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Genotypic Variation for Seedling Traits in Mustard (*Brassica juncea* L.) in Response to Salt Stress

Kassim Yahaya Mtilimbanya*, Mohan Lal Jakhar, Sarfraz Ahmad, Suman Chahar and Hari Ram Jat

Department of Plant Breeding and Genetics, S.K.N. Agriculture University, Jobner, Rajasthan-303029, India

*Corresponding author

ABSTRACT

Keywords

Brassica juncea, Indian mustard, Salt stress, Salt tolerance, Salinity, Seedling traits

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Soil salinity is one of the major factors responsible for poor germination and plant growth, ultimately limiting crop yield. This research was conducted to evaluate 10 genotypes of mustard at four levels of salinity based on electric conductivities (ECs) viz. 0.0 EC, 6 EC, 10 EC and 12 EC. Observations on twelve parameters were recorded including germination percent and early generation seedling traits. The genotypes exhibited significant differences among all the traits in all the levels of salinity concentrations. The mean values of all the parameters significantly reduced with the increase in the salinity level. The maximum reduction was observed in germination percentage followed by shoot length while and least affected trait was root dry weight. Genotypes RH – 406, RGN – 229, RGN - 145 and RGN - 48 showed tolerance and provided overall high stability results in higher salinities. These genotypes further can be exploited to increase the genetic base of existing lines of tolerant mustard genotypes and can be grown in marginalized salt affected land to increase productivity.

Introduction

Mustard, *Brassica juncea* (n = 18; AB) has derived from inter specific crosses between *B. nigra* (n = 8; B) and *B. campestris* (n = 10; A) as an amphidiploids 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 (FAO, 2011). 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. (Anonymous, 2018-19).

More than 43 percent of the area which produces mustard in India is contributed by Rajasthan which is semi-arid region, one of the obstacles which affects mustard production and productivity to a great extent

is salinity, this is due to the fact that majority sources of highly saline, medium to high sodicity is ground water. The main causes of the soil salinity are inappropriate irrigation and poor drainage practices. The harmful effects of salt on plants are results of both a water deficit that led from the relative high solute concentrations in the soil and a Na^+ , specific stress resulting from altered K^+/Na^+ ratios (Zhang *et al.*, 2001) and complete organic solute (soluble carbohydrates, amino acids, proline, betains, etc.) accumulations (Hasegawa *et al.*, 2000).

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 (Neto *et al.*, 2004). The critical stage in crop production is seedling establishment, which mainly depends on biochemical and physiological structures of the seed. Fast germination and good establishment of seedlings obtained when seeds with high vigour are provided with essential nutrients for seedling establishment and to enable them to photosynthesize independently.

The extent of the effect of salinity varied with the plant species, type and level of salinity. There are interspecies, intraspecies and intercultivar variability and even individual lines differ at various ontogenetic stages to salt tolerance, which gives opportunity for selection of genotypes for salt tolerance (Sharma *et al.*, 2013). Considering the cruciality of judicious and management of mustard group of the crops on economy and the adverse effect of salinity in many of the mustard growing areas, the present investigation was carried out to understand the effects of salinity stress on germination, seedling characteristics variation as well as on evaluation of tolerant genotypes for

stabilizing and boosting production and productivity.

Materials and Methods

The present study was conducted at the Plant Tissue Culture Laboratory of Department of Plant Breeding and Genetics, Sri Karan Narendra College of Agriculture, Jobner (Rajasthan) during *Rabi* 2019-2020. Ten mustard genotypes were obtained from Agriculture Research Station, Sri Ganganagar (Rajasthan). 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 ten genotypes of mustard.

Preparation of salinity solutions

Four levels of salinity concentrations L_0 , L_1 , L_2 , L_3 which had electric conductivities of 0.0 EC (control), 6 EC, 10 EC and 12 EC were prepared from the mixture of NaCl , NaHCO_3 , CaCl_2 , MgSO_4 and Na_2SO_4 . 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. 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° C temperature and 75% humidity. The germination was completed within 3 to 7 days of sowing.

Observations recorded

Observation on 12 traits *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 were recorded. Observations on 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^oC. 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 (Iqbal and Rahmati, 1992):

$$\text{Seedling Vigour Index (SVI)} = (\text{RL} + \text{PL}) \times (\text{GP})$$

Where,

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

percentage.

D – value

It is the measure of salinity stress intensity and it was derived by the following formula:

$$D = 1 - \left(\frac{Y_j}{Y_c} \right)$$

Where,

Y_j = Mean performance of a concerned character of all the genotypes in the salt stress environment

Y_c = Mean performance of a concerned character of all the genotypes in the normal environment.

Results and Discussion

The general statistical parameters for various characters are presented in table 1. Analysis of variance showed significant differences among the genotypes for all the characters at all the levels of salinity. Results from pooled analysis of variance portrayed existence of significant differences among genotypes, salinity concentrations and interactions between genotype x salinity concentrations, except for germination percentage under (genotype x salinity concentration) interaction, this demonstrating differential reaction of genotypes to saltiness for all the traits under the study.

Effect of salinity on mean performance of mustard genotypes

The extent of the germination percentage was decreased with increase in salinity concentration. Highest germination was observed in L₀ (95.83 per cent) then declined progressively in L₁ (93.33 per cent), L₂ (88.33 per cent) and L₃ (85.42 per cent). Perusal of table 1 showed significant differences among different genotypes of mustard for germination at all the levels of salinity.

A comparison between overall mean of different genotypes in control (L_0) and L_m (mean of L_1, L_2 & L_3) are presented in table 2. The mean values of all the parameters get reduced with the increase in the salinity concentrations. Values of the traits were higher in the control (0.0 EC) and were least at the highest salinity concentration. Same results were reported by Jamil *et al.*, (2005) and Sharma *et al.*, (2013). However, the magnitude of the salinity impact to all these characters were not the same as a result the genotypes showed fluctuation in the degree of variability towards the response to salinity concentrations (Houimli *et al.*, 2008). Some parameters like germination percentage, shoot fresh weight, seedling fresh weight and seedling vigour index have shown huge magnitude of variations in contrasts to shoot length, root length, seedling length, root fresh weight, shoot dry weight, root dry weight and seedling dry weight which showed less magnitude of variation in higher salinity concentrations, for the case of root to shoot ratio the variation was very less to all higher salinity concentrations. Similarly was observed by Kumawat and Gothwal (2019). The gradient of saltiness concentrations unfavorably influenced the mean estimations of practically all the characters, the derived character of root to shoot ratio has affected in very minimal magnitude in contrast to all other parameters. The impact was higher in L_3 concentrations where most of the characters attained low mean values as compared to L_2, L_1 , and Control (L_0) in increasing order. The reduction of these parameters might be because of lethal impacts of the salt ions in the root zone utilized just as lopsided supplement take-up by the seedlings and lower water accessibility (Xiong and Zhu, 2002). Such perception has additionally been accounted for in lentil (Sariye and Ercan, 2015). The parameters may be reduced due to oxidative stress, water stress, ion toxicity, genotoxicity, nutritional disorders, alteration

in the metabolic processes, membrane disorganization, reduction of cell division and expansion which caused by salinity concentrations (Munns 2002 and Zhu, 2007).

Based on the total ranking of the mean (L_m) of the different genotypes for L_1, L_2 and L_3 shown in table 3, it indicated none of the genotypes have uniform reaction under various saltiness concentrations for all particular parameters. The genotypes RGN – 236 followed by RGN – 303 were highly affected as they scored 10th and 9th position in ranking order respectively, this indicates that these genotypes are very sensitive to salinity conditions. The least affected genotype was RH – 406 which occupied position 1st followed by two genotypes (RGN- 229 and RGN- 145) which tied up in position 2nd of the total ranking. The description from the table 3 is in such a way those genotypes with least position all out will be the best genotypes and they have fewer score. It's an indication that these were perfect genotypes which are to be relied upon to give great reaction over various saltiness concentrations. The genotypes which showed medium response are RGN – 48, RGN- 73 and two genotypes which tied up in scoring (RGN- 13 and RGN- 298) were ranked in position 4th, 5th, and 6th, respectively.

General over view of the parameters on response to salinity concentrations based on D- value (table 4) it indicated that root dry weight, root fresh weight, root length and seedling fresh weight were highly affected by the salinity concentrations, thus marked them to be more sensitive to saline conditions.

These results indicate that root dry weight, root fresh weight, root length and seedling fresh weight cannot be used as a screening and selection criteria for evaluating the salt tolerance behavior among a large collection of plant accessions.

Table.1 General statistical parameters for various seedling traits in 10 mustard genotypes in response to salt stress

S.N.	Characters	Salinity levels (EC)	CV	CD	SEm
1	Germination Percent (%)	L ₀ (0 EC)	1.094	1.094	0.606
		L ₁ (6 EC)	4.891	4.891	2.636
		L ₂ (10 EC)	1.383	1.383	0.705
		L ₃ (12 EC)	4.494	4.494	2.216
2	Shoot Length (cm)	L ₀ (0 EC)	0.697	3.925	0.235
		L ₁ (6 EC)	0.774	4.756	0.260
		L ₂ (10 EC)	0.647	4.228	0.218
		L ₃ (12 EC)	0.333	2.409	0.112
3	Root Length (cm)	L ₀ (0 EC)	0.327	2.075	0.110
		L ₁ (6 EC)	0.499	4.365	0.168
		L ₂ (10 EC)	0.390	4.029	0.131
		L ₃ (12 EC)	0.365	4.282	0.123
4	Seedling Length (cm)	L ₀ (0 EC)	0.628	1.872	0.211
		L ₁ (6 EC)	1.016	3.670	0.342
		L ₂ (10 EC)	1.047	1.710	0.352
		L ₃ (12 EC)	0.483	2.161	0.163
5	Root to Shoot ratio	L ₀ (0 EC)	0.084	5.465	0.028
		L ₁ (6 EC)	0.069	5.726	0.023
		L ₂ (10 EC)	0.054	4.929	0.018
		L ₃ (12 EC)	0.057	5.326	0.019
6	Shoot Fresh wt (mg)	L ₀ (0 EC)	3.855	1.782	1.297
		L ₁ (6 EC)	5.199	3.076	1.750
		L ₂ (10 EC)	4.484	3.045	1.509
		L ₃ (12 EC)	4.232	3.333	1.424
7	Root Fresh wt (mg)	L ₀ (0 EC)	0.763	3.489	0.257
		L ₁ (6 EC)	0.643	4.637	0.216
		L ₂ (10 EC)	0.463	4.041	0.156
		L ₃ (12 EC)	0.377	4.039	0.127
8	Seedling Fresh wt (mg)	L ₀ (0 EC)	3.611	1.516	1.215
		L ₁ (6 EC)	5.322	2.91	1.791
		L ₂ (10 EC)	5.087	1.208	1.712
		L ₃ (12 EC)	9.765	1.617	3.287
9	Shoot Dry wt (mg)	L ₀ (0 EC)	0.661	4.382	0.222
		L ₁ (6 EC)	0.541	3.889	0.182
		L ₂ (10 EC)	0.305	2.378	0.103
		L ₃ (12 EC)	0.486	4.205	0.164
10	Root Dry wt (mg)	L ₀ (0 EC)	0.391	4.67	0.132
		L ₁ (6 EC)	0.163	3.531	0.055
		L ₂ (10 EC)	0.157	4.131	0.053
		L ₃ (12 EC)	0.181	5.711	0.061
11	Seedling Dry wt (mg)	L ₀ (0 EC)	0.800	3.412	0.269
		L ₁ (6 EC)	0.572	3.092	0.193
		L ₂ (10 EC)	0.344	2.067	0.116
		L ₃ (12 EC)	0.562	3.818	0.189
12	Seedling Vigour Index	L ₀ (0 EC)	67.542	2.102	22.735
		L ₁ (6 EC)	175.710	6.795	59.146
		L ₂ (10 EC)	86.081	3.903	28.976
		L ₃ (12 EC)	90.085	4.72	30.324

Table.2 The comparison between overall mean of different genotypes in control (L₀) vs L_m (mean of L₁, L₂ & L₃)

S. No.	Genotypes	EC Level	Germination (%)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Root - shoot 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)	Seedling vigor index
1	RH- 406	L ₀	100.00	10.37	9.51	19.89	0.92	121.40	12.13	133.53	8.40	5.87	14.27	1988.67
		L _m	93.06	9.40	5.96	15.35	0.63	111.33	7.56	118.89	8.00	2.40	10.40	1431.01
2	RGN- 48	L ₀	95.83	11.09	7.26	18.35	0.66	110.13	11.33	121.47	7.93	4.33	12.27	1758.45
		L _m	91.67	9.96	6.20	16.16	0.62	89.09	5.67	94.76	6.77	2.28	9.04	1483.83
3	RGN- 229	L ₀	100.00	10.55	9.48	20.03	0.90	126.73	15.40	142.13	8.13	3.70	11.83	2002.90
		L _m	95.83	8.60	5.86	14.46	0.68	90.75	12.78	103.53	7.52	2.27	9.79	1390.03
4	RH- 749	L ₀	100.00	9.87	9.62	19.49	0.98	155.13	10.87	166.00	7.73	4.20	11.93	1949.33
		L _m	93.06	7.65	5.39	13.04	0.71	79.52	7.28	86.80	7.42	1.97	9.39	1224.12
5	RGN- 236	L ₀	95.83	9.47	9.00	18.47	0.95	95.53	9.97	105.50	8.30	4.53	12.83	1770.40
		L _m	87.50	8.34	4.80	13.14	0.57	64.76	5.23	69.99	6.98	2.01	8.99	1156.84
6	RGN- 303	L ₀	91.67	9.39	8.69	18.07	0.93	91.07	9.97	101.03	7.93	4.47	12.40	1656.70
		L _m	84.72	7.48	5.73	13.21	0.77	76.67	5.40	82.07	7.14	2.66	9.80	1120.49
7	RGN- 73	L ₀	100.00	10.59	9.98	20.57	0.94	168.87	12.20	181.07	11.07	5.10	16.17	2057.00
		L _m	93.06	9.12	5.54	14.65	0.61	88.11	5.51	93.62	7.40	2.44	9.84	1365.97
8	RGN- 13	L ₀	91.67	10.99	9.95	20.94	0.90	159.67	17.13	176.80	11.67	7.00	18.67	1919.52
		L _m	83.33	9.24	5.67	14.92	0.61	81.20	5.27	86.47	8.47	2.29	10.76	1248.03
9	RGN- 298	L ₀	91.67	11.47	9.13	20.61	0.80	103.33	16.57	119.90	7.53	5.17	12.70	1888.96
		L _m	80.56	9.43	6.15	15.58	0.65	76.97	6.31	83.28	7.10	2.29	9.39	1260.70
10	RGN- 145	L ₀	91.67	9.68	9.33	19.01	0.96	128.93	11.87	140.80	9.20	4.47	13.67	1742.80
		L _m	87.50	9.01	6.26	15.27	0.69	103.02	6.39	109.41	7.62	1.87	9.49	1340.31
	Mean	L ₀	95.83	10.35	9.19	19.54	0.89	126.08	12.74	138.82	8.79	4.88	13.67	1873.47
		L _m	89.03	8.82	5.76	14.58	0.65	86.14	6.74	92.88	7.44	2.25	9.69	1302.13

Table.3 The rank total of different genotypes based on mean (L_m) of salinity levels L_1 , L_2 and L_3

Genotypes	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 Vigour Index	Total	Rank
RH- 406	2	3	4	3	6	1	2	1	2	3	2	2	31	1
RGN- 48	3	1	2	1	7	4	6	4	10	5	8	1	52	4
RGN- 229	1	7	5	7	4	3	1	3	4	6	5	3	49	2
RH- 749	2	9	9	10	2	7	3	6	5	8	7	8	76	8
RGN- 236	4	8	10	9	10	10	10	10	9	7	9	9	105	10
RGN- 303	5	10	6	8	1	9	8	9	7	1	4	10	78	9
RGN- 73	2	5	8	6	9	5	7	5	6	2	3	4	62	5
RGN-13	6	4	7	5	8	6	9	7	1	4	1	7	65	6
RGN-298	7	2	3	2	5	8	5	8	8	4	7	6	65	6
RGN-145	4	6	1	4	3	2	4	2	3	9	6	5	49	2

Table.4 D- value of different parameters of mustard grown under salinity levels

Characters	Control (0 EC)	Salt stress (L_m)	D – value	Rank
Germination percentage	95.83	89.03	0.071	12
Shoot length	10.35	8.82	0.148	11
Root length	9.19	5.76	0.373	3
Seedling length	19.54	14.58	0.254	9
Root to Shoot ratio	0.89	0.65	0.270	8
Shoot fresh weight	126.08	86.14	0.317	5
Root fresh weight	12.74	6.74	0.471	2
Seedling fresh weight	138.82	92.88	0.331	4
Shoot dry weight	8.79	7.44	0.154	10
Root dry weight	4.88	2.25	0.539	1
Seedling dry weight	13.67	9.69	0.291	7
Seedling vigour index	1873.47	1302.13	0.305	6

L_m = Mean of three salinity levels, viz; L_1, L_2 and L_3

The impact of salinity on seedling qualities demonstrated explicit pattern despite the fact that at higher salinities concentrations the mean values for all parameters were decreased in contrast with salinity concentrations from 0.0 EC, 6 EC, 10 EC and 12 EC. Genotypes RH – 406, RGN – 229, RGN - 145 and RGN - 48 showed tolerance and provided overall high stability results in higher salinities.

These genotypes can be exploited to increase the genetic base of existing lines of tolerant mustard genotypes. They hold a significant salinity tolerance potential that can be grown to increase productivity in marginalized salt affected land, arid and semi-arid areas of the world. Besides; introduction of salt tolerant mustard species is a promising choice to conquer salinity issues in the arid areas. However, in further breeding programmes the variability studies for germination and seedling growth traits to be conducted at higher salinity concentrations for the best identification of tolerant mustard genotypes.

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