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Genetic variability in Indian mustard for salt stress tolerance at seedling stage

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Abstract

Salinity is a major limitation to the cultivation of Indian mustard, particularly during the seedling stage in salt-affected areas. Developing salt-tolerant lines through plant breeding, supported by germplasm screening and genetic variability assessments, is a key strategy to enable cultivation in these regions. This study aimed to evaluate salinity tolerance and assess the genetic variability among 48 genotypes of Indian mustard at the seedling stage. The genotypes were grown in a greenhouse under control conditions and subjected to 8 dSm^{-1} and 12 dSm^{-1} salt stress. A two-way analysis of variance revealed highly significant ($p \leq 0.01$) effects of genotype, salinity level, and genotype x salinity interaction on most traits. Among the genotypes, RH 2066 and RH 0406 demonstrated a high degree of salt tolerance, comparable to known salinity-tolerant checks CS 52 and CS 58, at both stress levels based on morpho-physiological and biochemical parameters. Many traits, such as shoot length, root length, seedling length, fresh and dry weights, total soluble sugar content, and seedling vigour indices I and II, exhibited high genotypic and phenotypic variation. These traits also showed high broad-sense heritability and moderate to high genetic advance as a percentage of the mean, indicating their potential utility for breeding programs aimed at enhancing salinity tolerance in Indian mustard.

Keywords: *Salinity tolerance, Indian mustard, genetic variability, seedling stage, heritability*

Introduction

Brassica oilseeds are the third most important source of edible oil globally, following soybean and palm, and are cultivated in over 50 countries. In India, rapeseed-mustard ranks second only to soybean in terms of oilseed crops (FAO, 2011), contributing around 25% of the country's total oilseed production (Yadav *et al.*, 2019). Rapeseed-mustard also accounts for approximately 26.6% of India's total oil meal output and nearly 20% of the country's oil meal exports, positioning India as the world's largest exporter of oil meal (Sharma, 2017). In terms of cultivation, Brassica oilseeds occupy 23.4% of the total oilseed area and contribute to 24.2% of the total oilseed production in India (Jat et al*.,* 2019). Among Indian states, Haryana is a leading producer of mustard, ranking fifth in area (0.647 mha), second in production (1.31 million tons), and first in productivity (20.28 q/ha) (Anonymous, 2020-2021).

Despite its significance, India's mustard production is currently insufficient to meet the growing domestic demand for vegetable oils. To bridge this gap, the country imports nearly 60% of its vegetable oil (approximately 13.88 million tons), representing two-thirds of its edible oil requirements, at a cost of Rs 1.22 trillion (Anonymous, 2021). As per capita consumption of edible oil rises, there is a pressing need to increase mustard crop yields to meet national targets. However, production is often constrained by various biotic and abiotic stresses, with salt stress being a major abiotic challenge. Inappropriate irrigation and poor drainage practices are among the main causes of soil salinity, which affects over 800 million hectares globally, representing up to 33% of all agricultural land and nearly 50% of all irrigated land, the latter of which supplies about one-third of the world's food (Rengasamy, 2010; Coskun *et al*., 2016). High salinity levels have degraded approximately 95 million hectares of agricultural land worldwide (Szabolcs, 1994), with India having around 6.74 million hectares of salt-affected soils (Kumar and Sharma, 2020). In Haryana alone, 0.23 million hectares out of 4.42 million hectares are salt-affected (Mandal and Sharma, 2010), significantly impacting crop productivity and causing detrimental effects on germination, growth, physiology, and yield due to ionic, osmotic, and oxidative stresses (Iturbe-Ormaetxe *et al.*, 1998).

Salinity disrupts the photosynthetic process by damaging chloroplast thylakoid membranes, deactivating the electron transport system (ETS), and reducing photophosphorylation efficiency. It also lowers the concentration of chlorophyll and other photosynthetic pigments like carotenoids (Omoto *et al.*, 2010; Evelin *et al.*,

2012; Mittal *et al*., 2012). In Brassica oilseeds, salt stress negatively affects plant height, size, and yield, as well as the quality of the product. Under high salinity (EC 8 to 12 dS/m), plant dry weight declines substantially, with maximum weight recorded in lower salinity treatments (EC 4 dS/m) (Parti *et al.,* 2003).

The spatial and temporal variability in both resources and abiotic stressors creates significant environmental challenges for plant growth. Salt and osmotic stresses, in particular, are responsible for delayed and inhibited seed germination and seedling establishment (Almansouri *et al.*, 2001), impacting all stages of plant development, from germination through vegetative growth and reproduction (Machado and Serralheiro, 2017). However, it has been observed that once seedlings emerge in saline soils, they can continue growing to maturity with minimal yield loss. To develop salt-tolerant lines, rapid screening techniques have been developed to identify tolerant genotypes at the seedling stage under controlled conditions. These genotypes are then used in breeding programs to enhance salt tolerance. Tolerance in *Brassica juncea* at the seedling stage is often associated with traits such as higher shoot and root fresh and dry weight, minimal reduction in germination rates, and increased proline and soluble sugar accumulation under salinity stress.

Understanding genetic diversity is critical for any breeding program, and it plays a key role in sustaining plant populations. This study focuses on evaluating the genetic variability of Indian mustard at the seedling stage for salt stress tolerance, aiming to identify promising genotypes for future breeding efforts.

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 (Table 1).

Table 1: List of genotypes used in this study

Sr. No.	Genotypes	Source
1.	BPR 543-2	DRMR, Bharatpur/ India
2.	CS 52	CSSRI, Karnal/India
3.	CS 58	CSSRI, Karnal/India
4.	RGN 486	ARS, Sri Ganganagar/ India
5.	NPJ 244	IARI, New Delhi/ India
6.	DRMRIJ 18-62	DRMR, Bharatpur/ India
7.	DRMRCI 128	DRMR, Bharatpur/ India
8.	TM 264	BARC, Mumbai/ India
9.	RB 110	RRS Bawal, CCS HAU, Hisar/ India
10.	NRCHB 101	DRMR, Bharatpur/ India
11.	PM 28	IARI, New Delhi/ India
12.	RB 50	RRS Bawal, CCS HAU, Hisar/ India
13.	RH(0E) 1701	CCS HAU, Hisar/ India
14.	RH(0E) 1801	CCS HAU, Hisar/ India
15.	RH(0E) 1806	CCS HAU, Hisar/ India
16.	RH(0E) 1808	CCS HAU, Hisar/ India
17.	RH 1424	CCS HAU, Hisar/ India
18.	RH 1906	CCS HAU, Hisar/ India
19.	RH 1921	CCS HAU, Hisar/ India
20.	RH 1923	CCS HAU, Hisar/ India
21.	RH 1926	CCS HAU, Hisar/ India
22.	RH 1936	CCS HAU, Hisar/ India
23.	RH 1974	CCS HAU, Hisar/ India
24.	RH 1975	CCS HAU, Hisar/ India
25.	RH 1999-15	CCS HAU, Hisar/ India
26.	RH 1999-19	CCS HAU, Hisar/ India
27.	RH 1999-21	CCS HAU, Hisar/ India

28.	RH 1999-22	CCS HAU, Hisar/ India
29.	RH 1999-23	CCS HAU, Hisar/ India
30.	RH 1999-24	CCS HAU, Hisar/ India
31.	RH 2046	CCS HAU, Hisar/ India
32.	RH 2049	CCS HAU, Hisar/ India
33.	RH 2050	CCS HAU, Hisar/ India
34.	RH 2051	CCS HAU, Hisar/ India
35.	RH 2054	CCS HAU, Hisar/ India
36.	RH 2055	CCS HAU, Hisar/ India
37.	RH 2065	CCS HAU, Hisar/ India
38.	RH 2066	CCS HAU, Hisar/ India
39.	RH 2069	CCS HAU, Hisar/ India
40.	RH 2070	CCS HAU, Hisar/ India
41.	RH 30	CCS HAU, Hisar/ India
42.	RH 0406	CCS HAU, Hisar/ India
43.	RH 725	CCS HAU, Hisar/ India
44.	RH 0749	CCS HAU, Hisar/ India
45.	RH 761	CCS HAU, Hisar/ India
46.	RH 8812	CCS HAU, Hisar/ India
47.	RVM 2	RVSKVV, Gwalior, MP/ India
48.	Varuna	CSAUA&T, Kanpur/ India

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 2). The final salinity levels of 8 dSm^{-1} and 12 dSm^{-1} were chosen based on satisfactory germination results.

Table 2: Preparation of salt solution for salinity treatments:

Concentration (dSm^{-1})	Salts	Quantity (in 1 litre of water)
8 EC		
	NaCl	2.30 gm
	$CaCl_2$	1.13 gm
	$MgCl_2$	2.03 gm
	Na_2SO_4	1.32 gm
12 EC		
	NaCl	3.45 gm
	$CaCl_2$	1.70 gm
	$MgCl_2$	3.05 gm
	Na_2SO_4	1.98 gm

Preparation of salinity solutions

Salinity solutions were prepared using a mixture of four salts: NaCl, $CaCl_2$, $MgCl_2$, and Na_2SO_4, dissolved in distilled water. For the 8 dSm^{-1} solution, 2.30 g NaCl, 1.13 g $CaCl_2$, 2.03 g $MgCl_2$, and 1.32 g Na_2SO_4 were mixed in 1 liter of distilled water. Similarly, for the 12 dSm^{-1} solution, 3.45 g NaCl, 1.70 g $CaCl_2$, 3.05 g $MgCl_2$, and 1.98 g Na_2SO_4 were dissolved in 1 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 and layout

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}, 8dSm^{-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.

Trait assessment

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, water content, seedling vigour index I and II, total soluble sugars (Hansen and Moller, 1975), and proline content (Bates *et al.*, 1973). Seedling vigour indices were calculated as per ISTA (2001) guidelines:

Seedling Vigour Index I (SVI-I) = (Root Length + Shoot Length) × (Germination Percentage)

Seedling Vigour Index II (SVI-II) = (Seedling Dry Weight) × (Germination Percentage)

Statistical analysis

The data were analyzed using a two-factorial CRD test. The effects of treatments, genotypes, and their interaction were compared using critical difference (CD) at a 5% level of significance. Statistical analysis was performed using the OP-STAT online portal, CCS HAU, Hisar.

Results and Discussion

The two-way analysis of variance revealed highly significant effects ($p \leq 0.01$) of salinity levels, genotypes, and their interactions on most of the traits studied (Table 3).This indicates that both salinity stress and genetic background play crucial roles in influencing the phenotypic expression of traits in Indian mustard. The significant interaction effects suggest that the response of each genotype to varying salinity levels is not uniform, highlighting the complexity of salinity tolerance in these genotypes.

The phenotypic coefficient of variation (PCV) was consistently higher than the genotypic coefficient of variation (GCV) across traits and salinity levels, highlighting the influence of environmental factors on trait expression (Table 4). In this study, the PCV was slightly higher than the GCV at all salinity levels. GCV exhibited an increasing trend for most traits, except for root length, seedling length, total soluble sugar, and proline content. While root length and seedling length showed fluctuations in GCV, total soluble sugar and proline content increased with higher salinity levels. Notably, traits such as root fresh weight, seedling vigour index-II, and seedling dry weight displayed significantly higher GCV in the control (T_0) treatment. In the 8 dSm^{-1} salinity treatment (T_1), root fresh weight, seedling fresh weight, seedling dry weight, and seedling vigour index-II exhibited high GCV. In the 12 dSm^{-1} salinity treatment (T_2), traits such as shoot fresh weight, root fresh weight, seedling fresh weight, seedling dry weight, and seedling vigour index-II showed a pronounced increase in GCV. Similarly, significant high PCV values were observed for root fresh weight, seedling dry weight, total soluble sugar, and seedling vigourIndex-I under the control treatment (T_0). In the T_1 treatment, shoot fresh weight, root fresh weight, seedling fresh weight, seedling dry weight, and seedling vigour index-II exhibited higher PCV, while the T_2 treatment showed high PCV values for shoot fresh weight, root fresh weight, seedling fresh weight, seedling dry weight, seedling vigourIndex-I, and seedling vigour index-II. These findings align with previous studies by Das *et al*., (2001), Singh *et al*., (2007), and Singh *et al*., (2009).

However, it is important to note that GCV and PCV alone

Table 3: Two-way analysis of variance for various seedling traits in Indian mustard

SV	df	Mean squares												
		GN	SL	RL	SDL	R/S	SFW	RFW	SDFW	SDW	WC	TSS	PRO	SVI-I	SVI-II
Salinity level	2	3390.11**	230.39**	221.85**	903.36**	8.34**	95003.87**	225510.30**	613183.26**	538.58**	99.76**	15955.09**	16908.10**	12951424.90**	7369866.80**
Genotypes	47	121.15**	2.03**	11.57**	20.67**	0.17**	4203.82**	2589.47**	11078.54**	42.13**	6.24**	77.55**	47.91**	235009.56**	435811.77**
G x S	94	59.37**	0.70**	1.99**	3.55**	0.06**	575.63**	1225.70**	1939.67**	5.70**	2.52**	41.18**	35.86**	45186.38**	64026.12**
Error	288	24.23	0.08	0.19	0.27	0.01	130.88	28.95	141.55	0.52	0.29	1.84	2.46	6202.90	6328.88

*Significant at $p \leq 0.05$ and **Significant at $p \leq 0.01$; SV-Source of variation, df- degree of freedom, GN-Germination (%), SL-Seedling Length (cm), RL-Root Length (cm), SDL-Seedling Length (cm), R/S-Root to shoot ratio, SFW-Shoot fresh weight (mg), RFW- Root fresh weight (mg), SDFW- Seedling fresh weight (mg), SDW-Seedling dry weight (mg), WC- Water content, TSS- Total soluble sugar (µg GE g^{-1}DW), PRO- Proline content (µg g $^{-1}$DW), SVI-I- Seedling Vigour Index-I, SVI II- Seedling Vigour Index-II

Table 4: Genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) for various seedling traits in Indian mustard

Traits	Genotypic coefficient of variation (GCV)			Phenotypic coefficient of variation (PCV)		
	T_0	T_1	T_2	T_0	T_1	T_2
Germination (%)	2.74	3.76	7.20	4.32	6.23	10.44
Shoot length (cm)	8.57	8.88	13.30	9.15	10.50	14.80
Root length (cm)	17.79	15.57	18.55	18.70	16.58	19.69
Seedling length (cm)	12.43	11.43	15.27	12.84	12.19	15.96
Root/shoot	14.42	14.70	12.08	16.01	17.35	15.11
Shoot fresh weight (mg)	14.77	18.02	24.36	17.28	20.19	25.63
Root fresh weight (mg)	28.52	48.64	65.09	29.63	49.39	65.58
Seedling fresh weight (mg)	16.74	24.34	27.65	17.71	25.25	28.38
Seedling dry weight (mg)	25.55	24.19	26.40	26.61	25.37	27.65
Water content (%)	0.90	0.98	1.44	1.00	1.16	1.58
Total soluble sugar (µg GE g^{-1} DW)	23.55	16.17	12.36	24.50	16.82	13.26
Proline content (µg g^{-1} DW)	18.28	15.65	10.30	18.38	15.84	10.93
Seedling vigour index-I	12.43	12.92	19.14	12.95	14.47	21.29
Seedling vigour index-II	25.67	25.76	30.70	26.80	27.17	32.65

Table 5: Heritability (broad sense) and Genetic advance as per cent of mean for various seedling traits in Indian mustard

Traits	$Heritability_{(bs)}$			Genetic advance as per cent of mean		
	T_0	T_1	T_2	T_0	T_1	T_2
Germination (%)	40.35	36.47	47.56	3.59	4.68	10.23
Shoot length (cm)	87.63	71.44	80.75	16.52	15.46	24.63
Root length (cm)	90.53	88.14	88.69	34.88	30.11	35.99
Seedling length (cm)	93.63	87.85	91.48	24.77	22.07	30.09
Root/shoot	81.11	71.81	63.94	26.76	25.67	19.91
Shoot fresh weight (mg)	73.09	79.70	90.34	26.02	33.15	47.70
Root fresh weight (mg)	92.67	96.95	98.52	56.57	98.66	95.57
Seedling fresh weight (mg)	89.30	92.96	94.92	32.59	48.36	55.50
Seedling dry weight (mg)	92.20	90.96	91.13	50.55	47.54	51.92
Water content (%)	82.05	71.32	83.53	1.69	1.71	2.72
Total soluble sugar (µg GE g^{-1} DW)	92.42	92.46	86.87	46.65	32.04	23.74
Proline content (µg g^{-1} DW)	98.81	97.68	88.69	37.43	31.88	19.98
Seedling vigour index-I	92.20	79.78	80.84	24.60	23.78	35.46
Seedling vigour index-II	91.75	89.86	88.40	50.66	50.30	59.47

cannot determine the heritable variance. The combination of heritability estimates with genetic advance is crucial for predicting selection success. In this investigation, high heritability estimates (>60%) were found for all traits except germination percentage (Table 5), consistent with the findings of Meena *et al*., (2008) and Shahzad *et al*., (2012). Traits such as root length, seedling length, root-to-shoot length, shoot fresh weight, root fresh weight, seedling fresh weight, seedling dry weight, total soluble sugar, proline content, seedling vigourIndex-I, and seedling vigour index-II demonstrated high genetic advance as a percentage of the mean, indicating good potential for developing genotypes with enhanced performance under salinity stress.

Traits with high heritability coupled with high genetic advance, such as root fresh weight, seedling fresh weight, and seedling dry weight, offer the most promise for effective selection in salinity tolerance breeding. These findings are supported by earlier reports from Meena

et al., (2008) and Singh *et al*., (2009) in Indian mustard. Selection for these traits will likely be more efficient in developing salt-tolerant mustard cultivars.

Overall, this study highlights significant morphological and genetic variation among the Indian mustard genotypes for salt tolerance. The increasing salinity levels negatively affected most traits, except for total soluble sugar and proline content, which increased as salinity levels rose. Significant differences were observed in genotypes, salinity levels, and their interactions, with traits such as root fresh weight, seedling fresh weight, and seedling dry weight showing the highest potential for selection. The accessions RH 2066 and RH 0406 exhibited greater tolerance to salinity, performing comparably to the standard checks (CS 52 and CS 58), making them promising candidates for further breeding efforts to develop salt-tolerant Indian mustard cultivars.

Conclusion

In conclusion, this study identified significant genetic variation among Indian mustard genotypes in response to salinity levels, with traits such as root fresh weight, seedling fresh weight, and seedling dry weight showing high genotypic and phenotypic variability, strong heritability, and genetic advance, making them promising for selection in breeding programs. Notably, genotypes RH 2066 and RH 0406 exhibited superior salinity tolerance, comparable to standard checks CS 52 and CS 58, positioning them as valuable genetic resources for developing salt-tolerant mustard varieties. Overall, this research underscores the potential for breeding resilient Indian mustard varieties capable of thriving in saline environments, thereby enhancing mustard cultivation in affected regions.

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