



Assessment of salinity tolerance and trait associations in Indian mustard (*Brassica juncea* L.) under controlled salt stress conditions

Ankit Dhillon*, Neeraj Kumar, Ram Avtar, Amit Sharma, Shreya, Vishal Saini

Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar-125 004, Haryana, India

*Corresponding author: ankitdhill@gmail.com

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Abstract

Salinity poses a significant challenge to the cultivation of Indian mustard, especially during the seedling stage in salt-affected regions. One promising approach to overcome this limitation is the development of salt-tolerant varieties through plant breeding, supported by germplasm screening and genetic variability analysis. This study aimed to evaluate salinity tolerance and investigate the genetic variability among 48 Indian mustard genotypes at the seedling stage. The genotypes were grown in a greenhouse under controlled conditions and exposed to two levels of salt stress (8 dSm⁻¹ and 12 dSm⁻¹). Correlation analysis under controlled conditions showed significant positive correlation between shoot length and seedling dry weight, seedling length and seedling dry weight, shoot fresh weight and seedling dry weight, root fresh weight and seedling dry weight at 5 per cent level of significance. Path analysis under controlled conditions showed that seedling length has the maximum positive direct effect on seedling dry weight followed by root fresh weight then followed by germination, shoot fresh weight and seedling fresh weight. Under both salinity treatments, significant positive correlations were observed among most growth and biomass traits, whereas germination percentage was not significantly correlated with the other measured traits.

Keywords: *Brassica juncea* L., correlation and path analysis, genetic variability, salinity tolerance, seedling stage

Introduction

Brassica oilseeds hold a vital position in global agriculture, ranking as the third most significant source of edible oil after soybean and palm. Cultivated in over 50 countries, these crops are essential for both domestic consumption and international trade. In India, rapeseed-mustard is the second most important oilseed crop after soybean, contributing approximately 25% to the nation's total oilseed production (Yadav *et al.*, 2019; FAO 2011). Moreover, rapeseed-mustard constitutes around 26.6% of India's total oil meal output and nearly 20% of its oil meal exports, making India the largest global exporter of oil meal (Sharma 2017). Brassica oilseeds occupy 23.4% of India's total oilseed cultivation area and contribute 24.2% to the country's overall oilseed output (Jat *et al.*, 2019). Among the major producing states, Haryana plays a significant role, ranking fifth in area, second in production, and first in productivity (Anonymous, 2020-2021). Despite its strategic importance, mustard production in India remains insufficient to meet the increasing domestic demand for vegetable oils. The country currently imports nearly 60% of its edible oil, equivalent to approximately 13.88 million tonnes, fulfilling nearly two-thirds of national requirements at a cost of Rs. 1.22 trillion (Anonymous, 2021). With rising per capita

consumption, boosting mustard productivity has become a pressing agricultural and economic goal.

However, several biotic and abiotic stresses continue to hinder mustard cultivation, with soil salinity emerging as one of the most detrimental abiotic factors. Poor irrigation practices, excessive fertilizer use, and inadequate drainage systems are major contributors to salinization, affecting more than 800 million hectares of land worldwide—representing about 33% of global agricultural land and nearly 50% of irrigated land (Rengasamy 2010; Coskun *et al.*, 2016). Of this, approximately 95 million hectares have undergone permanent degradation (Szabolcs 1994). In India, nearly 6.74 million hectares are affected by salt stress (Kumar and Sharma 2020), and in Haryana alone, 0.23 million hectares out of 4.42 million hectares are salt-affected (Mandal and Sharma 2010). Salinity imposes complex stresses—ionic, osmotic, and oxidative—that severely impairs plant germination, growth, physiological function, and ultimately yield (Iturbe-Ormaetxe *et al.*, 1998). These challenges are particularly critical during the seedling stage, a sensitive phase in crop establishment. Therefore, the identification and development of salt-tolerant genotypes have become crucial components of sustainable mustard breeding programs. A thorough understanding of the genetic

variability and trait associations under salt stress conditions can facilitate the selection of promising genotypes, aiding the development of resilient varieties suited to saline environments.

This study aims to assess the salinity tolerance of 48 Indian mustard (*Brassica juncea* L.) genotypes at the seedling stage under controlled salt stress conditions and to analyze trait associations through correlation and path analysis. The findings are expected to contribute valuable insights for breeding programs focused on enhancing salt tolerance in Indian mustard.

Materials and Methods

This study was conducted in the greenhouse of the Department of Genetics and Plant Breeding at CCS Haryana Agricultural University, Hisar, India (29°10' N

latitude and 75°46' E longitude, with an elevation of 215.2 m above sea level). The plant material consisted of 48 genotypes of Indian mustard, obtained from the Oilseeds Section, Department of Genetics and Plant Breeding, CCS HAU, Hisar, and the Central Soil Salinity Research Institute, Karnal.

Experimental treatments

The experiment aimed to evaluate the effects of salinity stress on seed germination and early seedling development of the 48 mustard genotypes. Three salinity levels were applied: control (0 dSm⁻¹), 8 dSm⁻¹, and 12 dSm⁻¹. These salinity levels were selected after conducting four preliminary trials with varying electrical conductivities (ECs) from 2 dSm⁻¹ to 12 dSm⁻¹ (Table 1). The final salinity levels of 8 dSm⁻¹ and 12 dSm⁻¹ were chosen based on satisfactory germination results.

Table 1: Preparation of salt solution for salinity treatments

Concentration (dSm ⁻¹)	Salts	Quantity/L of water
8EC	NaCl	2.30 gm
	CaCl ₂	1.13 gm
	MgCl ₂	2.03 gm
	Na ₂ SO ₄	1.32 gm
12EC	NaCl	3.45 gm
	CaCl ₂	1.70 gm
	MgCl ₂	3.05 gm
	Na ₂ SO ₄	1.98 gm

Preparation of salinity solutions

Salinity solutions were prepared using a mixture of four salts: NaCl, CaCl₂, MgCl₂, and Na₂SO₄, dissolved in distilled water. For the 8 dSm⁻¹ solution, 2.30 g NaCl, 1.13 g CaCl₂, 2.03 g MgCl₂, and 1.32 g Na₂SO₄ were mixed in one liter of distilled water. Similarly, for the 12 dSm⁻¹ solution, 3.45 g NaCl, 1.70 g CaCl₂, 3.05 g MgCl₂, and 1.98 g Na₂SO₄ were dissolved in one liter of water. The electrical conductivity of each solution was verified using the Thermo Scientific Orion Star Benchtop pH and EC meter before being applied to the plants.

Experimental design, layout and trait assessment

The genotypes were evaluated using a completely randomized design (CRD) with three replications. Seedlings were irrigated with 10 ml of water at each salinity level (0 dSm⁻¹, 8 dSm⁻¹, and 12 dSm⁻¹) four times during the 15-day experimental period (at sowing, 4, 8, and 12 days after sowing). A total of 96 seedling trays, each with 40 slots, were used, and the experiment was terminated 15 days after sowing. The following traits were measured:

germination percentage, shoot length, root length, seedling length, root-to-shoot length ratio, shoot fresh weight, root fresh weight, seedling fresh weight, shoot dry weight, root dry weight, seedling dry weight.

Statistical analysis

Statistical analysis was performed using the R software and General R based Analysis Platform Empowered by Statistics (GRAPES).

Results and Discussion

The current study investigated the salinity tolerance and associated trait relationships among 48 Indian mustard (*Brassica juncea* L.) genotypes under controlled and salt stress conditions (8 dS/m and 12 dS/m). The findings provide valuable insights into the physiological and genetic mechanisms underlying early-stage salinity tolerance and reveal important considerations for future breeding strategies.

Correlation analysis under control conditions

Correlation analysis among nine seedling traits of 48 Indian mustard genotypes under controlled conditions

Table 2: Correlation matrix among seedling traits under control conditions

Traits	GER	SL	RL	SDL	RL/SL	SFW	RFW	SDFW	SDDW
GER	1	-0.081	0.058	0.012	0.097	0.139	0.09	0.14	0.187
SL	-0.081	1	0.527***	0.766***	0.11	0.223	-0.08	0.104	0.328*
RL	0.058	0.527***	1	0.929***	0.868***	0.199	-0.051	0.103	0.261
SDL	0.012	0.766***	0.929***	1	0.721***	0.246	-0.033	0.145	0.331*
RL/SL	0.097	0.11	0.868***	0.721***	1	0.136	0.021	0.101	0.155
SFW	0.139	0.223	0.199	0.246	0.136	1	0.279	0.822***	0.319*
RFW	0.09	-0.08	-0.051	-0.033	0.021	0.279	1	0.776***	0.304*
SDFW	0.14	0.104	0.103	0.145	0.101	0.822***	0.776***	1	0.394**
SDDW	0.187	0.328*	0.261	0.331*	0.155	0.319*	0.304*	0.394**	1

*** Correlation is significant at 0.001 level (two tailed); ** Correlation is significant at 0.01 level (two tailed); * Correlation is significant at 0.05 level (two tailed); GER- germination, SL- shoot length, RL- root length, SDL- seedling length, RL/SL- root length/shoot length, SFW- shoot fresh weight, RFW- root fresh weight, SDFW- seedling fresh weight, SDDW- seedling dry weight

revealed several significant interrelationships, particularly among growth and biomass parameters (Table 2). Shoot length exhibited a strong positive correlation with seedling length ($r = 0.766$, $p < 0.001$), whereas root length was highly correlated with both seedling length ($r = 0.929$, $p < 0.001$) and the root length/shoot length ratio ($r = 0.868$, $p < 0.001$), indicating a coordinated development between shoot and root systems. Shoot fresh weight and root fresh weight were positively associated with seedling fresh weight ($r = 0.822$ and $r = 0.776$, respectively; $p < 0.001$), suggesting their combined contribution to biomass accumulation. Seedling dry weight showed moderate positive correlations with shoot length, seedling length, shoot fresh weight, and root fresh weight (ranging from 0.304 to 0.394), all significant at either the 0.05 or 0.01 level. In contrast, germination exhibited weak and mostly non-significant correlations with other traits, indicating that seedling biomass traits under non-saline conditions may be influenced independently of initial germination ability.

Under control (non-saline) conditions, significant positive correlations were observed among growth and biomass traits, notably between shoot length, root length, seedling length, and biomass weights (shoot fresh weight, root fresh weight, and seedling fresh weight). These findings align with previous studies, such as those by Gyawali *et al.* (2019) and Kandil (2012) who also reported strong associations between seedling length and biomass accumulation traits under optimal growing conditions in mustard and other Brassica species. The coordinated development between shoot and root systems, as reflected by the high correlation between root length and seedling length, suggests a balanced allocation of resources during early growth, which is crucial for seedling vigour.

Path coefficient analysis under control conditions

Path coefficient analysis further clarified the relationships influencing seedling dry weight under control conditions (Table 2). Seedling fresh weight exhibited the highest direct positive effect (1.520) on seedling dry weight, followed by shoot fresh weight (1.247) and root fresh weight (1.186). Seedling length also had a considerable positive direct effect (0.583), emphasizing its role in seedling vigor. In contrast, shoot length (-0.803) and root length (-0.174) had negative direct effects but contributed positively via indirect effects through biomass traits. Germination exerted a small positive direct effect (0.174), whereas the root-to-shoot ratio contributed modestly (0.605), mainly through indirect pathways. Collectively, these findings highlight seedling fresh weight as the most crucial trait determining seedling dry weight under non-stress conditions.

Path coefficient analysis further clarified that seedling fresh weight had the highest positive direct effect on seedling dry weight, followed by shoot fresh weight and root fresh weight. This pattern emphasizes the importance of biomass traits over mere elongation traits (e.g., shoot or root length) in determining seedling dry matter accumulation. These results are consistent with findings in Brassica species reported by Ashraf and McNeilly (1990; 2004), who suggested that fresh biomass traits are more predictive of plant performance under both normal and stress conditions compared to shoot elongation alone. Interestingly, despite positive correlations, shoot length and root length had negative direct effects on seedling dry weight, suggesting indirect pathways of influence via fresh weights. This finding highlights a novel aspect

Table 3: Path coefficient analysis for seedling dry weight under control conditions

Traits	GER	SL	RL	SDL	R/S	SFW	RFW	SDFW
GER	0.173933	-0.06426	-0.01042	-0.00583	0.060543	-0.11954	-0.05729	0.21286
SL	-0.01391	0.803289	-0.09201	-0.44908	0.066597	-0.18784	0.050923	0.152043
RL	0.010436	0.425743	-0.17361	-0.5424	0.526724	-0.17077	0.031827	0.152043
SDL	0.001739	0.618533	-0.16146	-0.58323	0.435909	-0.21346	0.019096	0.21286
R/S	0.017393	0.088362	-0.15104	-0.41992	0.60543	-0.11954	-0.01273	0.152043
SFW	0.024351	0.176724	-0.03472	-0.14581	0.08476	-0.85383	-0.17823	1.246754
RFW	0.015654	-0.06426	0.00868	0.017497	0.012109	-0.23907	-0.63654	1.185937
SDFW	0.024351	0.080329	-0.01736	-0.08165	0.060543	-0.70014	-0.4965	1.520432

GER- germination, SL- shoot length, RL- root length, SDL- seedling length, RL/SL- root length/shoot length, SFW- shoot fresh weight, RFW- root fresh weight, SDFW- seedling fresh weight, SDDW- seedling dry weight

not commonly emphasized in earlier mustard studies and suggests that selection purely for elongation traits without considering biomass may not yield salt-tolerant phenotypes.

Genetic diversity analysis under control conditions

The dendrogram based on the genetic distance among 48 Indian mustard genotypes revealed six distinct clusters (Fig 1). Cluster I grouped closely related genotypes such as RH 725, RH 2069, and RH 2065. Cluster II contained genotypes like RGN 486 and RH 1384, representing a separate genetic lineage. Cluster III included RH 761, RH 8812, and NPJ 244, showing moderate diversity. The largest and most genetically diverse group was Cluster IV, which included elite lines like DRMRIC 128 and RH(OE) 1808. Varuna and RH 2054 formed an isolated Cluster V, while NRC-derived varieties formed Cluster VI. Notably, BPR 543-2 emerged as a distinct outlier within Cluster III, indicating its potential as a unique donor in breeding programs.

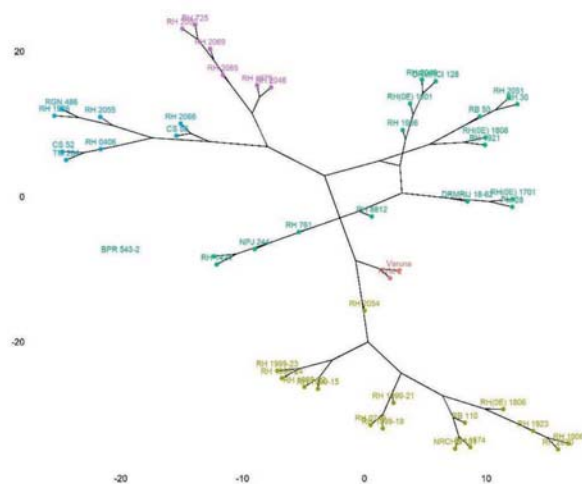


Fig. 1: Dendrogram showing genetic relationships among 48 Indian mustard genotypes under control conditions

Correlation analysis under salt stress (8 dS/m)

Under salt stress (8 dS/m), strong positive correlations among seedling traits were observed (Table 4). Germination continued to show weak correlations. Shoot length had significant positive correlations with root length ($r = 0.564^{***}$), seedling length ($r = 0.786^{***}$), shoot fresh weight ($r = 0.665^{***}$), seedling fresh weight ($r = 0.643^{***}$), and seedling dry weight ($r = 0.729^{***}$). Root length was strongly associated with seedling length ($r = 0.954^{***}$), shoot fresh weight, root fresh weight, and seedling dry weight. Seedling length, as a composite trait, showed the strongest correlations with biomass-related parameters. Biomass traits (SFW, RFW, and SDFW) were closely interrelated, with SFW and SDFW showing the highest correlation ($r = 0.851^{***}$), confirming that biomass traits are key indicators of salt stress tolerance.

A particularly interesting finding was the weak and non-significant correlations between germination and subsequent growth traits under both stress levels. This supports earlier observations by Alam et al., (2021), who suggested that germination tolerance under salinity is genetically independent of post-germination growth performance in tomato. Thus, for breeding programs, selection based on germination percentage alone may be insufficient to predict seedling vigour under salinity.

Path coefficient analysis under salt stress (8 dS/m)

Path coefficient analysis under salt stress conditions (Table 5) revealed shoot fresh weight (SFW) as the most influential trait with the highest positive direct effect (0.5732) on seedling fresh weight. Root fresh weight (RFW) also contributed positively (1.0053). In contrast, shoot length and root length exhibited negative direct effects (-0.9963 and -0.1996, respectively), despite positive correlations in bivariate analysis. Germination had a negative direct effect but showed positive indirect effects

Table 4: Correlation matrix among seedling traits under salt stress (8 dS/m)

Traits	GER	SL	RL	SDL	RL/SL	SFW	RFW	SDFW	SDDW
GER	1	0.035	0.052	0.052	0.052	0.019	0.155	0.105	0.016
SL	0.035	1	0.564***	0.786***	0.039	0.665***	0.416**	0.643***	0.729***
RL	0.052	0.564***	1	0.954***	0.579***	0.582***	0.442**	0.61***	0.689***
SDL	0.052	0.786***	0.954***	1	0.448**	0.678***	0.482***	0.69***	0.78***
RL/SL	0.052	0.039	0.579***	0.448**	1	0.238	0.379**	0.367*	0.403**
SFW	0.019	0.665***	0.582***	0.678***	0.238	1	0.428**	0.851***	0.675***
RFW	0.155	0.416**	0.442**	0.482***	0.379**	0.428**	1	0.839***	0.547***
SDFW	0.105	0.643***	0.61***	0.69***	0.367*	0.851***	0.839***	1	0.726***
SDDW	0.016	0.729***	0.689***	0.78***	0.403**	0.675***	0.547***	0.726***	1

*** Correlation is significant at 0.001 level (two tailed); ** Correlation is significant at 0.01 level (two tailed); * Correlation is significant at 0.05 level (two tailed); GER- germination, SL- shoot length, RL- root length, SDL- seedling length, RL/SL- root length/shoot length, SFW- shoot fresh weight, RFW- root fresh weight, SDFW- seedling fresh weight, SDDW- seedling dry weight

through RFW. Overall, biomass traits (especially SFW and RFW) played the most decisive roles under saline conditions. Under moderate salt stress (8 dS/m), positive correlations among biomass traits remained strong. Shoot length maintained significant positive associations with root length, seedling length, and biomass weights, similar to trends observed under control conditions. Previous

research, including Biswas et al., (2016), has emphasized that maintaining shoot and root growth under salinity is critical for crop tolerance. In the present study, shoot fresh weight showed the strongest direct positive effect on seedling fresh weight under salinity, followed by root fresh weight, highlighting the central role of biomass accumulation in salinity tolerance.

Table 5: Path coefficient analysis for seedling fresh weight under salt stress (8 dS/m)

Traits	GER	SL	RL	SDL	R/S	SFW	RFW	SDFW
GER	-0.13229	-0.5578	-0.73145	0.406992	-0.02704	0.326414	1.560842	-0.65567
SL	-0.00397	-0.59338	-0.39226	0.030477	-0.02163	0.634859	0.622211	-0.9963
RL	-0.00661	-0.41229	-0.62904	0.732853	-0.21361	0.665998	0.742316	-0.1996
SDL	-0.00661	-0.38877	-0.49759	0.739845	-0.24332	0.798066	0.682527	-0.32414
R/S	-0.00661	-0.74374	-0.08484	0.66293	-0.54071	0.916965	0.682	-0.42599
SFW	-0.00265	-0.45757	-0.48484	0.735095	-0.52977	0.320686	0.482264	0.573215
RFW	-0.02117	-0.80922	-0.23678	0.107126	-0.30547	0.017895	1.005264	-0.30765
SDFW	-0.01323	-0.89977	0.876	0.216493	-0.20006	0.872583	0.444422	-0.55672

GER- germination, SL- shoot length, RL- root length, SDL- seedling length, RL/SL- root length/shoot length, SFW- shoot fresh weight, RFW- root fresh weight, SDFW- seedling fresh weight, SDDW- seedling dry weight

Genetic diversity analysis under salt stress (8 dS/m)

The dendrogram generated under salt stress (8 dS/m) illustrated five major clusters among 60 genotypes (Fig 2). Cluster I comprised BPR 543-2 and elite genotypes like RH 2045 and Varuna, while Clusters II and III included genetically distinct lines like RH 725 and DRMRIC 128, respectively. Cluster IV included highly divergent genotypes such as CS 52 and RH 0406. These patterns suggest significant potential for identifying diverse and tolerant genotypes for breeding programs.

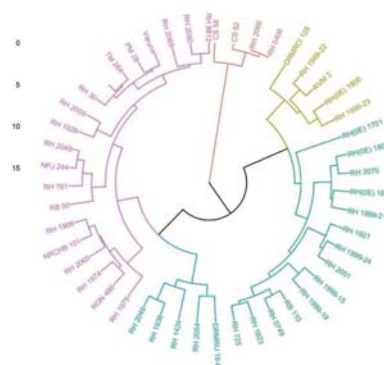


Fig. 2: Dendrogram showing genetic clustering of 60 Indian mustard genotypes under salt stress (8 dS/m).

Correlation analysis under severe salt stress (12 dS/m)

Under severe salinity stress (12 dS/m), significant positive correlations among major seedling traits were observed (Table 6). Shoot length remained highly correlated with root length ($r = 0.693^{***}$), seedling length ($r = 0.866^{***}$),

and seedling fresh weight ($r = 0.595^{***}$). Root length was strongly associated with biomass traits, particularly SDFW ($r = 0.703^{***}$). Fresh biomass accumulation continued to dominate seedling performance, with SFW and RFW strongly correlated with SDFW ($r = 0.936^{***}$ and $r = 0.835^{***}$, respectively). Germination maintained weak associations with other traits.

Table 6: Correlation matrix among seedling traits under severe salt stress (12 dS/m)

Traits	GER	SL	RL	SDL	RL/SL	SFW	RFW	SDFW	SDDW
GER	1	0.272	0.027	0.124	-0.152	0.023	0.229	0.111	0.171
SL	0.272	1	0.693***	0.866***	-0.247	0.512***	0.559***	0.595***	0.526***
RL	0.027	0.693***	1	0.961***	0.276	0.594***	0.67***	0.703***	0.523***
SDL	0.124	0.866***	0.961***	1	0.096	0.609***	0.68***	0.717***	0.566***
RL/SL	-0.152	-0.247	0.276	0.096	1	0.059	0.133	0.105	0.082
SFW	0.023	0.512***	0.594***	0.609***	0.059	1	0.59***	0.936***	0.627***
RFW	0.229	0.559***	0.67***	0.68***	0.133	0.59***	1	0.835***	0.478***
SDFW	0.111	0.595***	0.703***	0.717***	0.105	0.936***	0.835***	1	0.633***
SDDW	0.171	0.526***	0.523***	0.566***	0.082	0.627***	0.478***	0.633***	1

*** Correlation is significant at 0.001 level (two tailed); ** Correlation is significant at 0.01 level (two tailed); * Correlation is significant at 0.05 level (two tailed); GER- germination, SL- shoot length, RL- root length, SDL- seedling length, RL/SL- root length/shoot length, SFW- shoot fresh weight, RFW- root fresh weight, SDFW- seedling fresh weight, SDDW- seedling dry weight

Path coefficient analysis under severe salt stress (12 dS/m)

Path analysis under high salt stress revealed that seedling length (SDL), root length (RL), and shoot length (SL) had the strongest direct positive effects on seedling dry weight, with coefficients of 1.961, 1.344, and 1.101, respectively (Table 7). Among biomass traits, shoot fresh weight (1.481) had the highest direct contribution to seedling dry weight, affirming its importance in salinity tolerance. The model explained approximately 74% of the variance in seedling dry weight, demonstrating a strong relationship between selected traits and salt stress response.

At a higher stress level (12 dS/m), the relationships among seedling traits remained robust, although certain associations weakened slightly compared to 8 dS/m. Seedling length emerged as the trait with the highest direct positive effect on seedling dry weight, followed closely by root length and shoot length. Shoot fresh weight also maintained a significant positive contribution to seedling biomass. These findings are consistent with studies on other salt-tolerant crops like barley and wheat (Munns *et al.*, 2006; Bagwasi *et al.*, 2020), where maintenance of biomass production under salinity was key to tolerance. A notable novel trend observed was the consistent performance of biomass traits (especially shoot and seedling fresh weight) as the most critical determinants

Table 7: Path coefficient analysis for seedling dry weight under severe salt stress (12 dS/m)

Traits	GER	SL	RL	SDL	R/S	SFW	RFW	SDFW	Residual
GER	0.088029	0.297306	0.040308	-0.23532	-0.02528	0.029621	0.135486	-0.16015	0.508467
SL	0.023768	1.101133	0.927089	-1.70609	-0.04213	0.755327	0.329878	-0.85898	0.508467
RL	0.002641	0.759782	1.343608	-1.88258	0.047188	0.87381	0.394676	-1.01913	0.508467
SDL	0.010564	0.957986	1.289863	-1.96102	0.016853	0.90343	0.400566	-1.04824	0.508467
R/S	-0.0132	-0.27528	0.37621	-0.1961	0.168528	0.088862	0.076579	-0.14559	0.508467
SFW	0.001761	0.561578	0.792728	-1.19622	0.010112	1.481033	0.34755	-1.36854	0.508467
RFW	0.020247	0.616635	0.900217	-1.33349	0.021909	0.87381	0.589068	-1.20839	0.508467
SDFW	0.009683	0.649669	0.940525	-1.41193	0.016853	1.392171	0.488927	-1.45589	0.508467

GER- germination, SL- shoot length, RL- root length, SDL- seedling length, RL/SL- root length/shoot length, SFW- shoot fresh weight, RFW- root fresh weight, SDFW- seedling fresh weight, SDDW- seedling dry weight

of salinity tolerance across both stress levels. This trend has not been emphasized as strongly in previous Indian mustard studies, suggesting that breeding programs focusing on shoot and seedling biomass under stress could achieve greater gains in salt-affected environments.

Genetic diversity analysis under severe salt stress (12 dS/m)

The dendrogram (Fig 3) based on genetic distance under severe salt stress grouped 60 genotypes into five major clusters. Cluster I included elite and diverse lines like BPR 543-2, Varuna, and RH 2045, whereas other clusters contained highly divergent lines such as CS 52 and RH 0406. The heatmap (Fig 4) visually illustrated these genetic relationships, highlighting BPR 543-2 as the most genetically distinct genotype, reinforcing its potential utility in breeding programs aimed at enhancing salt stress tolerance.

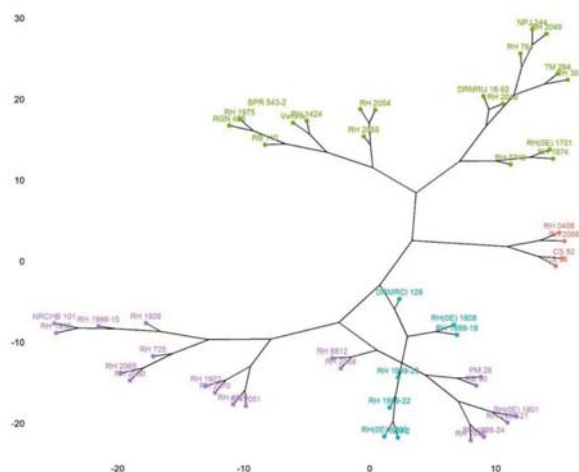


Fig. 3: Dendrogram of 60 Indian mustard genotypes under severe salt stress (12 dS/m)

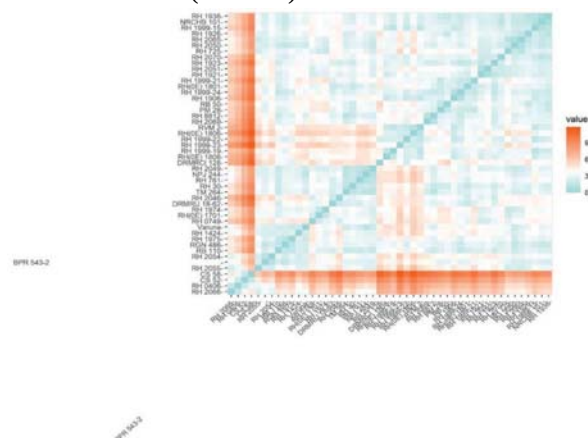


Fig. 4: Heatmap illustrating genetic divergence among Indian mustard genotypes under severe salt stress

Genetic diversity analysis revealed substantial clustering of genotypes under both control and stress conditions. The separation of distinct groups, particularly the consistent genetic divergence of BPR 543-2, suggests the presence of unique salinity tolerance mechanisms in certain genotypes. This supports the findings of Kumar and Saddhe (2018) and Kumawat *et al.*, (2024), who reported significant genetic variability for salt tolerance traits in mustard germplasm. The identification of genetically distant lines offers valuable material for hybridization programs to broaden the genetic base and enhance tolerance. The findings of the present study are further supported by the recent work of Verma *et al.*, (2023), who emphasized that salt stress in mustard causes a significant reduction in morpho-physiological traits, closely aligning with the patterns observed in our seedling growth traits under both moderate and severe salinity. Singh *et al.*, (2019) reported strong genotype $\times$ environment interactions and highlighted the importance of multi-trait indices for selecting salt-tolerant genotypes. Similarly, in our study, traits related to seedling biomass, particularly seedling fresh weight and shoot fresh weight, consistently demonstrated strong direct effects on seedling dry weight, confirming that multiple growth parameters must be considered simultaneously to effectively identify salt-tolerant mustard genotypes. Moreover, the observed genetic variability and clear clustering among genotypes in our study resonate with the high heritability and variation patterns reported by Singh *et al.*, (2019) further strengthening the argument for exploiting diverse genetic resources for salinity tolerance improvement in Indian mustard.

In summary, the study not only reaffirms the established importance of seedling biomass accumulation in salinity tolerance but also reveals novel insights, particularly the indirect and limited role of shoot and root length under salt stress, and the minor contribution of germination traits to later growth performance. These results emphasize the need to prioritize biomass traits over elongation traits in selecting and breeding salt-tolerant mustard varieties.

Conclusion

The present study highlights the critical role of seedling biomass traits, particularly seedling fresh weight and shoot fresh weight, as key determinants of salinity tolerance in Indian mustard. Strong positive correlations among growth and biomass traits under both control and salt stress conditions emphasize the importance of integrated trait performance over reliance on individual elongation traits like shoot or root length. Path coefficient analysis further revealed that biomass accumulation has

a more direct influence on seedling dry weight than germination ability or shoot elongation, especially under salt stress conditions. Significant genetic variability observed among genotypes, and the identification of distinct salt-tolerant lines such as BPR 543-2, offer promising opportunities for breeding programs aimed at enhancing salinity resilience. Overall, the findings underscore that breeding strategies should prioritize biomass traits for the effective development of salt-tolerant Indian mustard cultivars suited to saline environments.

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