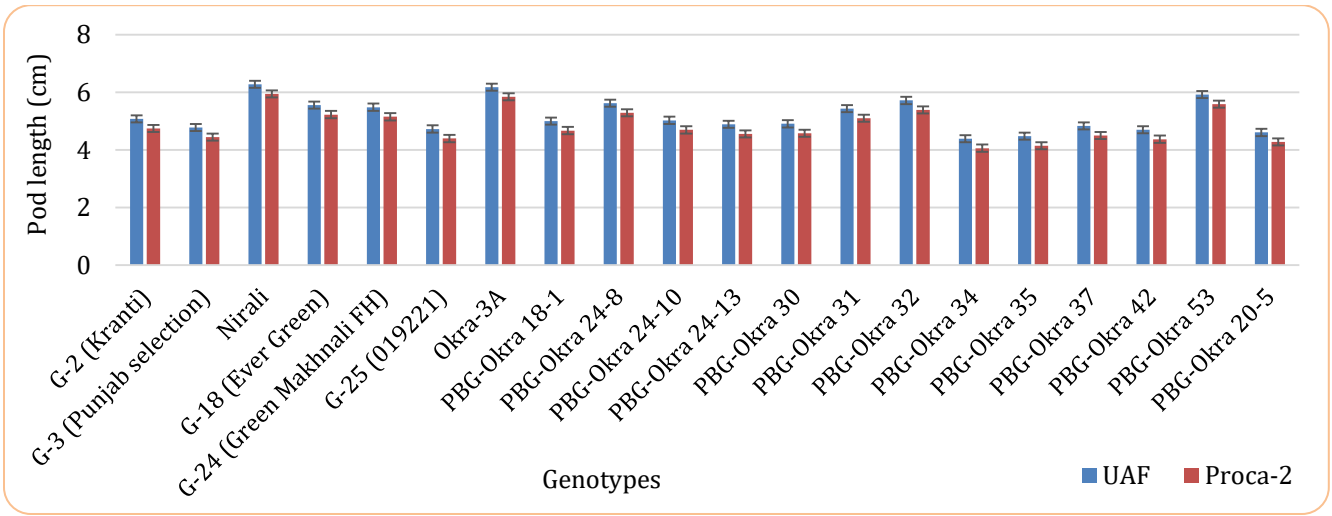


Figure 11. Effect of soil salinity on pod length in okra genotypes.

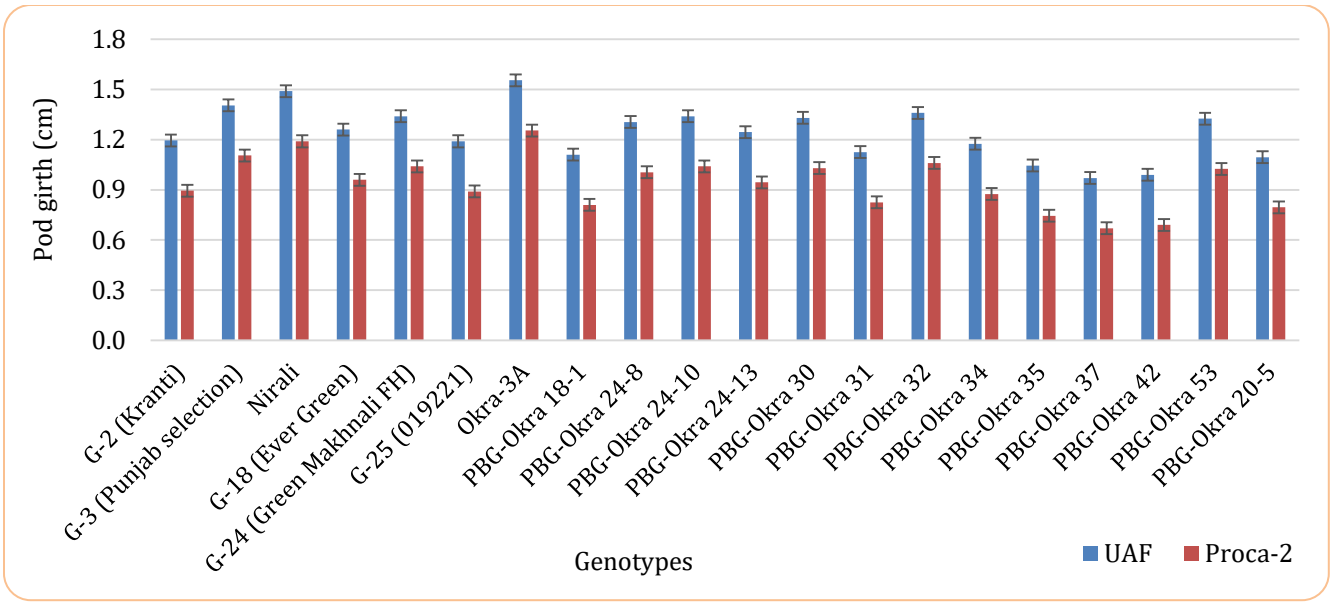


Figure 12. Effect of soil salinity on pod girth in okra genotypes.

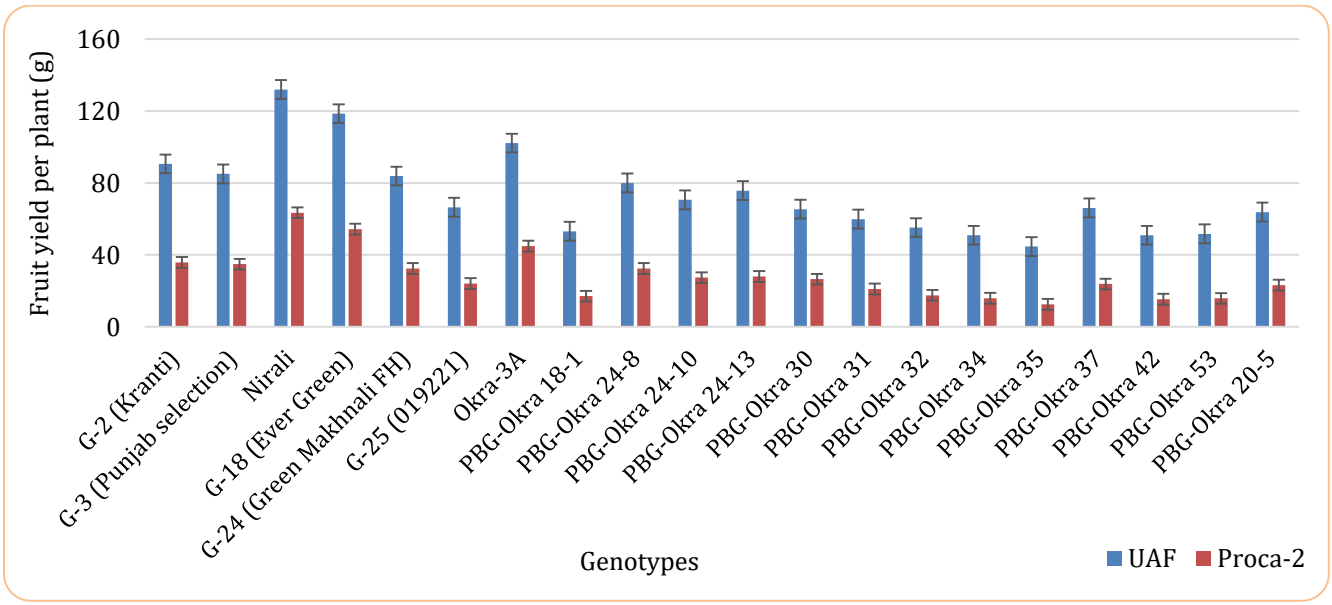


Figure 13. Effect of soil salinity on fruit yield per plant in okra genotypes.

Biochemical traits

The biochemical traits under normal and salt-affected soils also showed variations. Potassium (K^+) ion concentration decreased under salt stress compared to normal soil, and PBG-Okra 30 showed the minimum value of 26.16 mM (Figure 14). In contrast, sodium (Na^+) ion concentration increased under salt stress, with G-24 Green Makhnali FH showing the maximum value (17.71 mM) as shown in Figure 15. The K^+/Na^+ ratio also decreased under salt stress; however, G-3 Punjab Selection showed the maximum value (3.08), indicating a stronger resistance mechanism in okra genotypes (Figure 16).

Enzymatic activity

Enzymatic activities, including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), increased under salt stress. PBG-Okra 53 exhibited the highest enzymatic activity for SOD (8.22 U/mg), POD (11.26 U/mg), and CAT (14.29 U/mg) under salt stress (Figure 17, 18, 19).

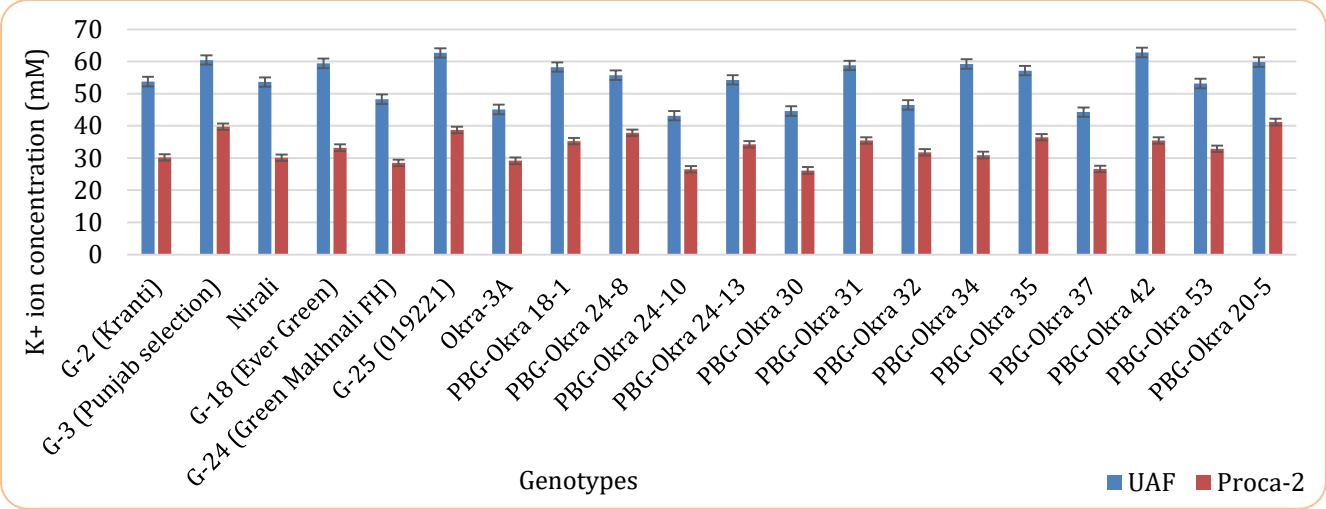


Figure 14. Effect of soil salinity on K^+ ion concentration in okra genotypes.

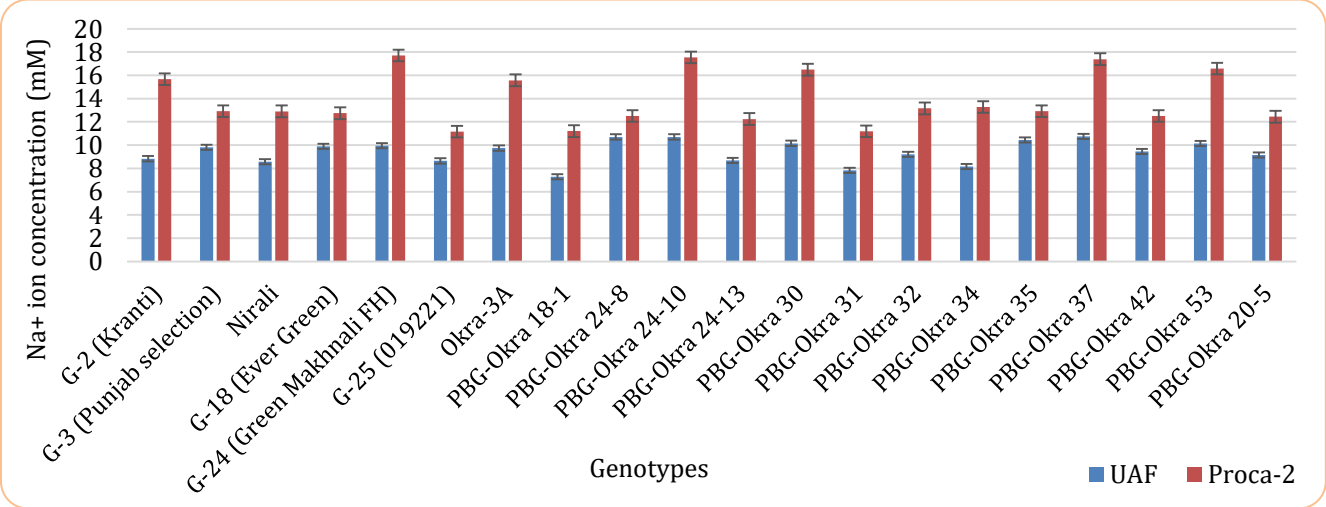


Figure 15. Effect of soil salinity on Na^+ ion concentration in okra genotypes.

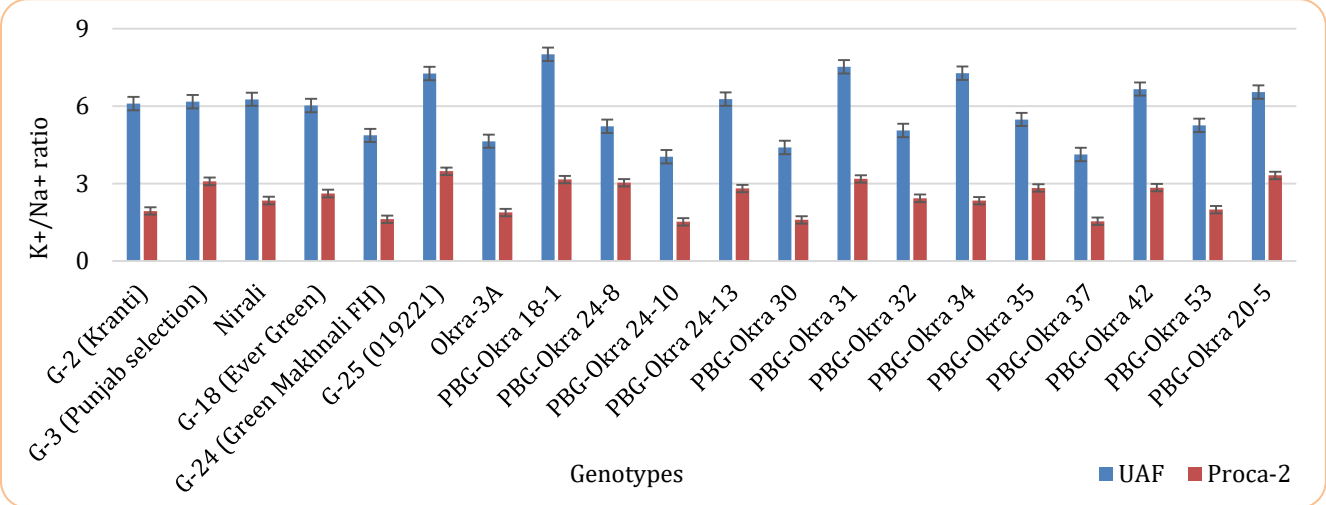


Figure 16. Effect of soil salinity on K^+/Na^+ ratio in okra genotypes.

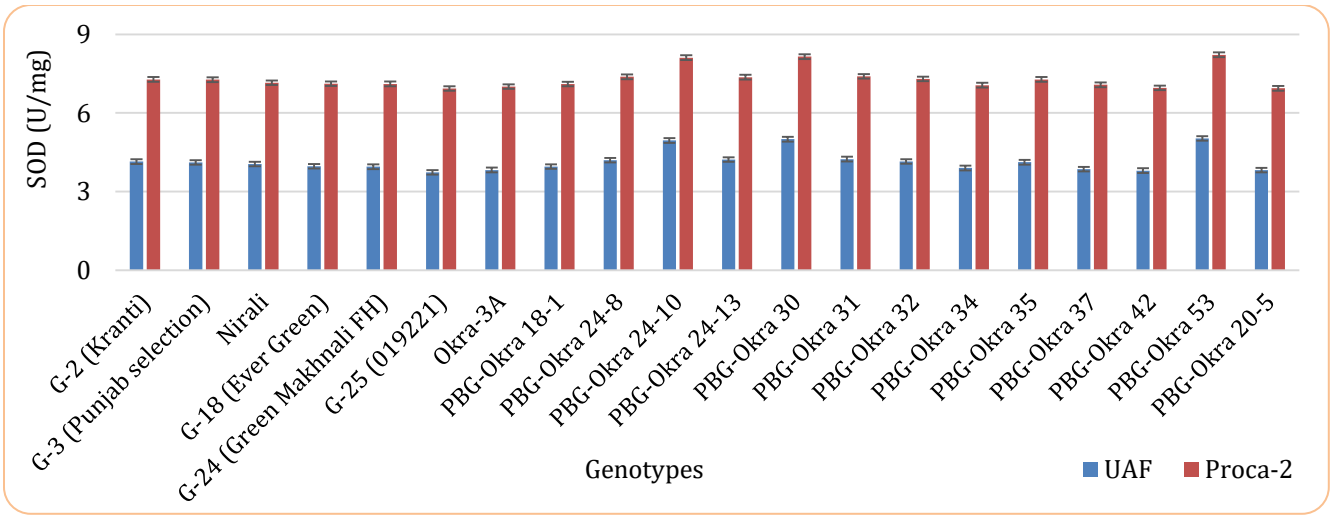


Figure 17. Effect of soil salinity on SOD activity in okra genotypes.

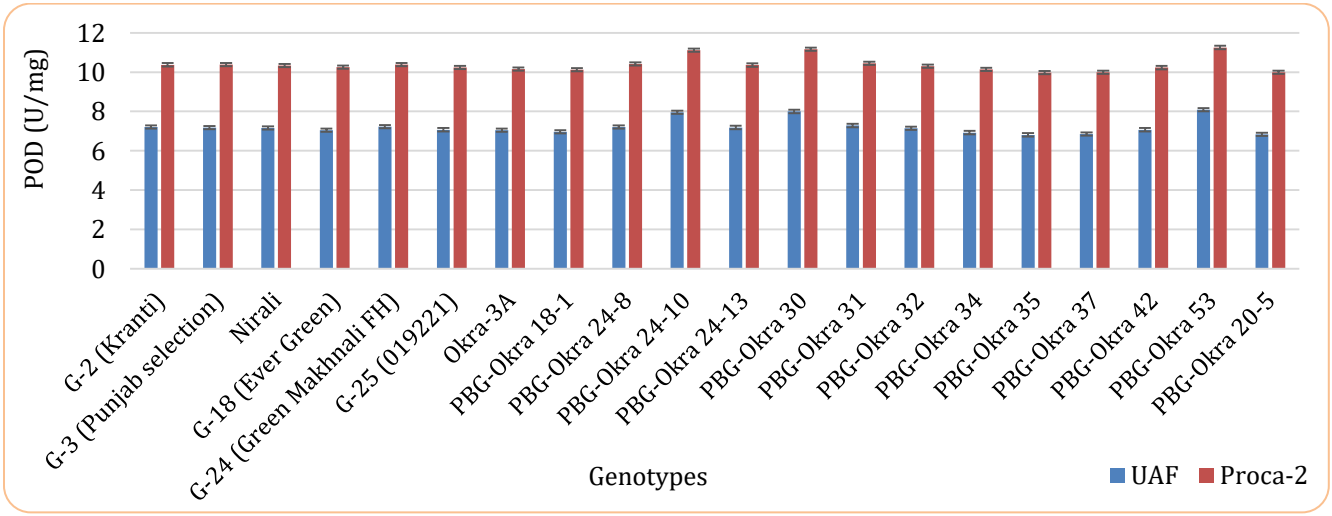


Figure 18. Effect of soil salinity on POD activity in okra genotypes.

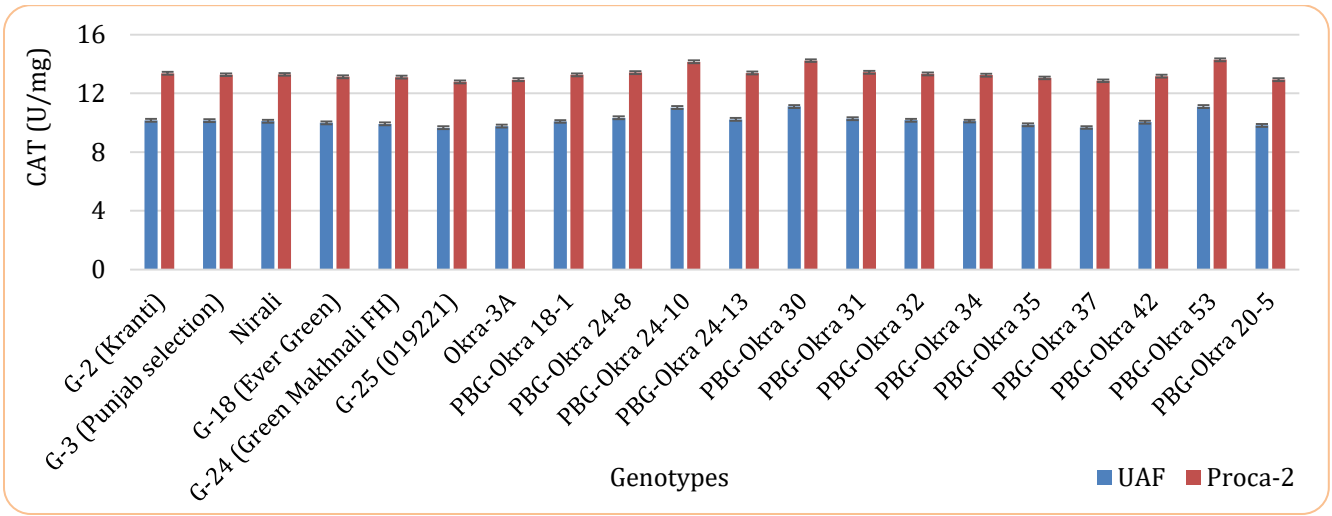


Figure 19. Effect of soil salinity on CAT activity in okra genotypes.

Correlation analysis

The correlation analysis (Figure 20) provided a comprehensive assessment of the relationships among morphological, yield-related, and biochemical traits in okra genotypes at the maturity stage. Days to emergence (DE) exhibited a negative, non-significant correlation with germination percentage (G%) and a significant positive correlation with days to flowering (DF). In contrast, DE showed significant negative correlations with plant height and most other traits, except sodium (Na^+) ion content. This indicates that early-emerging genotypes tend to perform better in terms of growth and yield attributes, suggesting a potential trade-off between delayed emergence and reduced growth vigor.

Germination percentage (G%) showed positive correlations with plant height, number of nodes per plant, internode distance, pod weight, pod girth, fruit yield per plant, potassium (K^+) ion content, and the K^+/Na^+ ratio, while a significant negative correlation was observed with Na^+ content. Days to flowering (DF) was positively and significantly correlated with fruit yield per plant and Na^+ content but exhibited significant negative correlations with most other traits.

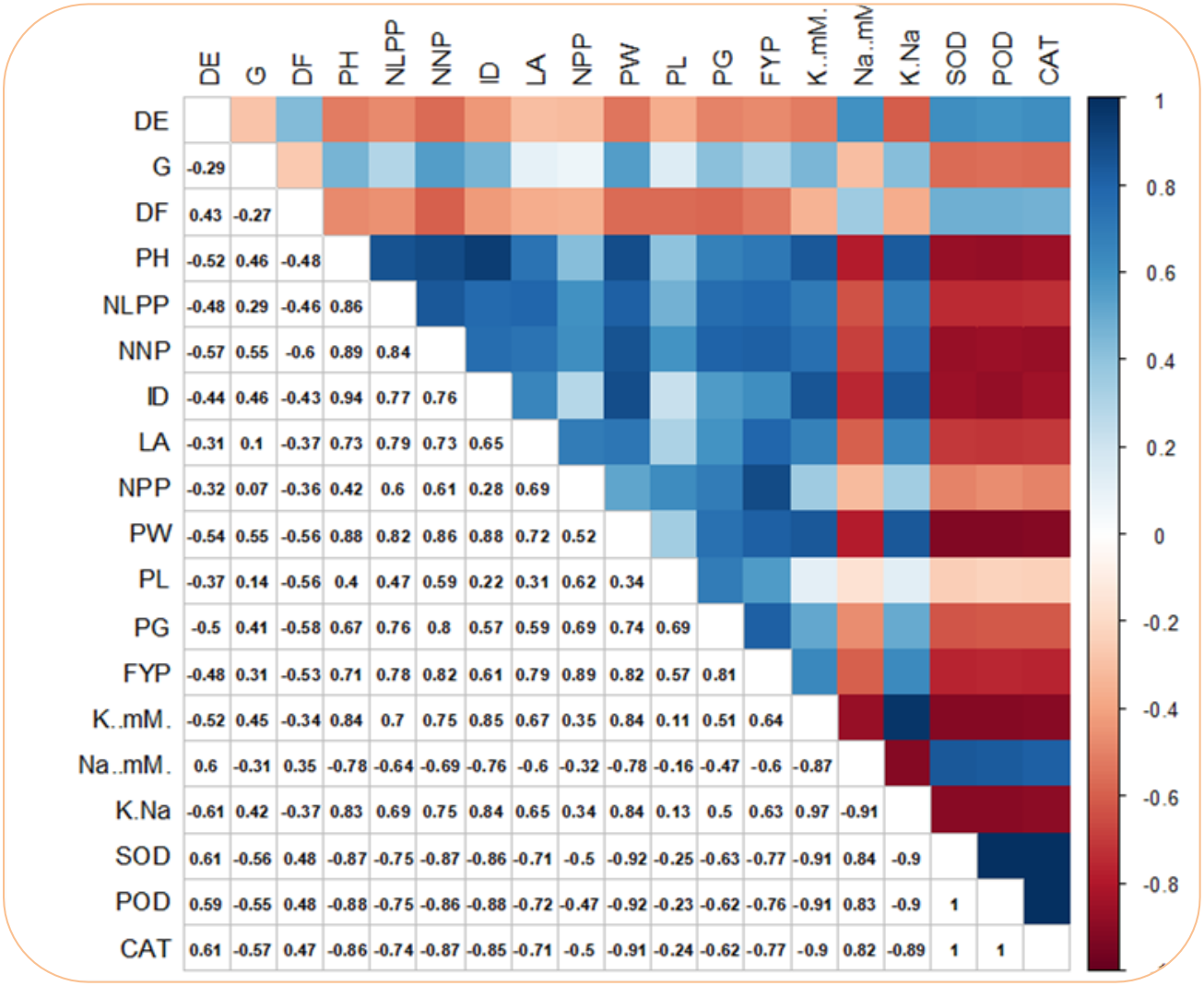


Figure 20. Correlation analysis of morphological, yield, and biochemical traits of 20 okra genotypes at the maturity stage. Abbreviations: DE: Days to emergence; G%: Germination percentage; DF: Days to flowering; PH: Plant height; NOLP: Number of leaves per plant; NONP: Number of nodes per plant; IND: Internodal distance; LA: Leaf area; NOPP: Number of pods per plant; PW: Pod weight; PL: Pod length; PG: Pod girth; FYP: Fruit yield per plant; K^+: Potassium ion; Na^+: Sodium ion; K^+/Na^+ ratio; SOD: Superoxide dismutase; POD: Peroxidase; CAT: Catalase.

Plant height (PH) was positively correlated with nearly all traits, except for number of locules per pod and Na^+ content. The number of leaves per plant (NLPP) showed significant positive correlations with most traits, except Na^+ content, and a negative, non-significant correlation with number of locules per pod. This suggests that an increase in leaf number is associated with improvements in node number, pod number, and related yield attributes. A strong positive correlation between NLPP and internode distance further indicates that genotypes with more leaves tend to have longer internodes, potentially enhancing light interception and overall plant architecture.

The number of nodes per plant also exhibited significant positive correlations with yield-related and other important traits, demonstrating strong interdependence among growth parameters. Internode distance (ID) showed non-significant correlations with number of pods per plant, pod length, and Na^+ content, but was positively correlated with all other traits. Leaf area (LA) was significantly and positively correlated with all measured traits, except number of locules per pod and Na^+ content.

The number of pods per plant (NPP) was positively correlated with pod weight, pod length, and other associated traits, indicating that increased pod number contributes to improved yield potential. Pod weight (PW) showed significant positive correlations with all traits except number of locules per pod. Pod length (PL) was positively and significantly correlated with pod girth and fruit yield per plant, while its correlations with other traits were non-significant. Pod girth (PG) also exhibited significant positive correlations with yield-related traits, highlighting the importance of pod characteristics in yield determination.

The relationship between potassium (K^+) and sodium (Na^+) ions was particularly noteworthy, showing a strong negative correlation. This inverse relationship underscores the importance of ionic balance under saline conditions, where higher K^+ levels are associated with reduced Na^+ accumulation, thereby contributing to improved salt tolerance. Furthermore, the correlation between the K^+/Na^+ ratio and days to emergence suggests a role of potassium in regulating early plant development.

Antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), exhibited positive correlations with days to emergence, days to flowering, and the K^+/Na^+ ratio. Moreover, strong positive interrelationships were observed among SOD, POD, and CAT, indicating coordinated activation of the antioxidant defense system under stress conditions.

Overall, these findings highlight the complex and interconnected nature of morphological, physiological, and biochemical traits in okra genotypes, providing valuable understandings for breeding programs aimed at enhancing yield performance and salinity tolerance.

Principal component analysis

The biplot (Figure 21) of 20 okra genotypes grown under control conditions in the field area of the Department of Plant Breeding and Genetics, based on variance in 16 morphological and physiological traits, was explained by two principal components (Dim1 and Dim2). These components accounted for 26.9% and 21.5% of the total variation, respectively. In Quadrant I, genotypes G-25 019221, PBG-Okra 42, and PBG-Okra 18-1 were identified as important, with vectors corresponding to DF, K^+ ion content, and the K^+/Na^+ ratio. In Quadrant II, genotypes Okra 3A and Nirali were positioned far from the origin, indicating strong associations with vectors such as LA, NOLP, NONP, NOPP, PL, PW, and FYP. In Quadrant III, PBG-Okra 33 and PBG-Okra 35 were prominent genotypes, associated with vectors including ID and G%. Quadrant IV comprised genotypes PBG-Okra 24-10, PBG-Okra 30, and PBG-Okra 53, with vectors representing DE, PH, SOD, POD, CAT, and Na^+ ion content.

Similarly, the biplot (Figure 22) of the same 20 okra genotypes grown under saline soil conditions in the Proka 2 field area of the University of Agriculture, Faisalabad, was constructed using the same 16 morphological and physiological traits. The first two principal components (Dim1 and Dim2) explained 27.1% and 20.4% of the total variation, respectively. In Quadrant I, genotypes Nirali, Okra 3A, and G-20 Irka Anamika were identified as important, particularly from a salinity tolerance perspective, as they were associated with vectors such as LA, NOLP, NONP, NOPP, PL, PW, and FYP. In Quadrant II, genotype G-25 019221 was located far from the origin, showing strong association with vectors of DE, K^+ ion content, and the K^+/Na^+ ratio. In Quadrant III, genotypes PBG-Okra 24-10, PBG-Okra 30, and PBG-Okra 53 were prominent and associated with vectors including G%, Na^+ ion content, SOD, POD, and CAT. In Quadrant IV, genotypes PBG-Okra 35, PBG-Okra 33, and PBG-Okra 42 were important, with vectors representing DE and ID.

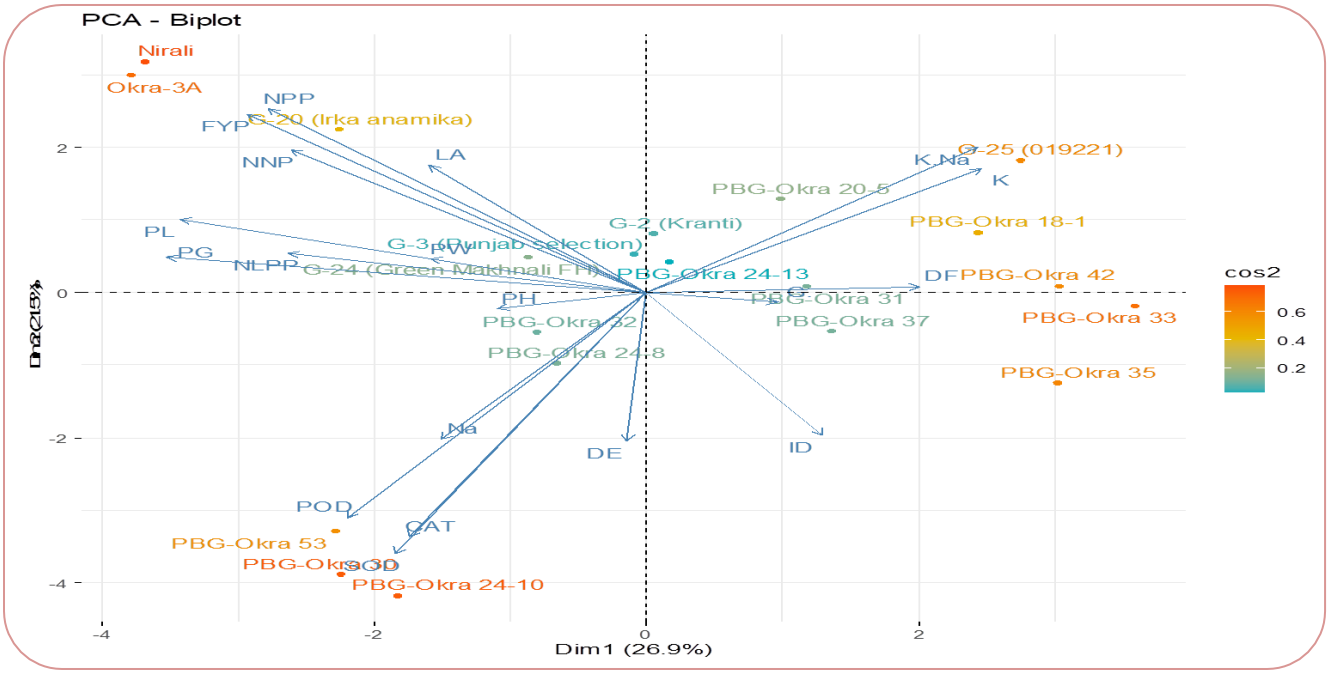


Figure 21. Principal component analysis biplot of 20 okra genotypes grown in normal soil at the maturity stage.
Abbreviations: DE: Days to emergence; G%: Germination percentage; DF: Days to flowering; PH: Plant height; NOLP: Number of leaves per plant; NONP: Number of nodes per plant; IND: Internodal distance; LA: Leaf area; NOPP: Number of pods per plant; PW: Pod weight; PL: Pod length; PG: Pod girth; FYP: Fruit yield per plant; K^+: Potassium ion; Na^+: Sodium ion; K^+/Na^+ ratio; SOD: Superoxide dismutase; POD: Peroxidase; CAT: Catalase.

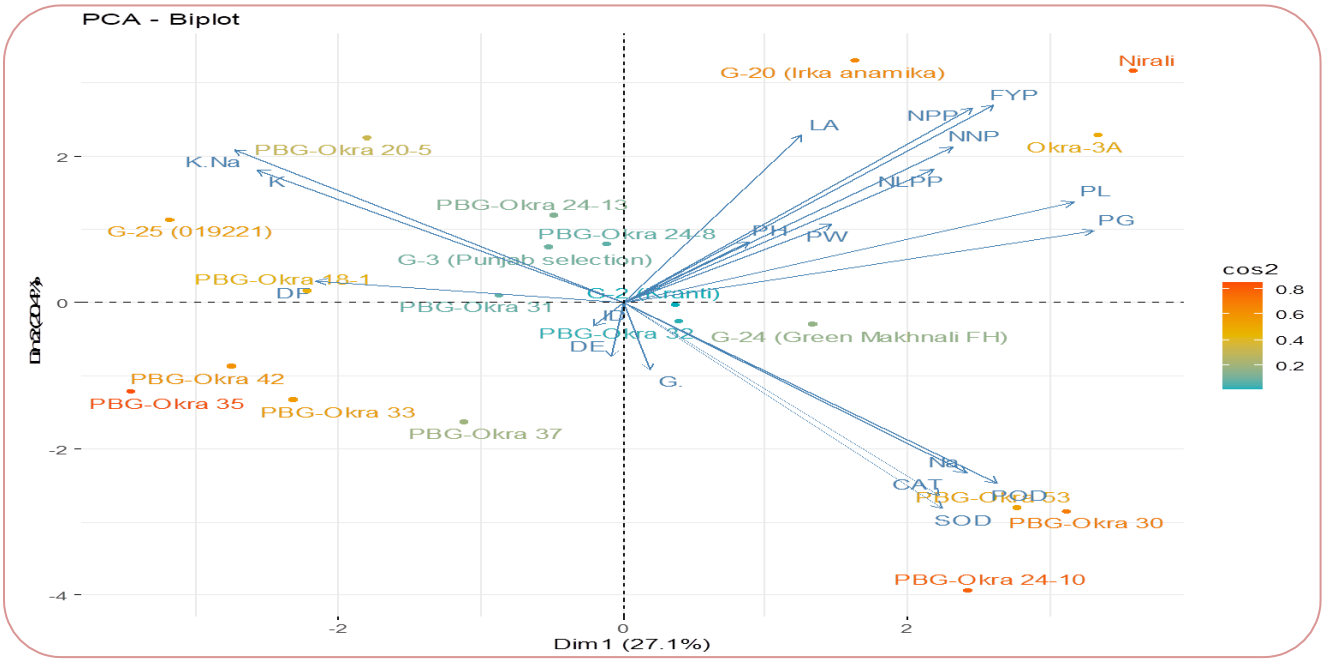


Figure 22. Biplot of 20 okra genotypes grown under saline soil conditions at the maturity stage.
Abbreviations: DE: Days to emergence; G%: Germination percentage; DF: Days to flowering; PH: Plant height; NOLP: Number of leaves per plant; NONP: Number of nodes per plant; IND: Internodal distance; LA: Leaf area; NOPP: Number of pods per plant; PW: Pod weight; PL: Pod length; PG: Pod girth; FYP: Fruit yield per plant; K^+: Potassium ion; Na^+: Sodium ion; K^+/Na^+: Potassium-to-sodium ratio; SOD: Superoxide dismutase; POD: Peroxidase; CAT: Catalase.

Discussion

Morphological and biochemical data were collected from 20 germinated genotypes and analyzed using split-plot ANOVA. The results indicated that replication effects were non-significant for all traits. Location effects were highly significant for all traits except days to emergence and number of locules per pod. The number of locules per pod remained uniform across all okra germplasm, while days to emergence was non-significant, possibly due to poor seed quality or the hard seed coat of okra. Genotypic effects were highly significant for all traits except number of locules per plant. The genotype × location interaction was highly significant only for internodal distance, Na^+ ion concentration, K^+ ion concentration, and the K^+/Na^+ ratio.

Ion homeostasis is a critical component of okra adaptation to salinity stress. Okra, like other moderately salt-tolerant glycophytes, relies on conserved regulatory mechanisms such as the Salt Overly Sensitive (SOS) signaling pathway to maintain Na^+ and K^+ balance under saline conditions (Ji et al., 2013). Under salinity, increased cytosolic Ca^{2+} levels activate the SOS signaling cascade. SOS3 (CBL4) interacts with SOS2 (CIPK24) to activate the plasma membrane Na^+/H^+ antiporter SOS1, facilitating Na^+ efflux from root cells (Ramakrishna et al., 2025). However, under severe salt stress (e.g., 100-150 mM NaCl), Na^+ influx may exceed the plant's capacity for extrusion and compartmentalization, resulting in cytosolic Na^+ accumulation, reduced K^+ levels, and inhibition of enzyme activity, ultimately leading to metabolic dysfunction. The ability of okra to delay these detrimental effects up to approximately 0.3% NaCl suggests a moderately efficient ion regulatory system, more robust than that of highly salt-sensitive species such as rice but less effective than that of true halophytes, which can tolerate NaCl concentrations exceeding 200 mM through specialized adaptations (Wei et al., 2025).

Under low salinity stress, okra initiates coordinated defense and acclimation mechanisms that limit cellular damage and sustain growth. Moderate increases in reactive oxygen species (ROS) act as signaling molecules, triggering antioxidant defenses and stress-responsive gene expression. This controlled ROS production enhances the activity of antioxidant enzymes, including SOD, CAT, and ascorbate peroxidase (APX), and activates redox-sensitive transcription factors, thereby priming plant defense systems (Van Zelm et al., 2020). Evidence from related species indicates that mild salinity enhances ROS-scavenging enzyme activity during early adaptation, suggesting a similar response in okra. Elevated levels of SOD, CAT, and POD have been reported in okra and other plant species under approximately 50 mM NaCl, indicating activation of enzymatic ROS detoxification pathways (Naseem et al., 2023). These antioxidant systems mitigate oxidative damage and preserve cellular integrity under controlled stress conditions (Das et al., 2024).

An experiment involving the collection of okra leaf samples from two regions of Faisalabad and one region of Pindi Bhattian demonstrated highly significant differences among locations for all biochemical and physiological traits (Sarwar et al., 2022). Similarly, salt stress adversely affected seed germination and early seedling growth in bread wheat, with progressive reductions observed at increasing salinity levels (Asad et al., 2024). Reduced germination under saline conditions has been attributed to increased osmotic potential of the soil solution, which restricts water uptake by seeds and lowers germination efficiency (Kafi and Goldani, 2001; Jamil and Rha, 2004).

Genotypic variation in germination response under salinity has also been reported. Accession No. 019217 (71.42%), Parbhani Kranti (65.13%), Chinese Red (58.67%), and Accession No. 015382 (15.50%) were ranked first, second, third, and thirty-ninth, respectively, based on germination percentage. Notably, Accession No. 019217 maintained 51.67% germination at 120 mmol L^{-1} NaCl, with a mean germination of 68.28% across salinity levels (Haq et al., 2012). Salinity stress also significantly reduced key morphological and yield-related traits in okra, including plant height, leaf area, number of leaves per plant, number of nodes per plant, internodal distance, number of pods per plant, pod weight, pod girth, pod length, and fruit yield per plant. Studies on eight okra landraces under varying salinity levels further confirmed a progressive decline in plant growth and yield parameters with increasing salt stress (Rady and Shama, 2023).

Correlation analysis among measured traits showed results consistent with previous studies (Haq et al., 2012). Similar positive correlations among most morphological traits were reported in okra genotypes (Komolafe et al., 2022). However, traits such as number of pods per plant, fruit yield per plant, and number of seeds per pod exhibited negative correlations with number of leaves per plant, plant height, and peduncle length (Komolafe et al., 2022). Additional studies also

supported these findings (Reswari et al., 2025). Conversely, some reports indicated a predominance of negative correlations among certain morphological and biochemical traits, suggesting trade-offs in trait expression under stress. This implies that morphological growth may be compromised when plants allocate resources toward defense mechanisms. Under salinity stress, morphological traits decline, whereas ionic regulation and antioxidant enzyme activities are enhanced (Ahmed et al., 2024).

Biplot analysis of field experiments effectively illustrated the performance of twenty okra genotypes under both normal and saline conditions. Similar approaches have been used to evaluate crosses and parental lines under varying salinity levels and to identify superior genotypes. Biplot analysis has also been successfully applied for selecting high-yielding and salt-tolerant genotypes in cotton under saline conditions (Zafar et al., 2023). Furthermore, it has been used to assess nutri-biochemical traits under biotic stress and to determine genotype-trait relationships in okra (Nathoo et al., 2025), as well as to evaluate genotype performance and combining ability across environments (Patel et al., 2025).

Conclusion

Okra exhibits moderate tolerance to salinity; however, salt stress significantly reduces germination, flowering, pod development, and seed formation, ultimately leading to yield losses. Under saline conditions, tolerant genotypes demonstrate enhanced antioxidant enzyme activities, including SOD, POD, and CAT, compared to those grown under normal conditions. Genotypes such as Okra-3A, G-20 Irka Anamika, and Nirali show promise and may be utilized in future breeding programs to develop salt-tolerant hybrids or improved varieties.

Author's contributions

KD conducted the field and laboratory experiments and performed data collection and analysis. AS and AS supervised the overall research work and provided critical guidance during the design, execution, and manuscript preparation. FSA contributed to the supervision of manuscript writing, critically reviewed the draft, and assisted in refining the final version of the manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger
SDG 12: Responsible Consumption and Production
SDG 15: Life on Land

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