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INFLUENCE OF SALT STRESS ON THE GROWTH OF BRASSICA SEEDLINGS

Original Article

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ABSTRACT

Background: Soil salinity is one of the leading abiotic stressors limiting agricultural productivity worldwide, particularly affecting the early growth stages of sensitive crops like *Brassica napus* L. (canola). Salinity induces osmotic and ionic stress, disrupting physiological and morphological processes vital to plant establishment and yield. In Pakistan, where over 6.22 million acres of land are salt-affected, improving salt tolerance in oilseed crops remains a critical priority for food security and import reduction.

Objective: The present study aimed to evaluate the response of ten *Brassica* genotypes under varying levels of salt stress and identify salt-tolerant candidates suitable for further breeding programs.

Methods: A glasshouse experiment was conducted using a factorial arrangement in a randomized complete block design with four treatments (0, 50, 100, and 150 mM NaCl) and three replications. Ten *Brassica* genotypes were evaluated for germination percentage, root length, shoot length, root-to-shoot ratio, seedling length, and seedling vigor index. Data were collected on the 15th day after sowing and subjected to analysis of variance to assess significance across genotypes, treatments, and interactions.

Results: Significant variation ($p < 0.001$) was observed among genotypes, treatments, and their interactions for most traits. Maximum germination (91.3%), shoot length (4.18 cm), and root length (2.04 cm) were recorded at 50 mM NaCl. Seedling vigor index peaked at 558.57 in UAF-11 and dropped to 182.51 at 150 mM NaCl. Genotypes UAF-11, AARI-Canola, and NIFA Gold showed consistently higher tolerance and growth performance across treatments.

Conclusion: Moderate salinity (50 mM NaCl) promoted early seedling growth in selected *Brassica* genotypes. UAF-11 emerged as the most salt-tolerant genotype, followed by AARI-Canola and NIFA Gold, indicating their suitability for use in future breeding programs targeting saline environments.

Keywords: Brassica napus, germination, salt stress, seedling vigor, shoot length, soil salinity, stress tolerance.

INTRODUCTION

Agriculture continues to play a pivotal role in sustaining the global population by providing essential food and fiber. However, the sector faces increasing challenges due to environmental stressors such as salinity, drought, and extreme temperatures. Among these, soil salinity represents one of the most pressing threats to agricultural productivity worldwide. Salinity disrupts plant physiological functions, mainly through osmotic stress that limits water uptake and ionic stress that interferes with cellular metabolism (1). High concentrations of sodium (Na^+) and particularly chloride (Cl^-) ions impair seed germination, photosynthesis, and enzyme activity, severely limiting plant growth and yield. The expansion of salt-affected soils is an escalating concern, with approximately 6.22 million acres of land in Pakistan alone—accounting for 29.2% of the country's arable land—being salinity-affected, thereby undermining national food security and crop-based industries (2). This issue is especially critical for oilseed crops like *Brassica napus* L. (canola), a primary source of edible oil in Pakistan. With a rapidly increasing population and rising consumer demand, Pakistan continues to face a widening gap between domestic edible oil production and consumption, relying heavily on costly imports (3,4). Brassica crops, valued for their high-quality oil and protein content, rank second only to cereals in global importance. *Brassica napus* L. is particularly significant not only for oil production but also for its multifaceted applications in food, animal feed, biofuels, industrial products like adhesives and lubricants, and as a sustainable alternative to vegetable wax (5). Within the Brassicaceae family, known for its adaptability to diverse climates and characteristic cross-shaped flowers, various species such as *Brassica juncea*, *Brassica campestris*, *Brassica carinata*, and *Eruca sativa* (Taramira) are cultivated for oilseed production (6).

Oilseed Brassicas face substantial yield constraints under salinity stress, with *Brassica napus* production particularly vulnerable. Though relatively tolerant to environmental stressors such as drought and high temperatures, salinity significantly diminishes its growth and oil yield (7). Notably, *Brassica napus*, an amphidiploid of *Brassica oleracea* and *Brassica campestris*, shows promise due to its adaptability, high oil content, and nutritional quality (8,9). However, the limited availability of high-yielding, salt-tolerant cultivars remain a critical bottleneck in achieving sustainable oilseed production. Addressing this deficit is vital not only for reducing dependence on imports but also for promoting agricultural diversification and food security, especially in regions like Punjab where such crops are well-suited for cultivation. The global interest in Brassica species has surged due to their capacity to serve both industrial and nutritional purposes. For instance, in Brazil, *Brassica napus* is cultivated in cooler climates for high-quality oil production, though current yields are insufficient to meet demand (10). Similarly, *Brassica juncea* and *Brassica rapa* are known for their high erucic acid content, useful in industrial applications (11). In South Asia, including Pakistan and India, poor crop management and low-fertility soils contribute to suboptimal yields. Therefore, a comprehensive understanding of genotype performance under saline conditions is essential to improve productivity. Given these challenges, the current study is designed to evaluate the genetic potential of *Brassica* genotypes under salt stress, with the objective of identifying salt-tolerant varieties (12). The research specifically aims to assess the effects of varying salt concentrations on Brassica seedlings and to compare the morphological response of salt-sensitive versus salt-tolerant genotypes. This investigation addresses a critical gap in the development of resilient Brassica cultivars and supports broader goals of sustainable agriculture and food self-sufficiency in salinity-affected regions.

METHODS

The present study was conducted during the spring season of 2022 in a controlled glasshouse environment at the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. The experiment aimed to evaluate the physiological responses of ten *Brassica* genotypes to varying levels of salinity stress. A randomized complete block design (RCBD) with a factorial arrangement was employed to ensure robust statistical validity and minimize environmental variability. The experiment consisted of four salinity treatments, including a non-saline control (0 mM NaCl) and three salt stress conditions (50 mM, 100 mM, and 150 mM NaCl), with each treatment replicated three times. Seeds of the selected genotypes were sown in polyethylene bags filled with a sterilized soil mixture under standardized moisture and temperature conditions. Each experimental unit consisted of one replication per treatment per genotype, and all units were randomly distributed across blocks to minimize positional bias. Saline solutions of NaCl were freshly prepared and applied consistently across treatment levels to maintain uniform salt stress. The inclusion criteria required genotypes to be previously characterized for oilseed productivity and viability under field conditions, whereas genotypes with known seed dormancy or extremely low germination rates were excluded to maintain data integrity.

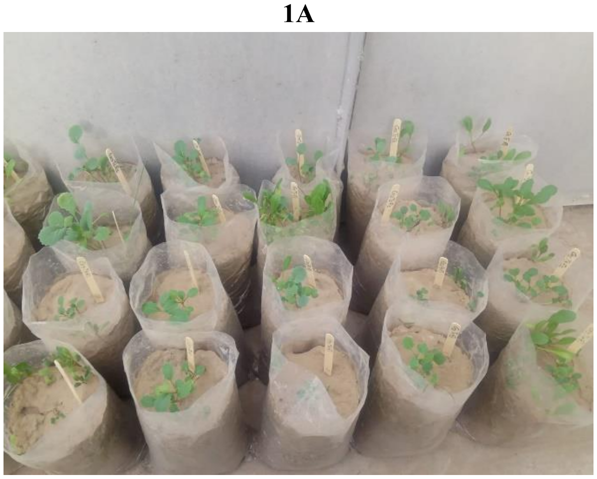
Germination percentage was recorded daily from the first day of emergence. The total number of germinated seeds in each treatment was recorded, and the germination percentage was calculated using the standard formula: Germination % = (Number of germinated seeds / Total number of seeds) × 100 (7). On the fifteenth day after sowing, four seedlings were randomly selected from each replication for morphological assessment. Seedling length was measured from the base of the neck region to the apex of the primary leaf using a calibrated ruler. Root length was measured from the base of the cotyledons to the tip of the main root. Shoot length was determined from the base of the hypocotyl to the tip of the primary leaf. All measurements were taken in centimeters, and the average length per replication was calculated to ensure consistency.

Seedling vigor index (SVI) was determined according to the method proposed by Abdul-Baki and Anderson (1973), which combines morphological growth with germination efficiency. The index was calculated as:

Seedling Vigor Index = (Mean root length + Mean shoot length) × Germination percentage (8). Additionally, the root-to-shoot ratio was calculated by dividing the average root length by the average shoot length for the same seedling: Root-to-shoot ratio = Root length / Shoot length. Data collected from all treatments and genotypes were subjected to statistical analysis using analysis of variance (ANOVA) to assess the significance of differences among treatments and genotypes. Post-hoc comparisons were carried out using the least significant difference (LSD) test at a 5% probability level, and data processing was performed using standard statistical software (e.g., SPSS, Statistix), although the specific software used was not indicated in the original documentation. Ethical approval for the experimental procedures was granted by the institutional research ethics committee of the University of Agriculture, Faisalabad. Since the study did not involve human or animal subjects, informed consent was not applicable.

1A

1B



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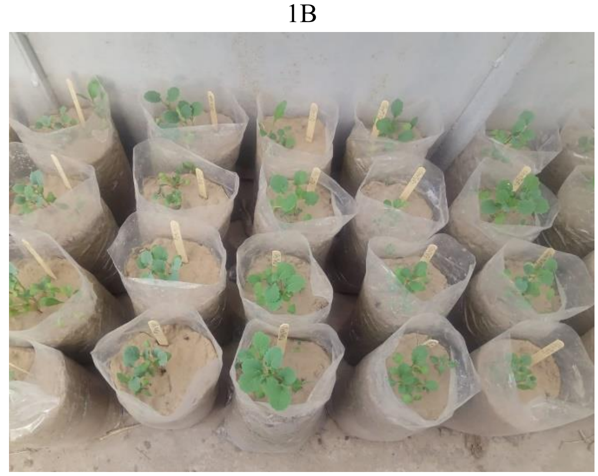

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Figure 1. Experimental setup showing Brassica seedlings grown under controlled salinity stress in polyethylene bags: (A) General view of setup, (B) Close-up of seedling emergence, (C) Early growth under salt stress, (D) Seedling variation among treatments.

RESULTS

Germination percentage varied significantly among *Brassica* genotypes in response to increasing salt concentrations. Under non-saline control conditions (0 mM NaCl), all genotypes exhibited optimal germination. At 50 mM NaCl (T2), germination percentage remained relatively stable or slightly increased in most genotypes. UAF-11, AARI-Canola, and NIFA Gold showed the highest germination rates under all conditions, particularly maintaining better performance at T2. Conversely, Faisal Canola, Super Canola, and KN-258 demonstrated markedly reduced germination under saline treatments compared to the control. Notably, genotypes such as Durr-e-NIFA, RBN-08002, NIFA-Sarson T20, and RBN-04021 exhibited minimal variation in germination across all salinity levels, indicating moderate salt resilience. Root length demonstrated a consistent decline with increasing salinity, with the highest values recorded under control conditions. The maximum root length of 2.04 cm was observed at T2 (50 mM NaCl), while the lowest, 1.10 cm, was recorded under T4 (150 mM NaCl). Among genotypes, UAF-11, AARI-Canola, and NIFA Gold maintained longer root lengths across treatments, suggesting better adaptability. In contrast, Faisal Canola, Super Canola, and KN-258 consistently displayed shorter roots, especially under higher salt concentrations.

Shoot length exhibited similar trends. The longest average shoot length, 4.18 cm, was observed under T2, while the shortest, 1.18 cm, was recorded at T4. UAF-11 again emerged as the most tolerant genotype with comparatively greater shoot length under stress. AARI-Canola and NIFA Gold also demonstrated moderate tolerance. Genotypes such as Faisal Canola, Super Canola, and KN-258 showed significantly reduced shoot growth as salinity increased. The root-to-shoot length ratio was influenced significantly by salt stress. The highest ratio of 0.49 was observed under control and T2 conditions, while the lowest ratio of 0.33 was recorded at T4. UAF-11, AARI-Canola, and NIFA Gold maintained higher ratios across treatments, highlighting efficient biomass allocation even under salinity. In contrast, the lowest ratios were recorded in Faisal Canola, Super Canola, and KN-258 under saline stress.

Seedling vigor index, calculated as a product of germination percentage and total seedling length, was maximally recorded in control and T2 treatments, with values reaching 558.57. In contrast, the lowest index was recorded at T4 (150 mM NaCl) with a value of 182.51. UAF-11, AARI-Canola, and NIFA Gold showed significantly higher vigor indices across treatments, whereas Faisal Canola, Super Canola, and KN-258 consistently showed lower indices. These findings emphasize the importance of genotype-specific responses in breeding programs aimed at improving salt tolerance in Brassica crops.

Table 1: Mean square values of different seedling traits

Source	DF	Germination%	Root Length	Shoot Length	Root Shoot Ratio	Seedling Vigor Index
Rep	2	20.8	0.32	1.44216	0.05	1430
Gen	9	634.3**	0.74**	1.8**	0.06**	98466**
T	3	10236.1**	4.65**	5.3 **	0.10 **	7676076**
Gen*T	27	660.5**	0.14	0.39**	0.04**	117829**
Error	78	101.0	0.10	0.13	0.009	19868
Total	119	62166.7				

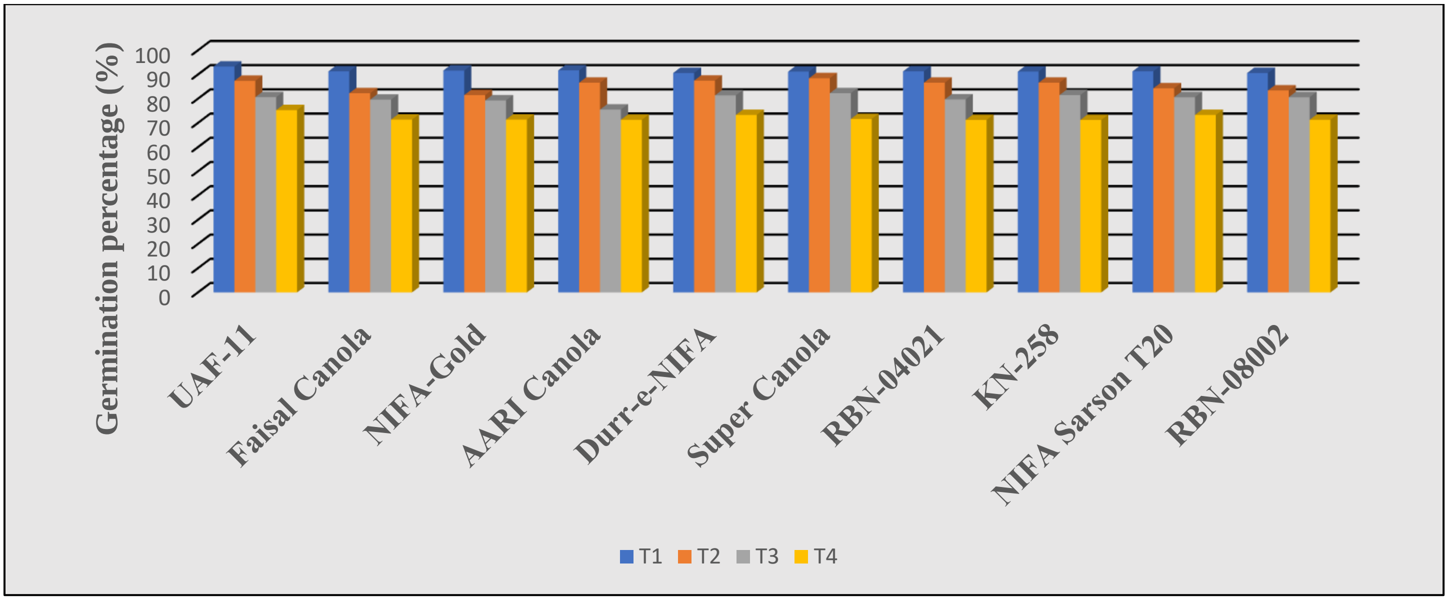

Figure 2 Effect of different salt treatments on germination percentage (%) under different genotypes

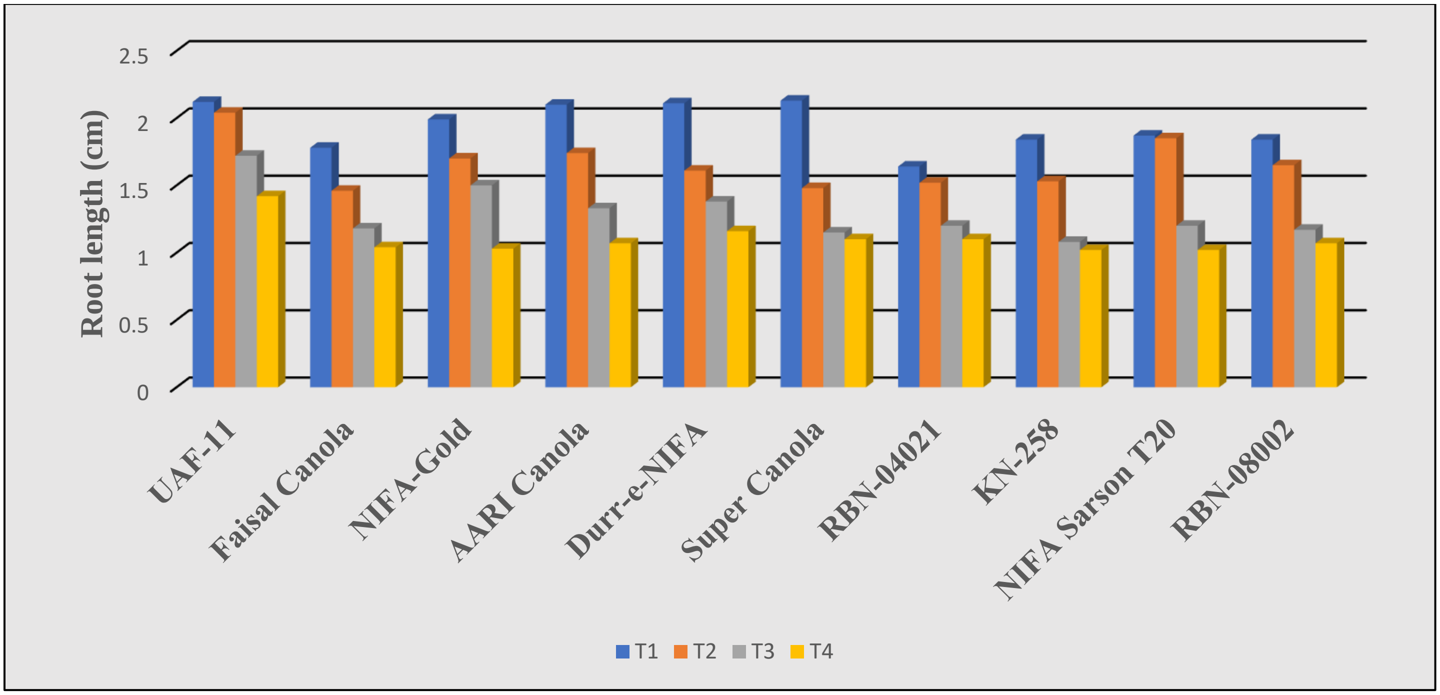

Figure 3 Effect of different salt treatments on root length (cm) under different genotypes

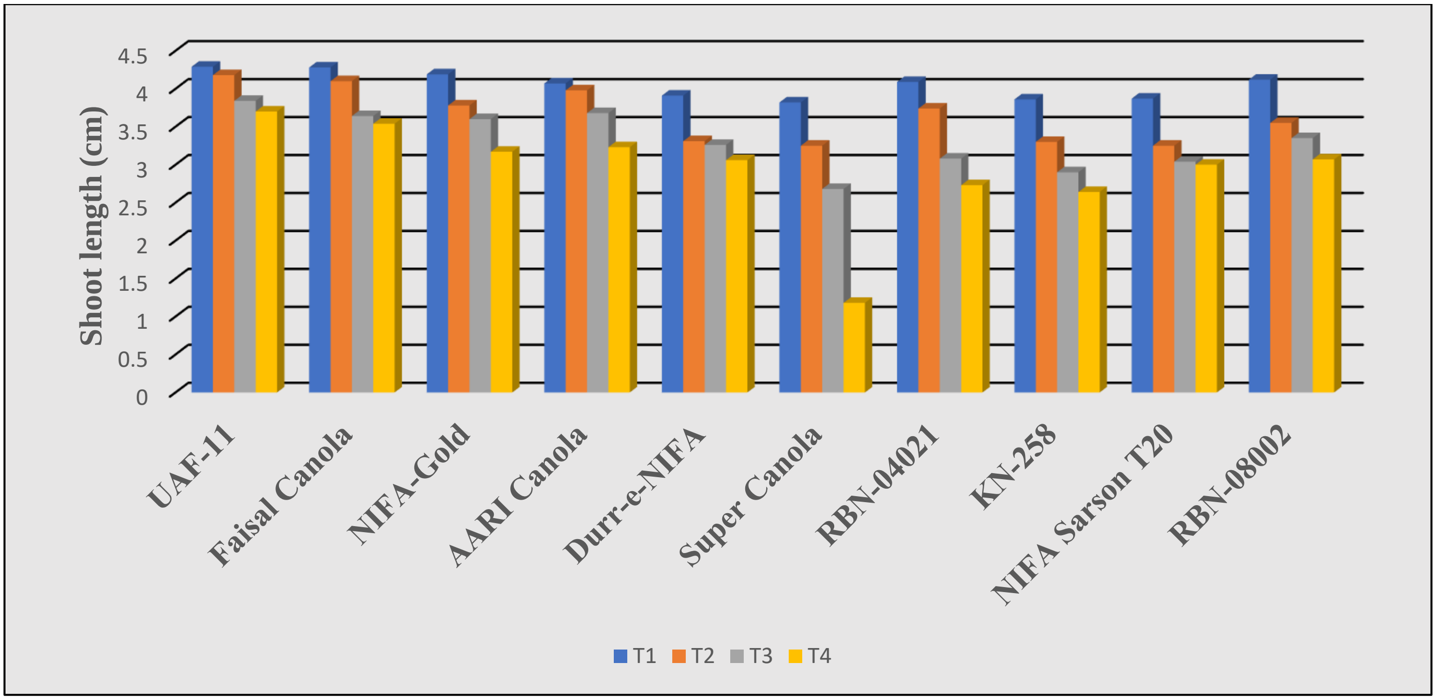

Figure 4 Effect of different salt treatments on shoot length (cm) under different genotypes

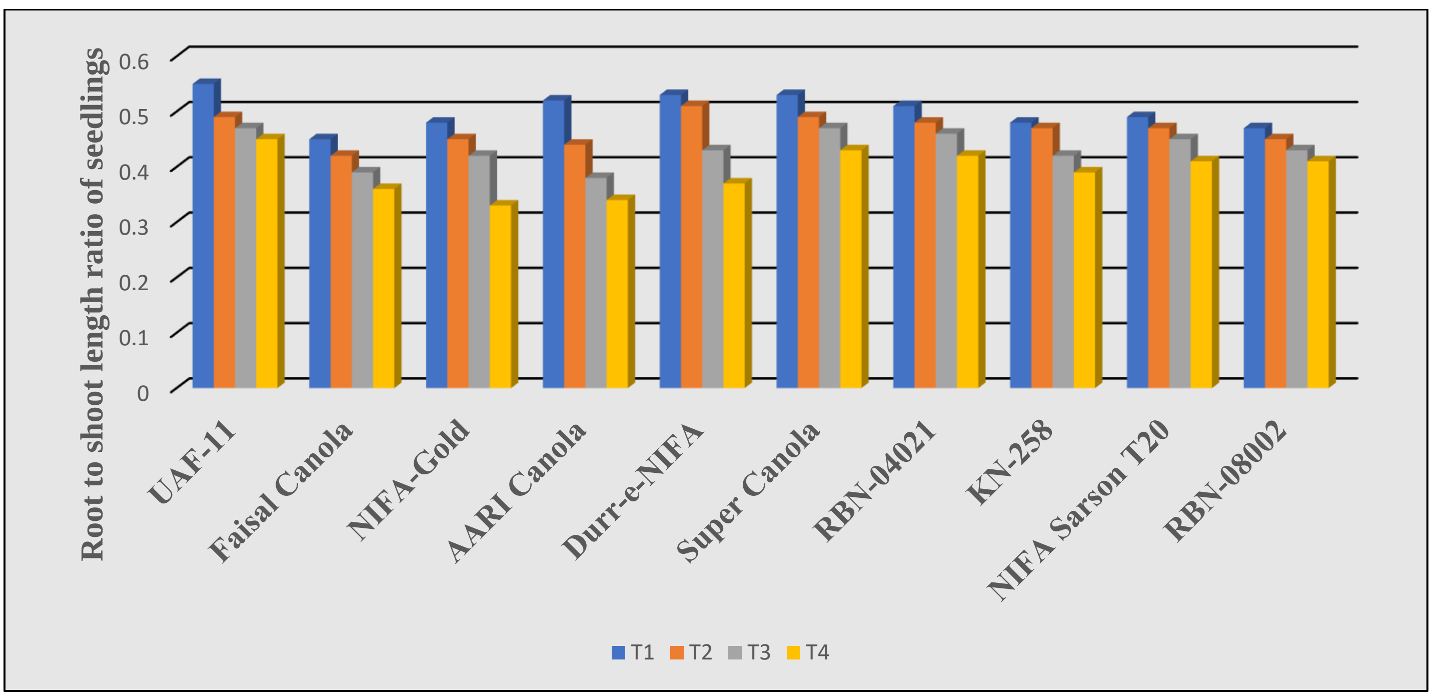

Figure 5 Effect of different salt treatments on root to shoot length ratio of seedlings under different genotypes

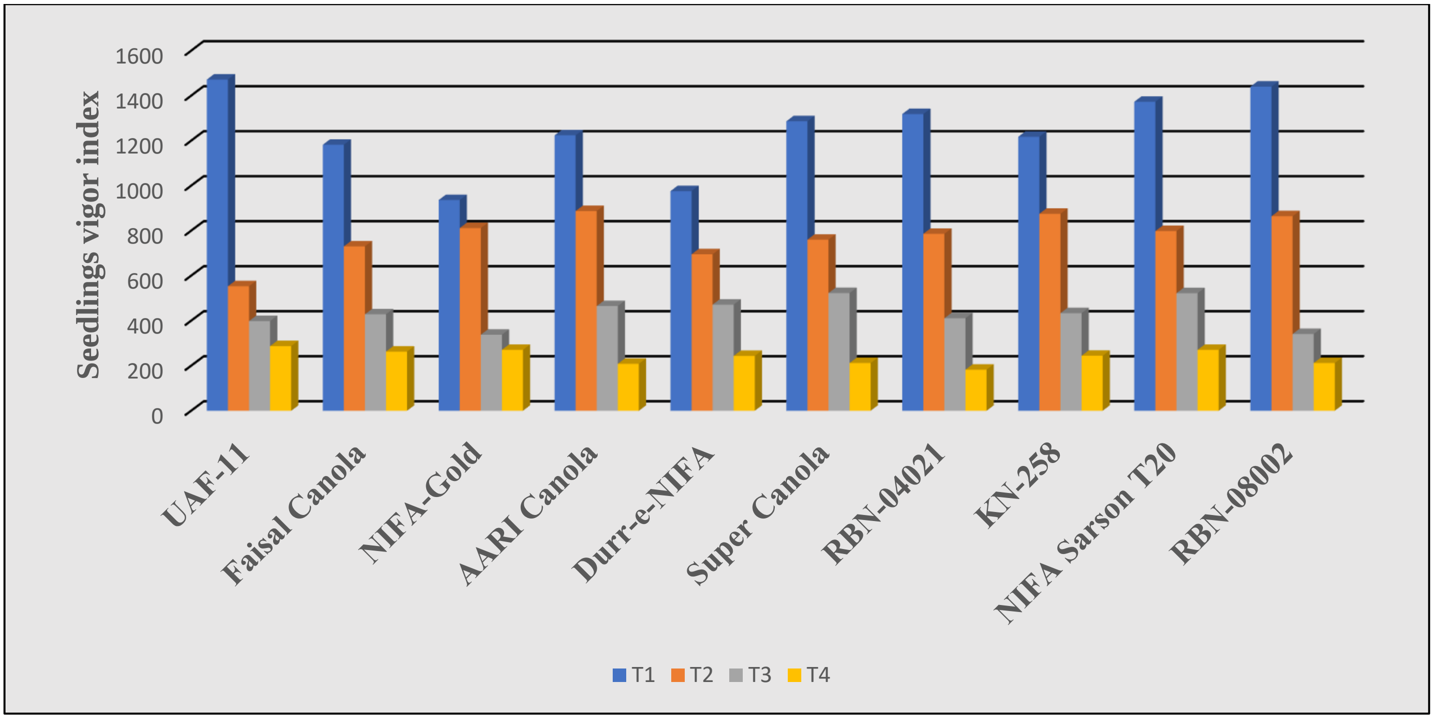

Figure 6 Effect of different salt treatments on seedlings vigor index under different genotypes

DISCUSSION

The findings of the present study offer valuable insights into the differential responses of *Brassica* genotypes to varying levels of salt stress, particularly during early seedling development. Germination percentage, as a fundamental indicator of crop establishment, demonstrated significant variation across genotypes and salt treatments. Genotypes such as UAF-11, AARI-Canola, and NIFA Gold consistently exhibited higher germination rates, even under moderate salt stress (50 mM NaCl), suggesting a degree of inherent tolerance (13). This trend aligns with previous observations where low levels of salt stress appeared to induce adaptive mechanisms in certain genotypes, temporarily enhancing physiological responses (13,14). In contrast, genotypes including Faisal Canola, Super Canola, and KN-258 exhibited pronounced declines in germination under stress, indicating their sensitivity and reduced adaptability to saline conditions. Root length, which reflects a plant's capacity to absorb water and nutrients under stress, also exhibited significant genotypic variation. Notably, the longest root length was recorded under the 50 mM NaCl treatment, while the shortest was observed at the highest stress level of 150 mM, illustrating a typical dose-dependent inhibitory effect of salinity on root elongation (15). Genotypes demonstrating superior root growth under stress maintained stronger establishment and potentially greater resilience, reinforcing the value of root traits in breeding programs targeting salt tolerance. Shoot length followed a similar pattern, with the 50 mM treatment yielding relatively higher values compared to higher salt concentrations. These results suggest that low salinity levels might act as a mild stimulant in some genotypes, possibly through osmotic adjustment or hormonal regulation, while higher levels lead to metabolic inhibition and growth retardation (16).

The root-to-shoot ratio served as a reliable index of biomass allocation and stress response. Higher ratios in tolerant genotypes indicated a strategic prioritization of root development to optimize water uptake under saline conditions. The control and 50 mM treatments maintained higher ratios, while significant reductions were recorded at 150 mM (17). These findings support earlier studies suggesting that root-to-shoot ratio adjustments under stress reflect physiological plasticity and can be used as a reliable indicator for screening salt-tolerant cultivars. Seedling vigor index, an integrated trait combining germination and growth metrics, revealed the most robust statistical differentiation across treatments and genotypes. The highest index values were observed under control and 50 mM conditions, while the lowest values occurred at 150 mM NaCl, highlighting the compounding negative effect of high salt levels on early growth. Genotypes such as UAF-11, AARI-Canola, and NIFA Gold again demonstrated higher vigor, suggesting their superior metabolic efficiency under stress. The observed genotype-by-treatment interactions underscore the genetic diversity and potential for selection of salt-tolerant lines within *Brassica* species (18,19).

A key strength of this study lies in its multifactorial design and the assessment of multiple physiological parameters, which provided a comprehensive evaluation of genotype performance under controlled salt stress. The use of early seedling traits allowed for rapid screening and differentiation of salt tolerance, contributing to the development of salt-resilient cultivars for saline-prone regions. However, the study is not without limitations. The experiment was conducted in a glasshouse under controlled conditions, which may not fully replicate the complex interactions present in field environments. Furthermore, the absence of molecular or biochemical profiling limits the understanding of underlying stress tolerance mechanisms. The lack of detailed data on ion accumulation or photosynthetic efficiency may restrict the translational value of the results for advanced physiological or genetic research. Future studies should expand on these findings by incorporating molecular markers, gene expression profiling, and field-based evaluations to validate and refine the selection of tolerant genotypes. Evaluating additional traits such as proline content, chlorophyll fluorescence, and membrane stability under salt stress could also provide deeper insights into the physiological pathways involved (20). Moreover, expanding the range of genotypes and including more severe or fluctuating salt conditions would enhance the robustness of selection and adaptability analysis. In conclusion, the study confirms that moderate salt stress may not universally inhibit germination and growth and, in some genotypes, may even act as a stimulatory signal. The observed genotype-specific responses, particularly those of UAF-11, AARI-Canola, and NIFA Gold, indicate promising avenues for breeding and agronomic improvement. These findings contribute to a more strategic approach to addressing salinity challenges in agriculture through targeted genotype selection and stress-resilient crop development.

CONCLUSION

This study concluded that moderate salt stress conditions supported more favorable seedling growth in certain *Brassica* genotypes, demonstrating their potential for cultivation in saline-prone environments. Among the genotypes tested, UAF-11 consistently exhibited superior performance across multiple traits, followed closely by AARI-Canola and NIFA Gold, highlighting their adaptability under salt stress. These findings underscore the value of identifying salt-tolerant genotypes early in the growth cycle, which can serve as promising candidates for future breeding programs aimed at improving crop resilience. The results contribute meaningful insights toward enhancing agricultural productivity in salt-affected areas and offer a foundation for selecting and developing more stress-resilient oilseed varieties through targeted research and expert validation.

Author Contributions

Author	Contribution
Sana Ghaffar	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Muhammad Azam Khan*	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Muhammad Ahsan Khan	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Umara Sahar Rana	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Mubashar Nadeem	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Hafiz Saad Bin Mustafa	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Misbah Zulfqar	Contributed to study concept and Data collection Has given Final Approval of the version to be published
Maria Ghias	Writing - Review & Editing, Assistance with Data Curation
Sajida Habib	Writing - Review & Editing, Assistance with Data Curation
Salsabeel Rauf	Writing - Review & Editing, Assistance with Data Curation
Muhammad Rizwan Bashir	Writing - Review & Editing, Assistance with Data Curation

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