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Assessment of variability parameters for germination and seedling traits in mustard (*Brassica juncea* L.) under salinity stress condition

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Abstract
The experiment investigates the effects salinity on seed germination and seedling characteristics of ten diverse genotypes of *Brassica juncea* L. The genotypes were evaluated at four levels of salinity based on electric conductivities (ECs) *viz*. 0.0 EC, 6 EC, 10 EC and 12 EC in completely randomized design (CRD) with three replications. Twelve parameters were recorded on germination percent (%), shoot length (cm), root length (cm), seedling length (cm), root to shoot length ratio, shoot fresh weight (mg), root fresh weight (mg), seedling fresh weight (mg), shoot dry weight (mg), root dry weight (mg), seedling dry weight (mg) and seedling vigour index. The genotypes exhibited significant differences in all the salinity concentrations for all the parameters. High heritability was observed for shoot fresh weight, root fresh weight and seedling fresh weight, while the least value was found for germination percentage. The higher values of genetic advance were obtained for shoot fresh weight, root fresh weight and seedling fresh weight. Selections based on these high heritability estimate coupled with high genetic advance, further can be used in mustard improvement program.

Keywords: *Brassica juncea*, coefficient of variation, genetic advance, heritability, salinity, seedling traits, variability parameter

Introduction
The *Brassica juncea* (n = 18; AB) commonly known as Indian mustard has derived from inter specific crosses between *B. nigra* (n = 8; B) and *B. campestris* (n = 10; A) as an amphidiploid species. Mustard seed is the second most crucial oil seed crop in India after soybean and it is the third considerable source of edible vegetable oils in the world, after soybean and oil palm [1]. It contributes for nearly 20–22 percent of the total oil seeds produced in the country. India's Mustard seed production in 2018-19 is estimated at around 86.93 lakh MT which is marginally higher from around 83.22 lakh MT produced in 2017-18 [2]. However, In India mustard production still remains inadequate to satisfy even daily demand of its people. This deficit is brought about due to the several factors of biotic and abiotic stresses, among which a fearsome concern is the salt stress. High salinity deteriorated about 95 million hectares of land worldwide [3], and largely affects germination, growth, physiology and productivity by producing ionic and osmotic stresses as well as oxidative damage [4]. Though the relationship between osmotic regulation and salt tolerance is not well clear, there is attestation evidence that the osmotic adjustment appears at least partially to be involved in the salt tolerance of certain plant genotypes [5]. Easily noticeable spartial and temporal variability of both resources and abiotic factors create major environmental limitations. Salt and osmotic stresses are accountable for inhibition and delayed seed germination and also seedling establishment [6]. Considering the cruciality of judicious and management of mustard group of the crops on Indian economy and the adverse effect of salinity in many of the mustard growing districts gave the motivation to conduct this basic and strategic research which will help in understanding the effects of salinity stress on germination, seedling characteristics variation of mustard as well as on evaluation of tolerant genotypes for stabilizing and boosting production and productivity. Therefore, the present investigation was carried out with the following objectives to assess variability for germination and seedling traits in mustard under different salinity levels to identify suitable traits for selection of tolerant genotype under salt stress.

Materials and methods
The laboratory experiment was carried out during the rabi period from November, 2019 to March, 2020 at department of Plant Breeding and Genetics of S. K. N. College of Agriculture,

Sri Karan Narendra Agriculture university Jobner, Jaipur (Rajasthan). A total of ten diverse mustard genotypes were evaluated at four levels of salinity, including control. The genotypes were as RH-406, RGN-48, RGN-229, RH-749, RGN-236, RGN-303, RGN-73, RGN-13, RGN-298 and RGN-145. The experiment was laid out under completely randomized design (CRD) with three replications. The experiment investigates the effects of different levels of salinity solution on seed germination and subsequent early seedling development characteristics of the genotypes of mustard.

Preparation of salinity solutions

Four levels of salinity concentrations L_0, L_1, L_2, L_3 were prepared from the mixture of NaCl, $NaHCO_3$, $CaCl_2$, $MgSO_4$ and Na_2SO_4 which had electric conductivities of 0.0 EC (control), 6 EC, 10 EC and 12 EC. For making solution of 6 EC, the mixture comprising of NaCl, $NaHCO_3$, $CaCl_2$, $MgSO_4$ and Na_2SO_4 with the weights of 8.775 gm, 8.1 gm, 8.325 gm, 5.4 gm and 4.26 gm respectively was dissolved in 10 liters of double distilled water. For 10 EC stock solution, weights of the mixture were 14.62 gm, 13.50 gm, 13.80 gm, 9.90 gm and 7.10 gm for NaCl, $NaHCO_3$, $CaCl_2$, $MgSO_4$ and Na_2SO_4 respectively, and for 12 EC solution the weights were 17.55 gm, 16.20 gm, 16.54 gm, 10.80 gm and 8.52 gm for NaCl, $NaHCO_3$, $CaCl_2$, $MgSO_4$ and Na_2SO_4 respectively were dissolved in 10 liters of double distilled water. Disposable PVC pots of 8.5 cm x 9.5 cm in size were used to carry out an experiment. In each treatment 100 ml of the solution was used to irrigate the pot for germination. Eight seeds of each genotype were placed in one plastic pot filled with sandy soil at equal depth and placed in germination chamber maintained at 25^{o} C temperature and 75% humidity.

Observations recorded

Twelve seedling parameters were recorded *viz.* germination percent, shoot length (cm), root length (cm), seedling length (cm), root to shoot length ratio (cm), shoot fresh weight (mg), root fresh weight (mg), seedling fresh weight (mg), shoot dry weight (mg), root dry weight (mg), seedling dry weight (mg) and seedling vigour index. Germination percentage were recorded on 7th day after planting, while other characters and parameters were recorded on 15th day onwards after sowing on five randomly selected seedlings from each pot in each replication. The shoot length, root length and seedling length were recorded by using a measuring scale in centimeter and average were used in analysis. The root to shoot length ratio of seedling was calculated by dividing root length to the shoot length. The fresh weight of shoot and fresh weight of root were measured in milligram by using a sensitive electronic balance and averaged. For obtaining seedling fresh weight (mg), shoot fresh weight and root fresh weight were added. The data on shoot dry weight (mg) and root dry weight (mg) were recorded after drying fresh shoot and root in hot air oven for 48 hours at 65^{0}C. For obtaining seedling dry weight, shoot dry weight and root dry weight were added. The seedling vigour index was determined by multiplying the sum total of mean length of shoot and root of a seedling with concerned germination percentage by the following formula [7]:

Seedling Vigour Index (SVI) = (RL+PL) X (GP)

Where, RL= Mean radical (root) length, PL= Mean plumule (shoot) length and GP= Germination percentage.

Statistical analysis

The data obtained from this study were subjected to appropriate statistical analysis. Genetic parameters *viz;* Coefficients of variation (GCV and PCV) were calculated as suggested by [8], Heritability in broad sense and expected genetic genetic advance were calculated as per formulae suggested by [9]. Standard procedures were followed to estimate the various parameters as described by [10].

Results and Discussions

Significant differences were observed to all genotypes in all the salinity levels, *viz;* 0.0 EC, 6 EC, 10 EC and 12 EC for all the traits, this demonstrating presence of inherent differences among the genotypes under the study (table 1). Results from pooled analysis of variance portrayed existence of significant differences among genotypes, salinity concentrations and interactions between genotype x salinity concentrations, exceptional was only for germination percentage under (genotype x salinity concentration) interaction, this demonstrating differential reaction of genotypes to saltiness for all the traits under the study.

The variability parameters of different characters including mean, range, genotypic variation (GV), phenotypic variation (PV), genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability and genetic advance (%) in each salinity level are presented in table 2. The mean values of all the parameters get reduced with the increase of salinity concentrations. Mean values were higher in the control (0.0 EC), reduced subsequently (in 6 EC and 12 EC) and were least at the highest salinity concentration (12 EC). A wide range of variability was found for the entire parameters studied. The trends of observations showed that the values of phenotypic variance (PV) were greater than the values for genotypic variance (GV) in all the levels of salinity. The genotypic variance showed increasing trend with salinity for germination percentage only and fluctuations were observed for other parameters, whereas fluctuations were observed for all the traits studied with respect to salinity gradient. The values of GCV were lower than PCV values for all the characters, demonstrating a positive outcome of environment on the character articulation. The increasing trend with salinity concentrations for GCV were seen for the characters; germination percentage, shoot length, seedling length, root fresh weight and seedling vigour index and for the case of PCV the increasing trend with salinity concentrations were seen shoot length and root fresh weight. The highest and the lowest values for both GCV and PCV were observed for the traits; root fresh weight and shoot dry weight. It is demonstrating that these highest values and lowest values to be the most and the least variable parameters. But the distinctions were nonetheless, low for all the attributes. Similarly, some of the previous researchers reported pertaining fluctuations of genotypic and phenotypic variability for most of the characters were [11] in Indian mustard, [12] in cotton and [10] in sugarcane for germination percent under water logging conditions.

In case of heritability in broad sense, irregular pattern were observed for various parameters. Higher heritability were reported for the traits; shoot fresh weight, root fresh weight, seedling fresh weight and root dry weight at all the level of salinity. These results were in collaborating with the findings of [13]. High heritability on these traits indicated that environmental effects created by salinity were less and major portion of the variations governed by genetic variance [14]. This may be attributed by the presence of tolerant genes for salinity as reported by [15, 16]. Genetic advance as percentage of mean also portrayed irregular pattern across the salinity

concentrations, the increasing trends were seen for seedling length and root fresh weight. The higher values were obtained for shoot fresh weight, root fresh weight and seedling fresh weight, while the least value was found for germination percentage. Selections based on heritability estimate coupled with genetic advance are more helpful in predicting the gain under selection than selection based on heritability alone as high genetic advance was an indicative of additive gene effects [17]. In the present study shoot fresh weight, root fresh weight and seedling fresh weight were shown high heritability and high genetic advance, indicate that improvement can be made through direct selection of these traits.

Table 1: Analysis of variance for various traits of *Brassica juncea* L. showing mean sum of square

Salinity levels (EC)	Source of variation	d.f.	Germination (%)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Root to shoot ratio	Shoot fresh wt (mg)	Root fresh wt (mg)	Seedling fresh wt (mg)	Shoot dry wt (mg)	Root dry wt (mg)	Seedling dry wt (mg)	Seedling vigor index
0.0 EC	Variety	9	46.297**	1.568**	1.871**	3.168**	0.029**	2254.69**	21.104**	2402.007**	6.204**	2.738**	14.39**	53245.188**
	Error	20	1.1	0.166	0.037	0.134	0.002	5.051	0.197	4.431	0.149	0.052	0.218	1550.791
06 EC	Variety	9	71.76*	1.467**	0.918**	3.78**	0.009**	502.74**	18.163**	586.82**	4.524**	0.303**	5.664**	51603.719**
	Error	20	20.842	0.204	0.085	0.351	0.002	9.187	0.141	9.628	0.1	0.009	0.112	10494.874
10 EC	Variety	9	78.704**	2.256**	0.486**	3.031**	0.018**	451.312**	15.777**	528.641**	0.314**	0.333**	0.843**	41935.893**
	Error	20	1.492	0.142	0.052	0.238	0.001	6.836	0.073	7.257	0.032	0.009	0.04	2518.733
12 EC	Variety	9	95.486**	3.376**	0.728**	6.386**	0.011**	776.989**	17.233**	842.17**	1.642**	0.229**	1.299**	59724.287**
	Error	20	14.734	0.038	0.046	0.079	0.001	6.088	0.048	6.138	0.081	0.012	0.108	2758.704
Pooled ANOVA	Genotypes (G)	9	264.61**	6.71**	1.33**	10.64**	0.04**	2206.39**	48.67**	2446.16**	5.34**	1.33**	9.77**	137693.23**
	Salinity levels (S)	3	667.97**	27.75**	103.04**	233.16**	0.47**	14963.42**	305.38**	19513.76**	22.94**	55.72**	143.42**	3230991.97**
	G X S	27	9.21	0.65**	0.89**	1.90**	0.01**	593.08**	7.87**	637.83**	2.45**	0.76**	4.14**	22887.05**
	Pooled Error	80	9.54	0.14	0.05	0.2	0.001	6.79	0.115	6.86	0.09	0.02	0.119	4330.134

* and ** represent significant at 5 per cent and 1 per cent level of significance, respectively

Table 2: Variability parameters of germination and seedling traits of *Brassica juncea* L.

S. N.	Characters	Salinity level	Mean	Range	GV	PV	GCV	PCV	h^2 (%)	GA (%)
1.	Germination percent	L_0 (0 EC)	95.83	91.67-100.00	15.07	16.17	4.05	4.20	93.20	8.05
		L_1 (6 EC)	93.33	87.50-100.00	16.97	37.81	4.41	6.59	44.88	6.09
		L_2 (10 EC)	88.33	79.17-95.83	25.74	27.23	5.74	5.91	94.52	11.50
		L_3 (12 EC)	85.42	75.00-91.67	26.92	41.65	6.07	7.56	64.63	10.06
2.	Shoot length (mm)	L_0 (0 EC)	10.35	9.39 - 11.47	0.47	0.63	6.61	7.69	73.79	11.69
		L_1 (6 EC)	9.49	7.85 - 10.37	0.42	0.62	6.84	8.33	67.36	11.56
		L_2 (10 EC)	8.92	7.33 - 10.11	0.70	0.84	9.41	10.32	83.23	17.69
		L_3 (12 EC)	8.06	6.18 - 9.66	1.11	1.15	13.09	13.31	96.70	26.51
3.	Root length (mm)	L_0 (0 EC)	9.20	7.26 - 9.98	0.61	0.65	8.51	8.76	94.29	17.02
		L_1 (6 EC)	6.66	5.62 - 7.34	0.28	0.37	7.91	9.04	76.56	14.26
		L_2 (10 EC)	5.64	4.86 - 6.13	0.14	0.19	6.74	7.86	73.56	11.91
		L_3 (12 EC)	4.97	3.93 - 5.55	0.23	0.28	9.59	10.52	83.17	18.02
4.	Seedling length (mm)	L_0 (0 EC)	19.54	18.07 - 20.94	1.01	1.14	5.15	5.48	88.30	9.96
		L_1 (6 EC)	16.14	14.00 - 17.37	1.14	1.49	6.62	7.57	76.51	11.94
		L_2 (10 EC)	35.69	12.67 - 15.97	0.93	1.17	6.63	7.43	79.64	12.18
		L_3 (12 EC)	13.04	19.68 - 15.15	2.10	2.18	11.12	11.33	96.38	22.49
5.	Root to shoot ratio	L_0 (0 EC)	0.89	0.66 - 0.98	0.01	0.012	10.66	11.78	81.82	19.86
		L_1 (6 EC)	0.70	0.62 - 0.79	0.01	0.012	6.90	9.40	53.85	10.43
		L_2 (10 EC)	0.64	0.55 - 0.80	0.01	0.011	11.76	12.76	85.00	22.34
		L_3 (12 EC)	0.62	0.55 - 0.73	0.02	0.021	9.31	10.62	76.92	16.82
6.	Shoot fresh weight (mg)	L_0 (0 EC)	126.08	91.07 - 168.87	749.88	754.93	21.72	21.79	99.33	44.59
		L_1 (6 EC)	98.53	77.00 - 121.4	164.52	173.71	13.02	13.38	94.71	26.1
		L_2 (10 EC)	85.87	70.20 - 109.07	148.16	155.00	14.17	14.50	95.59	28.55
		L_3 (12 EC)	74.03	47.07 - 103.53	256.94	263.03	21.65	21.91	97.69	44.08
7.	Root fresh weight (mg)	L_0 (0 EC)	12.74	9.97 - 17.13	6.97	7.17	20.72	21.01	97.25	42.09
		L_1 (6 EC)	8.09	5.47 - 14.07	6.01	6.15	30.30	30.65	97.71	61.69
		L_2 (10 EC)	6.69	4.77 - 12.37	5.23	5.30	34.20	34.44	98.62	69.96
		L_3 (12 EC)	5.44	3.60 - 11.90	5.73	5.78	44.00	44.18	99.17	90.25
8.	Seedling fresh weight (mg)	L_0 (0 EC)	138.82	101.03 - 181.07	799.19	803.62	20.36	20.42	99.45	41.84
		L_1 (6 EC)	106.61	83.33 - 130.33	192.40	202.03	13.01	13.33	95.23	26.16
		L_2 (10 EC)	245.44	75.97 - 118.00	173.79	181.05	14.24	14.54	95.99	28.75
		L_3 (12 EC)	352.05	50.67 - 108.33	278.68	284.82	21.00	21.23	97.84	42.80
9.	Shoot dry weight (mg)	L_0 (0 EC)	8.79	7.53 - 11.67	2.02	2.17	16.16	16.75	93.13	32.13
		L_1 (6 EC)	8.10	7.20 - 11.50	1.47	1.57	14.99	15.49	93.65	29.89
		L_2 (10 EC)	7.48	7.13 - 8.20	0.09	0.12	4.10	4.75	74.60	7.29
		L_3 (12 EC)	6.74	4.93 - 7.53	0.52	0.60	10.70	11.51	86.53	20.51
10.	Root dry weight (mg)	L_0 (0 EC)	4.88	3.70 - 7.00	0.90	0.95	19.39	19.94	94.51	38.83
		L_1 (6 EC)	2.69	2.07 - 3.07	0.10	0.11	11.64	12.16	91.59	22.94
		L_2 (10 EC)	2.21	1.73 - 2.73	0.11	0.12	14.87	15.48	92.31	29.43
		L_3 (12 EC)	1.84	1.47 - 2.40	0.07	0.08	14.62	15.78	85.77	27.89

11.	Seedling dry weight (mg)	L_0 (0 EC)	13.67	11.83 - 18.67	4.72	4.94	15.90	16.26	95.59	32.02
		L_1 (6 EC)	10.79	9.87 - 14.57	1.85	1.96	12.61	12.98	94.29	25.22
		L_2 (10 EC)	9.69	8.87 - 10.80	0.27	0.31	5.34	5.72	87.00	10.26
		L_3 (12 EC)	8.58	7.07 - 9.47	0.40	0.51	7.34	8.28	78.61	13.41
12.	Seedling Vigour Index	L_0 (0 EC)	1873.47	1656.70-2057.00	17231.47	18782.26	7.01	7.32	91.74	13.83
		L_1 (6 EC)	1507.58	1224.85-1662.85	13702.95	24197.82	7.76	10.32	56.63	12.04
		L_2 (10 EC)	1286.01	1146.59-1463.91	13139.05	15657.78	8.91	9.73	83.91	16.82
		L_3 (12 EC)	1112.81	917.85-1325.33	18988.53	21747.23	12.38	13.25	87.31	23.84

Where, GV= genotypic variance, PV= phenotypic variance, GCV= genotypic coefficient of variation, PCV= phenotypic coefficient of variation, h^2 = heritability in broad sense, GA = genetic advance as percentage of mean.

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