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Phenotypic and Biomass Profiling Reveals Differential Salinity Tolerance among Diverse *Brassica* Genotypes

Waqas Mushtaq[1], Amna Sarwar[2], Muhammad Munem Khan[2], Muhammad Talha Aslam[1*], Saira Ghafoor[1], Ahmed Mahmoud Ismail[3*], Hossam S. El-Beltagi[4], Adel A. Rezk[4], Saleh Mbark Alturki[5], Tarek A. Shalaby[5]

[1]Key Laboratory of Crop Physiology, Ecology, and Genetic Breeding, Ministry of Education/College of Agronomy, Jiangxi Agricultural University, Nanchang, 330045, China.
[2]Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad 38040, Pakistan.
[3]Pests and Plant Diseases Unit, College of Agricultural and Food Sciences, King Faisal University, Al-Ahsa-31982, Saudi Arabia.
[4]Agricultural Biotechnology Department, College of Agricultural and Food Sciences, King Faisal University, Al-Ahsa 31982, Saudi Arabia.
[5]Department of Arid Land Agriculture, College of Agricultural and Food Sciences, King Faisal University, Al-Ahsa 31982, Saudi Arabia
[*]Corresponding authors. E-mail: taslamuaf@gmail.com; amismail@kfu.edu.sa

ABSTRACT

***Brassica* species are an imperative source of vegetable oil. However, salt stress significantly limits their productivity in the world. An understanding of genetic diversity and inter-trait relationships under salinity is therefore essential for developing salt-tolerant cultivars. In this study, fifteen *Brassica* genotypes were evaluated under varying salinity levels for growth and yield-related traits. Principal component and cluster analyses revealed significant genotypic variation. These analyses explored the trait associations and classifying the genotypes based on their salinity stress responses. Considerable genetic variability was observed across the studied traits, confirming substantial diversity among the accessions. The first two principal components accounted for 90.02% of the total variation, with plant height, root length, and fresh biomass being major contributors. Cluster I contained genotypes UAF11, 8003, 25047, and 25062, which were distinguished by their improved shoot and root performance under salinity stress. This study identifies promising genetic material for breeding salt-tolerant, high-yielding cultivars to meet global vegetable oil demand.**

Keywords: crop improvement, salinity stress, trait-based selection.

INTRODUCTION

Oilseed crops are the main source of edible oils, proteins, and biofuels. The seeds of oilseed crops can deliver 20-40% of dietary protein and 20-50% of fat necessary for human nutrition (Gupta and Singh, 2024) therefore these crops possess significant industrial utilization (Khan, M.N. et al., 2024). Globally, *Brassica* species among other oilseed crops, majorly contributing to fulfil the consumption requirement of vegetable oil (Tan et al., 2024). Among the *Brassica* genus, different species i.e., *B. napus*, *B. juncea*, and *B. rapa* (canola/rapeseed, mustard, turnip rape, respectively) displayed an significant genetic variation and adaptability, making them ideal for cultivation in various and challenging environmental conditions. Thus, cultivation of improved and high-yielding Brassica genotypes is dispensable to promote the crop productivity and securing the global oil supplies (Zandberg et al., 2022).

Climate induced environmental factors severely decrease the global productivity of oilseed crops (Attia et al., 2021). Escalating soil salinization affecting one billion hectares (ha) of arid and semi-arid region, reducing 20% productivity of the cultivated land and half of all irrigated regions (Syed et al., 2021). In Pakistan, salinity has degraded 14% of irrigated land, causing 64% yield loss, diminishing the 23 million ha of country's arable land (Aslam et al., 2026 a). Increasing salinity stress is a primarily constraints for

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the *Brassica* oilseed crop production, which are regularly cultivated in these regions.

The presence of high concentration of salt ions (Na^+, Cl^-) in soil solution cause osmotic and ionic stresses in plants (Aslam et al., 2023). These ions disrupt water uptake and cause nutrient imbalances in plants, which in turn decrease photosynthetic production and respiration processes. This functional damage significantly reduced plant growth and cause yield loss (Aslam et al., 2023). A substantial variation in salinity tolerance depends upon the genetic material (Chattha et al., 2022; Khan. I. et al., 2024; Aslam et al., 2026b) and presence of salt tolerant genes (He et al., 2021), which modify the physiological adaptation under stress conditions (Dai et al., 2024). Many authors confirmed the genotypic variability in salinity stress through observing germination percentage, and photosynthetic production rate, and crop biomass production (Pavlović et al., 2019; Zhu et al., 2022). Likewise, Petcu et al. (2007) demonstrated that alfalfa genotypes exhibited distinct responses to salt and water stress, highlighting the importance of selecting tolerant germplasm for sustainable forage production under adverse conditions.

Despite this progress, a critical gap persists. Many studies constrain their scope to a limited number of genotypes and impose short-term abiotic stress, which limits the applicability of their findings to practical breeding programmes. The previous investigations have restricted their scope to a narrow genetic base, often comparing fewer commercial cultivars in stressful environments (Khan, M.O. et al., 2024). These studies lacks and commonly overlook the entire adaptive variation present within the wider *Brassica* gene pool and fail to display the physiological effects of prolonged salt stress on long-term growth and crop biomass yield, traits of paramount importance for breeders aiming to develop a variety for stable yield in saline field (Panjabi et al., 2019; Dai et al., 2024). Furthermore, narrow focus limit the applicability of their findings to practical breeding programs, those requiring a strong understanding of the heritable variation in complex, yield-related traits (Panjabi et al., 2019).

Therefore, the present study was conducted in greenhouse to address the current research gap thoroughly assessing the diverse *Brassica* genotypes. This research was designed to quantify the *Brassica* genotypic responses through a comprehensive set of growth and plant biomass-related traits, which are true representative of the overall stress response of plants. Using multivariate statistical models, including principal component and cluster analyses, our research aims to 1) identify the promising salt-tolerant *Brassica* genotypes and 2) determine the key morphological traits indicative of salt tolerance. Our findings will provide a reliable, trait-based framework to select salt-resilient parent material for improving breeding efforts for saline agriculture.

MATERIAL AND METHODS

Experimental site and growing conditions

The controlled experiment was performed under greenhouse conditions at the Department of Plant Breeding and Genetics (PBG), University of Agriculture Faisalabad (UAF), Pakistan. The geographical location of this site is 31°44′ N latitude, 73°07′ E longitude, at an elevation of 184.4 m above sea level, and had an arid climatic zone. During experiment, 16 hours photoperiod followed by 8 hour dark period was maintained at greenhouse. The diurnal temperature was set at 26±2°C while ±21±2°C temperature was set at night with relative humidity between 50-70% and 180 μmol photons/m²/s photon flux density.

Plant Material and Experimental Design

Before the execution of experiment, soil physiochemical properties were analysed and given in Table 1.

Table 1. Soil physicochemical properties of the experimental site

Parameter	Unit	Value
Soil texture	–	Sandy loam
pH (1:2.5 soil-water)	–	7.8
Electrical conductivity (EC)	dS/m	1.2
Organic matter	%	0.7
Total nitrogen (N)	%	0.06
Available phosphorus (P)	mg/kg	8.5
Exchangeable potassium (K)	mg/kg	145
Bulk density	g/cm	1.35

Fifteen diverse genotypes of *Brassica* were used for the experiment (Table 2). Before sowing, seeds were treated with 1% sodium hypochlorite solution for five minutes, followed by washing with distilled water. The experiment was arranged in a completely randomized design (CRD) with replicates considering genotypes and salt stress levels as fixed factors in greenhouse controlled conditions. The seeds of *Brassica* genotypes were sown in large sized polyethylene bags containing a sterilized soil mixture that was received from the university's agricultural field. The experimental unit comprised of 3 pots/genotypes/replication with 5 plants/pot, comprising total 15 plants/genotype for each treatment and replication. A recommended dose of NPK fertilizers was applied prior to sowing. For this, N, P, and K were given at 100 mg, 50 mg and 50 mg per kg of soil, respectively. While, all other agronomic management practices were kept constant for better crop growth. Sixty-day salt stress imposition was initiated at two leaf stage (20 days after sowing). Salt stress levels were maintained through irrigation with sodium chloride solution calibrated to achieve and sustain electrical conductivity (EC) of 0, 5, 10, and 15 dS/m in soil. The soil EC was monitored and adjusted every five days to maintain consistent stress levels.

Data Recording

Five plants of each genotype were selected from every replication and the data of the following traits were recorded. The fresh weight (g) of shoot (SFW) and root (RFW) was measured in grams using an electronic balance (Setra BL-410S) from the uprooted *Brassica* plants in every replicate and then averaged. The root and shoot were separated from the harvested plants, placed in paper bags and dried in an oven (Fisher Scientific, Model 655 F, US) at 65°C for 72 hours. Later, electronic balance was used to weigh the root and shoot separately by standard protocol mentioned in Altaf et al. (2020). Length (cm) was recorded with a measuring scale for each replicate in standard unit and then averaged. Plant height (cm) was recorded with the help of measuring tape from the base of the plant. Data were recorded and the mean was calculated. Number of leaves/plant and branches/plant were counted manually of selected plant from each replication and averaged.

Table 2. Passport Data and Key Traits of Brassica Genotypes used in study

Sr. No.	Code/genotype	Brassica species	Origin / Types	Key known traits / remarks
1	G01/UAF-11	*B. rapa*	UAF	High-yielding; non-shattering; improved seedling vigor under moderate salinity
2	G02/8003	*B. napus*	NARC	Early maturity, drought tolerance
3	G03/25047	*B. napus*	-	Canola type, high-yielding line
4	G04/25062	*B. napus*	-	Canola type, medium duration
5	G05/61-A	*B. napus*	NARC	
6	G06/85-A	*B. napus*	NARC/AARI	
7	G08/88	*B. napus*	NARC/AARI	
8	G09/ 68	*B. napus*	NARC/AARI	
9	G10/53	*B. napus*	NARC/AARI	Standard check line used in yield trials
10	G11/0259	*B. napus*	NARC	Advanced line for high oil content
11	G07/25052	*B. napus*	Double-zero line	High protein content, low erucic acid, low glucosinolate
12	G12/Super Canola	*B. napus*	ORI	High-yielding, double-zero (00) type in RBN breeding
13	G13/36	*B. rapa*	Local breeding line	
14	G14/25007	*B. rapa*	Local breeding line	Higher biomass and better water retention
15	G15/RBN-08002	*B. rapa*	ORI	Seedling salinity screening studies (specific pedigree not found in public articles)

UAF: University of Agriculture Faisalabad-Pakistan; NARC: National Agricultural Research Centre-Islamabad, Pakistan; AARI: Ayub Agricultural Research Institute; ORI: Oilseeds Research Institute (AARI).

Statistical analysis

All recorded data was first examined for normality and homogeneity of variance. Where required, percentage data were arcsine-transformed, and non-normal data were log-transformed prior to analysis of variance (ANOVA). The significance level among treatment means were analysed using the least significant difference (LSD) test at the 5% probability level (Steel et al., 1996). Furthermore, Pearson’s correlation analysis was performed to determine the interrelationships among the studied traits. All statistical analyses were carried out using Statistix 8.1 (Analytical Software, USA), and graphs were generated in Microsoft Excel 2019.

RESULTS AND DISCUSSION

Descriptive statistics

Descriptive statistics revealed significant phenotypic variation among he evaluated Brassica genotypes under salt-stress conditions, with shoot fresh weight and number of leaves per plant showing the highest coefficients of variations under salt-stress treatment (Table 3). The highest variability was noticed in shoot fresh weight and number of leaves/plant. Conversely, root fresh and dry weight (CV=8.79% and 6.66%, respectively) showed a minimal fluctuation amongst evaluated genotypes, suggesting the better trait stability. Most traits followed a normal distribution; however, root dry weight and root length showed the moderate peak distribution. Additionally, shoot fresh and dry biomass displayed a more platykurtic distribution, reflecting a broader spread of values around the mean (Table 3).

Analysis of variance

ANOVA revealed highly significant ($p<0.001$) effects of treatment, genotypes and their interaction for the majority of the studied parameters, indicating strong genetic differences and differential salinity responses among the accessions (Table 4).

Table 3. Descriptive statistics of growth and biomass traits of *Brassica* genotypes in salinity stress

Trait	Mean	SD	CV (%)	Minimum	Maximum	Kurtosis	Skewness
SFW	0.69	0.09	13.64	0.54	0.85	-0.46	0.15
RFW	0.62	0.05	8.79	0.52	0.71	-0.27	-0.23
SDW	0.61	0.07	11.37	0.50	0.74	-0.27	0.36
RDW	0.57	0.04	6.66	0.49	0.64	0.44	0.17
RL	17.68	1.58	8.96	15.18	20.85	-0.30	0.35
SL	10.21	1.12	11.01	7.98	12.13	-0.24	-0.36
PH	17.68	1.59	8.96	15.18	20.85	-0.30	0.35
No. LP	9.88	1.32	13.32	7.50	12.25	-0.12	-0.23
No. BP	8.80	0.89	10.12	7.37	10.36	-0.91	0.03

SFW: shoot fresh weight, RFW: root fresh weight, SDW: shoot dry weight, RDW: root dry weight, RL: root length, SL: shoot length, Ph: plant height, No. LP: number of leaves/plant, No. BP: number of branches/plant, SD: standard deviation, CV: coefficient of variance.

Table 4. Mean squares from two-way analysis of variance of *Brassica* accessions checked under salinity stress

SOV	df	SFW	RFW	SDW	RDW	RL	Ph	SL	No. LP	No. BP
Genotype (G)	14	119.23**	53.25**	5.39***	0.790	57.90**	66.50**	156.47***	55.81***	42.05***
Treatment (Trt)	3	741.43**	718.16***	8.86**	10.07**	833.82**	895.64**	1337.04**	366.65***	552.58***
G × Trt	42	4.716**	2.90***	0.88	1.07	2.82**	4.06**	6.578***	2.439**	3.16***
Error	60	0.00038	0.00030	0.00583	0.00254	0.21	0.203	0.04	0.16	0.09
Total	119									

SOV: Source of Variation, SFW: shoot fresh weight, RFW: root fresh weight, SDW: shoot dry weight, RDW: root dry weight, RL: root length, Ph: plant height, SL: shoot length, No. LP: number of leaves/plant, No. BP: number of branches/plant. '*' indicates significance at p ≤ 0.05; '**' indicates significance at p ≤ 0.01. '***' indicates significance at p ≤ 0.001.

Principal component analysis (PCA)

PCA showed that the first two components (PC1 and PC2) explained 90.02% of the total variation (Table 5). Additionally, PC1 explains the vegetative growth related traits (fresh weight, plant height, and root and shoot length) of genotypes, while PC2 was strongly influenced by root dry weight (Figure 1). PC3 explained the the contribution of shoot dry weight to trait variability. A moderate loading from the No. LP (0.71) and the No. BP (-0.55) in PC4 indicated the morphological differences in branching and leaf production. PC5 showed the trait divergence linked to plant stature and branching by explaining the number of branches per plant (0.69) and the negative loading (-0.47) of plant height. While, PC6 was defined by shoot fresh weight (-0.76) and number of leaves per plant (0.49), indicating additional variability contributed by shoot biomass and leaf production.

Table 5. Multivariate Structure of Quantitative Traits Based on the first six principal components (PCs) for 9 quantitative characters of 15 Brassica accessions checked under salinity stress.

Traits	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	7.29	0.81	0.57	0.10	0.07	0.05
Variance %	80.99	9.03	6.38	1.08	0.77	0.60
Cumulative %	80.99	90.02	96.40	97.48	98.25	98.85
SFW	-0.36	0.09	-0.01	0.23	0.13	-0.76
RFW	-0.36	0.02	-0.19	-0.13	-0.23	-0.24
SDW	-0.25	0.07	0.95	-0.09	-0.06	0.03
RDW	-0.17	-0.98	0.04	0.05	0.05	-0.01
RL	-0.36	0.02	-0.14	-0.22	-0.42	0.12
Ph	-0.36	0.04	-0.12	-0.18	-0.48	0.23
SL	-0.36	0.09	-0.09	0.17	0.18	-0.06
No. LP	-0.36	0.09	-0.05	0.71	0.14	0.49
No. BP	-0.36	0.06	-0.11	-0.55	0.69	0.22

SFW: shoot fresh weight, RFW: root fresh weight, SDW: shoot dry weight, RDW: root dry weight, RL: root length, Ph: plant height, SL: shoot length, No. LP: number of leaves/plant, No. BP: number of branches/plant.

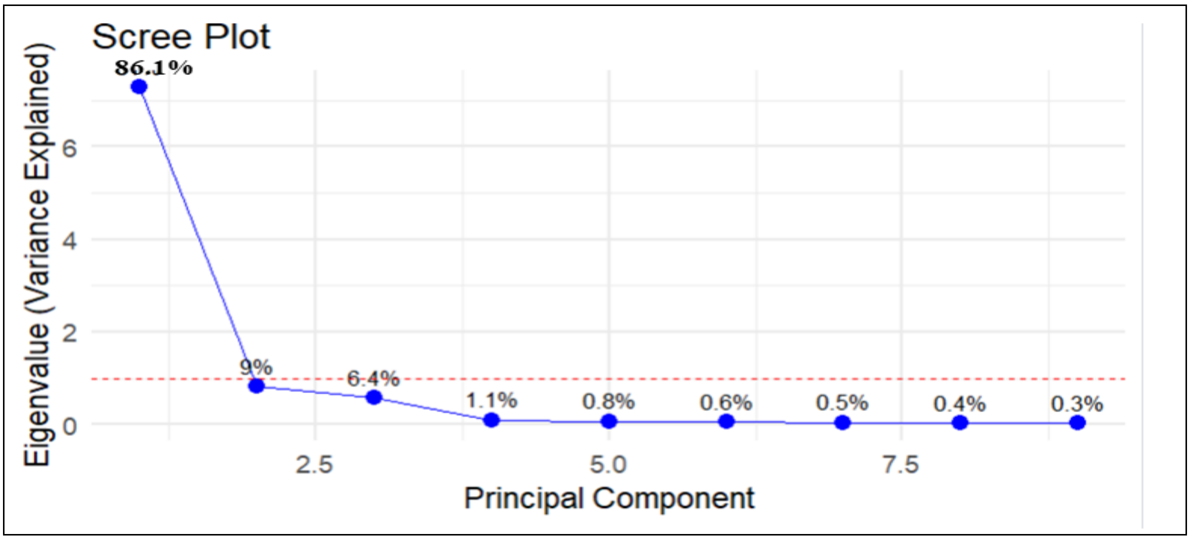

*Figure 1***.** Scree plot reflecting the eigenvalues of principal components obtained using nine quantitative traits in fifteen *Brassica* genotypes

Trait-based grouping of genotypes using principal component analysis

The biplot observation confirmed that most of studied growth and biomass related traits clustered together, strongly affecting the distribution of genotypes along the PC1 axis (Figure 2). PC1 accounting for 86.1% of the total variation, with PC2 explaining an additional 9.1%. Most of the studied traits were closely clustered along PC1. G09, G13, G14, and G15 grouped positively along PC1, showing a strong association with overall plant growth traits.

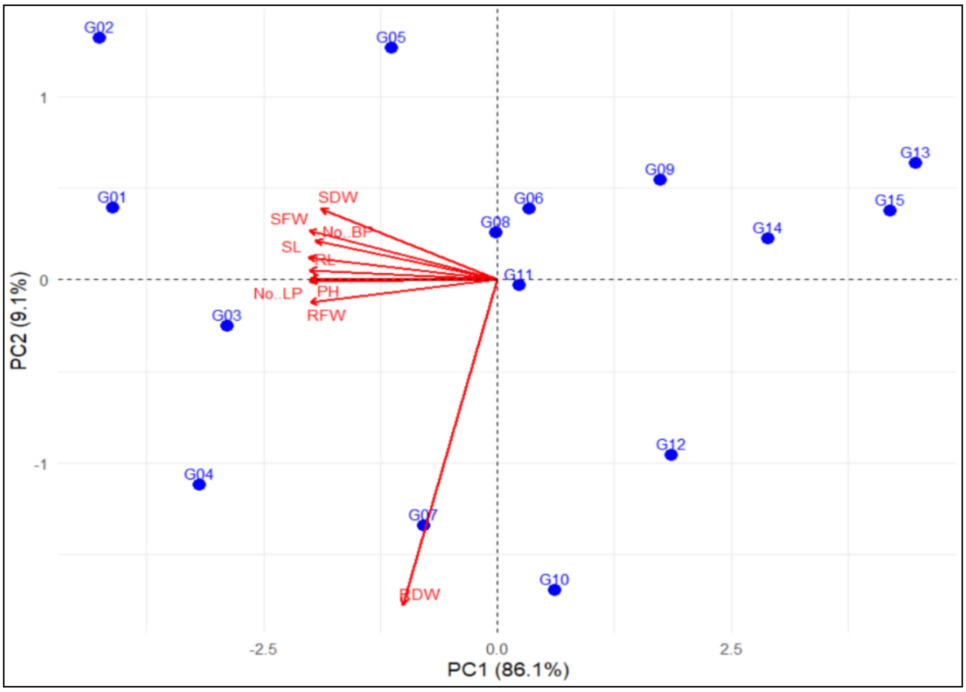

Figure 2. Principal component analysis (PCA) biplot showing nine quantitative traits and fifteen *Brassica* genotypes

Pearson correlation analysis

To support the PCA findings, a Pearson correlation analysis was carried out to examine how the nine agronomic traits were related to each other. Pearson correlation analysis described strong positive correlation (r>0.90) between the most studied growth and biomass related traits, particularly between shoot fresh weight, plant height and root length (Figure 3).

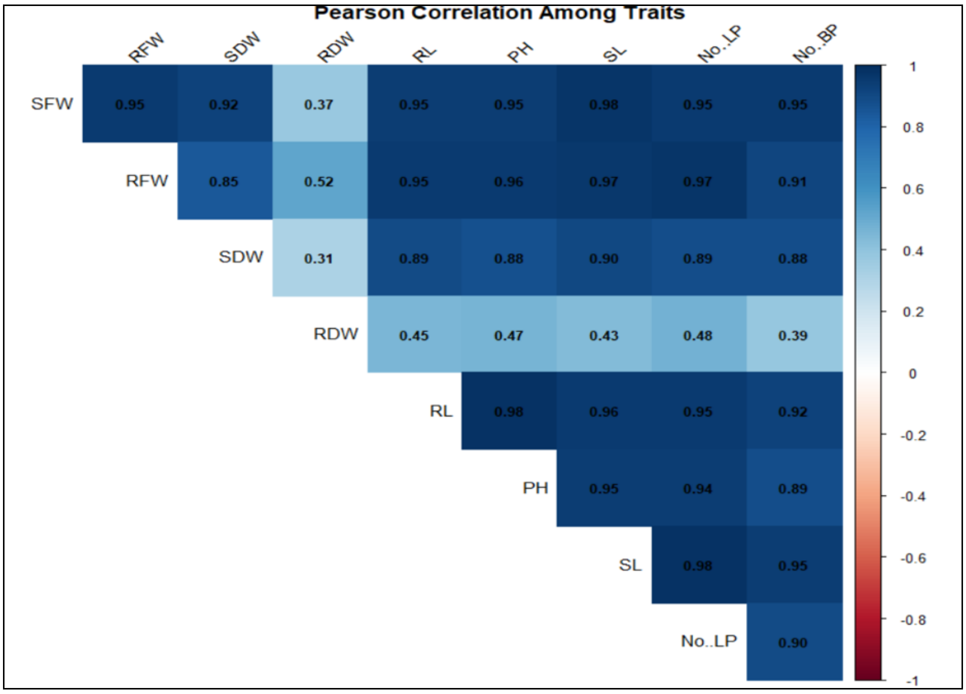

Figure 3. Pearson correlation matrix of nine quantitative traits measured across fifteen *Brassica* genotypes.

Cluster analysis

Hierarchical cluster analysis, validated by silhouette width, grouped the fifteen genotypes into three distinct clusters (Figure 4 and 5).

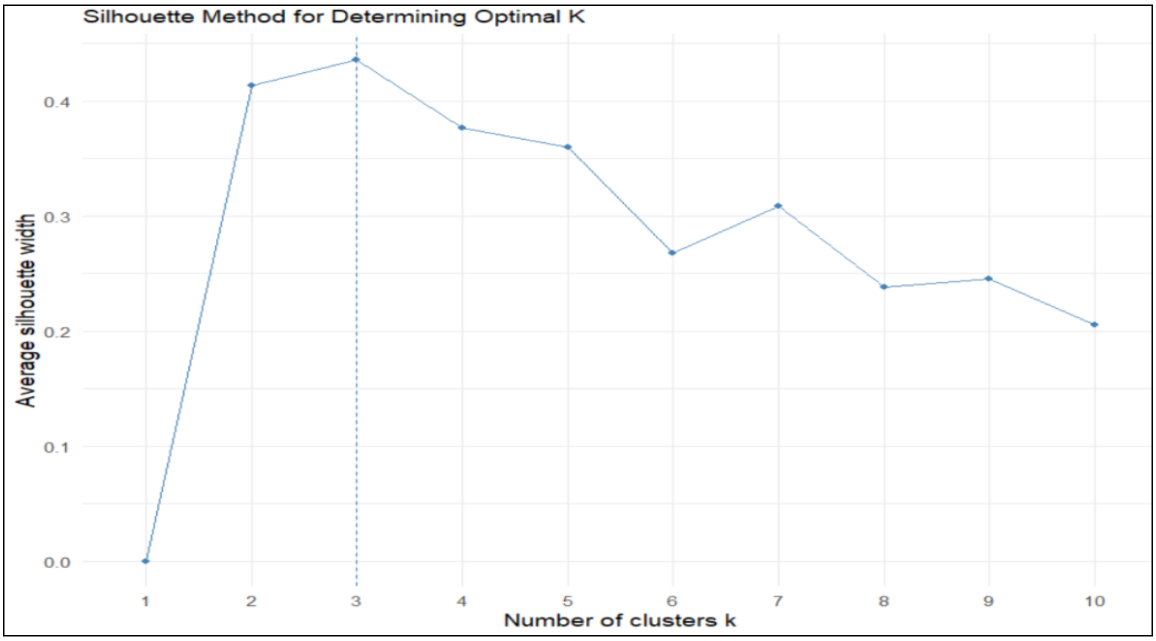

Figure 4. Cluster validation using silhouette width in quantitative data

Cluster I contained four genotypes (G01/UAF-11, G02/8003, G03/25047, G04/25062), Cluster II comprised six (G05/61-A, G06/85-A, G07/25052/ G08/88, G10/25007, G11/0259), and Cluster III included five (G09/68, G12/Super Canola, G13/36, G14/25007, G15/RBN-08002). The genotypes in cluster I exhibited consistently superior performance, with significantly higher mean values for most traits compared to Cluster II and III (Table 6).

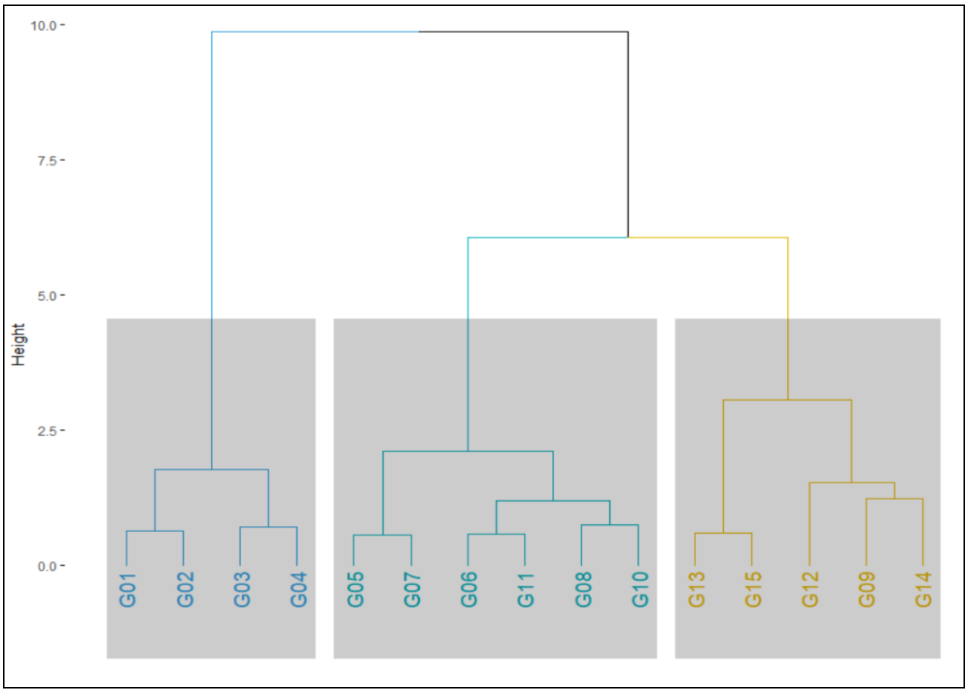

Figure 5. Dendrogram showing genetic relationships among fifteen *Brassica* genotypes derived from nine quantitative traits

Table 6. Cluster means for nine quantitative traits among *Brassica* genotypes

Traits	Clusters			Grand mean	P value
	1	2	3		
SFW	0.68	0.584	0.503	0.589	0.00000357
RFW	0.585	0.531	0.480	0.532	0.00000434
SDW	0.369	0.283	0.227	0.293	0.0000336
RDW	0.261	0.255	0.241	0.252	0.132
RL	16.7	15.1	13.8	15.20	0.00000548
PH	16.7	15.1	13.7	15.17	0.0000105
SL	9.87	8.76	7.76	8.80	0.0000325
No. LP	9.69	8.54	7.32	8.52	0.0000493
No. BP	8.41	7.62	6.8	7.61	0.00000873

Intra and inter-cluster distance

According to the cluster analysis based on quantitative parameters, the average intra-cluster distance of the genotypes varied from 1.81 to 2.34; clustering of the genotypes in each cluster was more or less similar. The average intra-cluster distances recorded lower than all inter-cluster distances, with the maximum divergence noticed among Cluster I and Cluster III (Table 7).

Table 7. Average intra-cluster and inter-cluster distances among three groups of *Brassica* genotypes based on nine quantitative traits

Comparison	Average distance
Intra-cluster (Cluster 1)	2.34
Intra-cluster (Cluster 2)	1.81
Intra-cluster (Cluster 3)	2.22
Inter-cluster (Cluster 1 vs Cluster 2)	3.24
Inter-cluster (Cluster 1 vs Cluster 3)	5.64
Inter-cluster (Cluster 2 vs Cluster 3)	3.31

The substantial genetic variability observed for morphological and growth traits under salinity stress highlights a strong potential for the selective improvement of salt tolerance in *Brassica*. The analyses of variance verified that salt stress treatment caused a significant variation which aligns with the established knowledge that salinity severely damage plant physiological functions. The significant reduction in shoot fresh weight and plant height can be attributed to osmotic and ionic stress, which inhibits water uptake and leads to toxic ion accumulation, thereby decreasing photosynthetic capacity (Kumar et al., 2021).

The interactive effect highlighted an imperative variation among different genotypes and salt stress levels, which is a prerequisite to identify the promising lines for salt stressed areas (Shahzad and Raza, 2021). The resistant genotypes encompasses intrinsic mechanism of osmotic adjustment and ion exclusion that helps to maintain normal plant functioning under saline stressed conditions (Aslam et al., 2023). Additionally, multivariate analyses explained the relationship between plant structure and traits underlying genetic diversity. The first two components of PCA accounted the majority of the total variation. The high laodings for root length on PC1; plant height, and shoot biomass remains the primary indicators of phenotypic performance under salinity stress. Our observations are align with Afzal et al. (2023) who reported the vegetative growth as a major axes of variation among genotypes under abiotic stress conditions (Atta et al., 2023). The present study showed a coordinated growth response with positive correlation between plant height and shoot fresh biomass. It implies that focusing on the increase in plant height could promise the breeding program for better crop biomass, yield and related traits under stressful environment (Rehman et al., 2022).

The cluster analyses grouped the genotypes based on their phenotypic performance. The genotypes in Cluster I (UAF-11, 25062, 25047, and 8003) produced high biomass compared to other clusters under salt stress. This could be due to the tolerance mechanisms of these genotypes, marking them as a promising candidate for use as donor parents in breeding programs (Ashraf and Munns, 2022). Likewise, Mondal et al. (2023) reported the promising role of improved plant growth and biomass towards better crop development under climate change induced abiotic stresses (Usman et al., 2026). The distance between Cluster I and III represent a significant genetic variation between the tolerant and sensitive *Brassica* accessions. This genetic divergence caused heterosis and genetic recombination in subsequent generations (Javed et al., 2022), thereby enhancing the selection of salt-tolerant progeny (Raboanatahiry et al., 2021). The superiority of Cluster I genotypes could be due to the improved physiological mechanisms (Zhang et al., 2024). This is supported by the observations of Hao et al. (2021) reported the plant response and associated salt-resistant mechanism. High salt tolerance is due to the selective ion uptake (Seleiman et al., 2022) and osmotic adjustment (Muneeba et al., 2024). Moreover, the identification of promising quantitative trait loci (QTL) and genes also helps improving plant stress resistance (He et al., 2021). Likewise, increased root length recorded from tolerant genotype is an imperative trait to access water from deeper soil layers, thus, reducing the osmotic stress (Zhu et al., 2022). The present study bridges the gap between physiology and breeding through identifying these morphological parameters for underlying genetic tolerance.

CONCLUSIONS

A significant genetic variability was observed between different *Brassica* genotypes for salinity tolerance. Traits such as plant height, fresh weight and root length were promising to screen the genotypes. High salt tolerance was identified in Cluster I (8003, 25062, 25047 and UAF-11), while both Cluster I and the sensitive Cluster III (sensitive) showed potential for developing the best recombinants by strategic crossing and provides a practical roadmap for breeding programmes for improving crop stability and high yield under salinity stress. Therefore, future work should integrate these traits with transcriptomic and metabolomics analyses to further elucidate the molecular mechanism underlying salt tolerance. This integrative approach would facilitate marker-assisted selection and accelerate the development of Brassica cultivars with enhanced salinity tolerance.

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