



Germination and early growth of Indian mustard (*Brassica juncea* L.) genotypes under saline conditions

Sarita^{1*}, Anita Kumari¹, Ram Avtar², Babita Rani¹, Vinod Goyal¹, Pooja Ahlawat¹

¹Department of Botany and Plant Physiology, CCS Haryana Agricultural University, Hisar 125004, Haryana, India

²Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar 125004, Haryana, India

*Corresponding author: anitahsr@gmail.com

(Received: 31 March 2022; Revised: 20 June 2022; Accepted: 27 June 2022)

Abstract

Salinity is a prominent abiotic stress in many parts of the world. It is the most important element that causes crop damage and lower yields. Mustard is one of the most salt-sensitive crops. An experiment was designed comprising fifteen genotypes of Indian mustard under varied saline levels (control, 8 dSm⁻¹, 10 dSm⁻¹, 12 dSm⁻¹, and 14 dSm⁻¹) to screen and identify resistant genotypes at the early seedling stage. Out of 15 genotypes, four genotypes (two tolerant genotypes viz., RH 725 and RH 1512 and, two susceptible genotypes viz., RH 1520 and RH 8812) were chosen for further studies at vegetative and flowering stages up to maturity, and grown in pots under greenhouse conditions with various salinity levels viz. control, 8 dSm⁻¹, 10 dSm⁻¹, and 12 dSm⁻¹ compare the physiology and yield of these genotypes. As saline levels increased, negative impacts on germination percentage, seedling dry weight, seedling length, seedling vigor-I, seedling vigor-II, photosynthetic rate, transpiration rate, total biomass and 1000 seeds weight were detected. Lower salinity level (8 dSm⁻¹) had no effect, whereas higher salinity levels resulted in a significant drop. The RH 1520 genotype showed the greatest reduction up to 50% under salinity levels, while the RH 725 genotypes demonstrated resilience (~15%) to salinity levels.

Keywords: Indian mustard, photosynthetic rate, salinity, seedling stage, seedling vigor, transpiration rate

Introduction

Indian mustard (*Brassica juncea* L.) is the second most important oil seed crop in India after the soybean. Increasing demand of edible oil will definitely lead to more acreage under the rapeseed-mustard around the world, especially in areas where some soils are prone to becoming saline (FRANCOIS, 1994). Plants growing under field conditions are exposed to various environmental factors; any deviation in these factors from the optimal levels is deleterious to plants and leads to stress. Salinity is a major factor which limits the growth and productivity of plants throughout the world due to increasing use of poor quality of water for irrigation and soil salinization. Salinity limits the productivity of crop due to adverse effects on germination, seedling vigor and crop yield (Munns and Tester, 2008). The seed germination is strongly affective trait of Indian mustard by salinity whereas root dry weight is least affective trait (Mtilimbanya *et al.*, 2020). Higher salts cause toxic effect on embryo development. The high salt concentration in soil and irrigation water decreases the germination rate of almost all *Brassica* species. If the plants germinate under stress condition, they may show stunted growth and poor development (Zamani *et al.*, 2010). Salinity

decreases germination percent, root length, callus size, coleoptiles length and seedling growth (Agnihotri *et al.*, 2006). It was also observed that plant height, stem diameter, dry weight also declines with increasing levels of salinity (Asha and Dhingra, 2007). Salt stress not only affects the germination of the seeds but it also affects other growth parameters like seedling length, seedling fresh and dry weight, and seedling vigor index (Bawa *et al.*, 2019). It has been claimed that selecting salt tolerant genotypes is the most efficient strategy to reduce the negative effects of salt stress on crops (Pervaiz *et al.*, 2002). This method is far less expensive and more feasible to implement on a wide scale in under developed nations than other management practices (e.g., leaching salt from the soil surface etc.). Agronomists and plant breeders regard salt tolerant genotype screening based on grain yield to be the ultimate goal, although it is time consuming and costly. Early examination of salt tolerance during the growth stage saves time and money. However, this is especially true if the degree of salinity tolerance of genotypes at the early growth stage is associated with other growth stages and yield (Hu *et al.*, 2005). The goal of this experiment was to identify salt-tolerant cultivars among those with unknown tolerance. This experiment

was motivated by the fact that no systematic study has been done to screen Indian mustard genotypes for salt resistance, and no physiological feature has been evaluated in *Brassica* genotypes under salinity stress. Knowledge of genetic association between selection indices and morpho-physiological traits can be useful to improve the efficiency of breeding programs in saline irrigation conditions.

Materials and Methods

The experiment was setup in screen house at Chaudhary Charan Singh Haryana Agriculture University, Hisar, located at 29°10' N latitude and 75°46' E longitude, with an elevation of 215.2 m above mean sea level. Seeds of all genotypes for the experiment was taken from Oilseed Section of Genetics and Plant Breeding, CCS Haryana Agriculture University, Hisar.

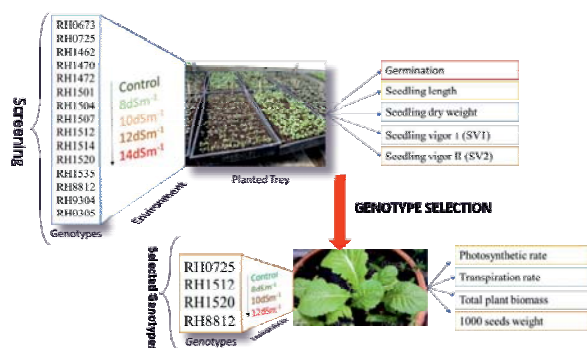


Fig. 1: Experimental layout

Fifteen *Brassica juncea* L. genotypes with variable salinity stress resistance were sown in a variety of salinity solutions, namely control (tap water), 8 dSm⁻¹, 10 dSm⁻¹, 12 dSm⁻¹, and 14 dSm⁻¹ as shown in Fig.2. The genotypes were taken during the experiment are named as RH 0673, RH 725, RH 1462, RH 1470, RH 1472, RH 1501, RH 1504, RH 1507, RH 1512, RH 1514, RH 1520, RH 1535, RH 8812, RH 9304 and RH 0305 (Fig. 2).

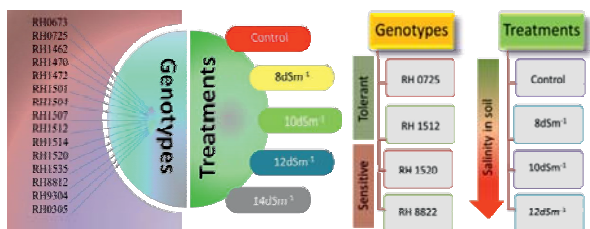


Fig. 2: Evaluation of Indian mustard genotypes under different salinity levels

Desired salinity levels were developed by saturating soil with saline water of respective conductivities which was prepared by using the mixture of NaCl, MgCl₂, MgSO₄ and

CaCl₂, Na: Ca + Mg (1:1), Ca: Mg (1:3) and Cl: SO₄ (7:3) on mEqbasis (chloride dominated salinity). Each treatment had three sets of trays in the experiment design. Each tray set contained all genotypes and was served as a replicate of each treatment. Eighteen seeds of each genotype were sown in lines in each plastic tray as shown in Fig. 1. The selected genotypes were grown in pots under greenhouse condition with the salinity levels viz. control, 8 dSm⁻¹, 10 dSm⁻¹ and 12 dSm⁻¹ (Fig. 1). After one week of sowing, the germination percentage (%) was recorded and after 15 days of sowing, seedling length, seedling dry weight, seedling vigor-I and seedling vigor-II were observed. The photosynthetic rate, and transpiration rate was observed at vegetative and flowering stages; whereas, total plant biomass and 1000 seeds weight were recorded at maturity. Five fresh seedlings were taken from each set of treatment’s trays and dried in oven at 70°C. The weight of dried seedling was taken. Mean of dry weight of these five seedlings was considered as one replicate. Five seedlings were taken from each set of treatment’s trays along with root, and length was measured with the help of scale. Mean of five seedlings length from each set of trays was considered as a replicate. The seedling length was expressed in terms of centimeter (cm). The fresh seedlings dried in oven at 75°C and weighed. The seedling vigor-I and seedling vigor-II of each genotype was calculated by formula as suggested by ISTA, (2001)-

$$\text{Seedling vigor-I} = \text{Germination percentage} \times \text{mean dry weight}$$

$$\text{Seedling vigor-II} = \text{Germination percentage} \times \text{mean seedling length}$$

The fully expanded leaves were selected for the measurement of photosynthetic rate and transpiration rate. The photosynthetic rate and transpiration rate was measured using ADC-LCi-SD portable infra-red gas analyzer (IRGA) instrument on cloudless days (i.e in the presence of more intensity light) between 10.00AM-01.00PM with the interval of 2-3 minutes.

DATA was analyzed using two factorial CRD (Completely Randomized Design) test. Treatments, genotypes and interaction between treatments and genotypes were compared using critical difference (CD) at 5% level of significance with using OP-STAT, online statistical portal, CCS, HAU, Hisar.

Results and Discussion

Germination percentage significantly decreased in all genotype with gradual increase in salinity levels viz.

control, 8 dSm⁻¹, 10 dSm⁻¹, 12 dSm⁻¹ and 14 dSm⁻¹ salinity solutions. Among the 15 genotypes, RH 725 and RH 1512 germinated up to 95 % under all salinity levels, whereas, germination of genotypes RH 1520 and RH 8812 was substantially affected with 37 and 35 % reduction (Fig. 3). As demonstrated in Fig. 4, germination % declined as salinity increased, and the effect increased as salinity increased. The shade of the colors and varied colors in the heatmap of Fig. 4 reflect the range of germination % among genotypes. The yellow tint represents the maximum percentage of germination, while the dark blue color represents the lowest. The lowest germination percentage was found at a salinity level of 14 dSm⁻¹. As illustrated in Fig. 4, the genotype RH 725 had a yellowish orange coloration which showed 95 % germination, but the genotype RH 8812 had a dark blue color showed 55 % germination.

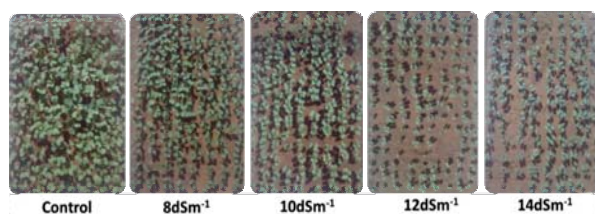


Fig. 3: Growth of Indian mustard under different salinity levels

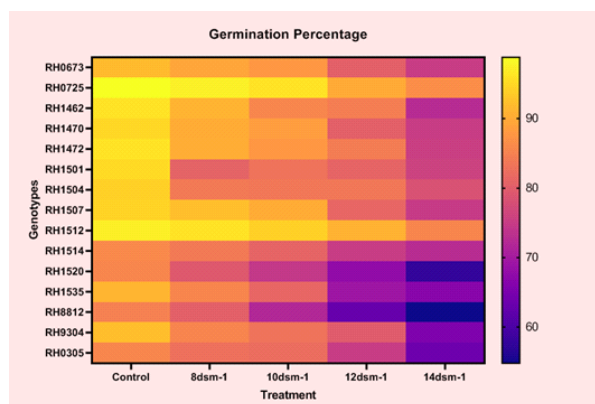


Fig. 4: Heatmap generations using GraphPad Prism. Here the colors and shade of color represent the variation of traits and effect of salinity among the genotypes. This represents the effect of various salinity levels on seed germination (%)

The rate of numerous metabolic processes is slowed by salinity, which reduces the production of various enzymes essential for the germination process. The lower the salt level, the lower the synthesis of certain enzymes will be. Similar effects were seen by Kandil *et al.* (2016) in

cowpea, Nikolai *et al.* (2016) in *Salicornia europaea* L., and Mtilimbanya *et al.* (2020) in *B. juncea* genotypes under salinity stress.

One of the most essential parameters in determining salt tolerance genotypes is dry weight. The dry weight of all genotypes reduces as the saline level rises. The genotypes RH 725 and RH 1512 had less reduction in their dry weight with gradual increase in salinity level, while, maximum reduction was seen in genotype RH 1520 followed by RH 8812 (Fig. 5).

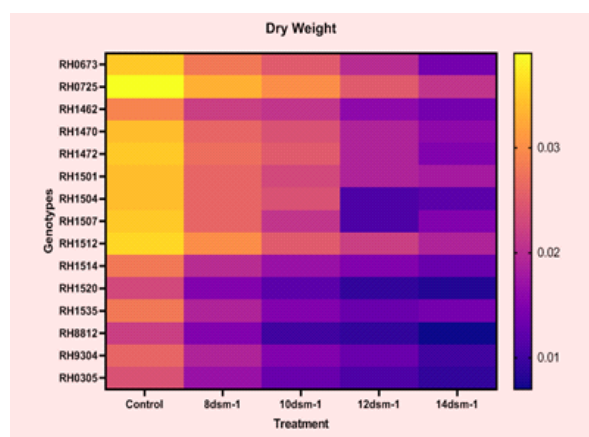


Fig. 5: Heatmap generations using GraphPad Prism. Here the colors and shade of color represent the variation of traits and effect of salinity among the genotypes on dry weight (g)

In heatmap of Fig. 5, the shade of the color and different colors shows the range of the dry weight of seedlings among the genotypes. The highest dry weight was observed in genotype RH 725 (0.039 g) shown in Fig. 5 as yellowish color in control condition and decreased to 0.025 g and 0.021 g with 12 and 14 dSm⁻¹ respectively (shown as purple color in heatmap), whereas the minimum dry weight was observed in genotype RH 8812 (0.022 g) shown as orange bluish color in heatmap under control condition and decrease to 0.009 g and 0.008 g with 12 and 14 dSm⁻¹ respectively (shown as dark blue color in heatmap) considered on the basis irrespective of genotypes. The decrease in seedling dry weight could be related to a decrease in the production of enzymes involved in various metabolic processes, as well as a decrease in seedling length. High salt concentrations increase Na⁺ and K⁺ ions, reducing cell wall flexibility and causing membrane damage, affecting seedling dry weight. Singh *et al.* (2010) found similar results in two *Brassica* species cultivars (Kranti and HC2), while Kandil *et al.* (2016) found similar results in two cowpea cultivars. Another factor contributing to the loss in dry weight is

an osmotic imbalance in root cells, which limits water intake and, as a result, photosynthetic rate (Hooks *et al.*, 2019). The difference in dry weight across genotypes could be attributed to genetic makeup and metabolic processes, which have a direct impact on seedling fresh and dry weight. Nabipour *et al.* (2009) found similar results in *Brassica napus*; Sharma *et al.* (2013) found similar results in 25 Indian mustard genotypes; and Hooks *et al.*, 2019 found similar results in *B. juncea* under salinity stress.

Seedling length also significantly decreased in all genotype with gradual increase in salinity levels (control, 8 dSm⁻¹, 10 dSm⁻¹, 12 dSm⁻¹ and 14 dSm⁻¹) but every genotype shows different effect (Fig. 6). Kumar *et al.* (2008) in different *Brassica* species, and Mojarad *et al.*, (2014) observed different seedling lengths among the 16 *Brassica rapa* cultivars and Pandey *et al.* (2020) observed comparable results in 40 *B. juncea* germplasm. The experimental results are shown as heatmap (Fig. 6), here the color shows the range of seedling length. Among the genotypes, the maximum height retained by the seedling of genotype, RH 725 (6.85 cm and 5.94 cm at 12 dSm⁻¹ and 14 dSm⁻¹ respectively) followed by RH 1512 (5.92 cm and 5.33 cm at 12 dSm⁻¹ and 14 dSm⁻¹ respectively) shown yellow in color in heatmap in Fig. 6, whereas, minimum height retained by the genotype RH 1512 (4.43 cm and 3.72 cm at 12 dSm⁻¹ and 14 dSm⁻¹ respectively) followed by RH 8812 (4.57 cm and 3.40 cm at 12 dSm⁻¹ and 14 dSm⁻¹ respectively) shown in dark blue color.

The toxic effect of NaCl and uneven nutrient intake by seedlings could explain the reduction in seedling length caused by salt. Salinity raises the rate of respiration while lowering the rate of photosynthesis. Salinity reduces cell

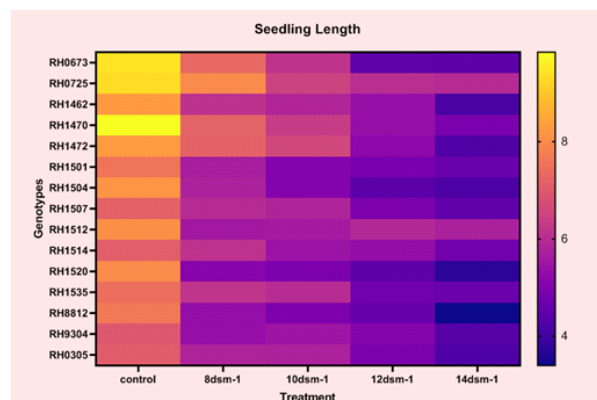


Fig. 6: Heatmap generations using GraphPad Prism. Here the colors and shade of color represent the variation of traits and effect of salinity among the genotypes on seedling length (cm)

division and differentiation, which has a negative impact on physiological processes and may shorten seedling lifespan. El-Hendaway *et al.* (2005) found similar results in wheat genotypes, and Mtilimbanya *et al.* (2020) found similar results in *Brassica juncea* L. genotypes. Shoots of wheat seedlings (*Triticum aestivum* L.) were more sensitive to salt than roots, according to Keiffer and Ungar (1997), Kaya *et al.* (2008), and Moud and Maghsoudo (2008).

On the basis of seedling dry weight, seedling vigor-I was observed. In seedling vigor-I (SV1) similar decreasing pattern was detected as it was in germination % and dry weight under salinity stress. Variation in SV1 was seen in all genotypes, which could be attributable to changes in their genetic makeup (Fig. 7). Similar variation of SV1 among the genotypes/cultivars have also been observed by Ahmed *et al.* (2012) in chickpea cultivars, Ashkan *et al.* (2013) in *Agropyron* species and Shareif *et al.* (2016) in cowpea cultivars. The experimental results are shown as heatmap (Fig. 7); here the color shows the range of seedling vigor-I. The genotypes RH 725 and RH 1512 had maximum values of SV 1 (2.26 and 1.79 at 12 dSm⁻¹ and 14 dSm⁻¹ respectively; 1.99 and 1.67 at 12 dSm⁻¹ and 14 dSm⁻¹ respectively represented as reddish color in heatmap in respective genotypes) at the higher level of salinity stress while the genotypes RH 8812 and RH 1520 (0.58 and 0.39 at 12 dSm⁻¹ and 14 dSm⁻¹ respectively; 0.63 and 0.43 at 12 dSm⁻¹ and 14 dSm⁻¹ respectively represented as dark blue color in heatmap in respective genotypes) showed minimum values.

Shareif *et al.* (2012) recorded similar results in cowpea cultivars, Saeid (2015) in Chicory, Cumin and Fennel, and

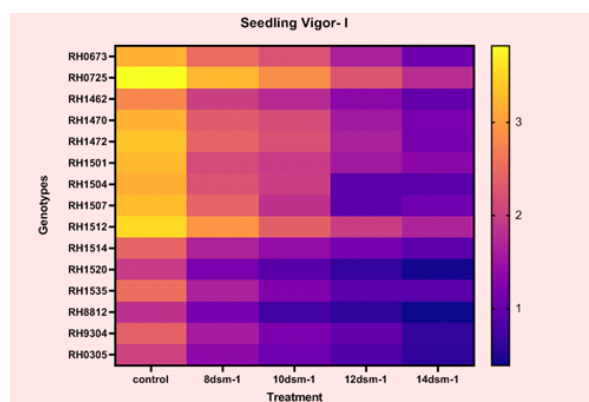


Fig. 7: Heatmap generations using GraphPad Prism representing the effect of various salinity levels on seedling vigor-I (seedling length basis). Here the colors and different shade of colors shows the effect of salinity among the genotypes

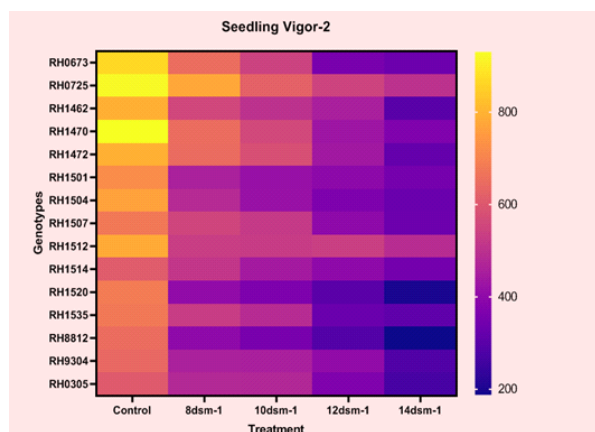


Fig. 8: Heatmap generations using GraphPad Prism representing the effect of various salinity levels on seedling vigor-II (seedling dry weight basis). Here the colors and different shade of colors shows the effect of salinity among the genotypes

Pandey and Penna (2017) in *B. juncea*. Seedling vigor-II (SV2) is based on the seedling length. As mentioned above (Fig.7), seedling length decreased with the increasing level of salinity. However, the SV2 also decreased under different salinity levels (Fig. 8).

The seedling length and germination percentage vary among different genotypes due to above given possible reasons. So, this is obvious that SV2 differed in different genotypes and this type of variations also have been observed by Ahmed *et al.* (2012) in chickpea cultivars, Ashkan *et al.* (2013) in *Agropyron* species and Shareif *et al.* (2016) in cowpea cultivars. Minimum decrease in SV2 was shown at 14 dSm⁻¹ because the high salt concentration affects the seedling length and germination percentage by changing physiological and metabolic processes as observed by El- Shaieny (2015) in *Vigna unguiculata*, Ashebir *et al.* (2013) in cow pea, Rao *et al.* (2002) in chickpea, Lobata *et al.* (2009) in cowpea and Pandey and Penna (2017) in *B. juncea*. The experimental results are shown as heatmap (Fig. 8), here the color shows the range of seedling vigor-II. The genotype showed maximum SV2 was RH 725 with 544.0 at 12 dSm⁻¹ and 501.6 at 14 dSm⁻¹ followed by RH 1512 with 537.2 at 12 dSm⁻¹ and 489.6 at 14 dSm⁻¹ shown as reddish color in Fig. 8 whereas maximum decrease at 12 dSm⁻¹ and 14 dSm⁻¹ in genotype RH 8812 (286.8 and 186.2 at 12 dSm⁻¹ and 14 dSm⁻¹ respectively) followed by RH 1520 (299.6 and 199.6 at 12 dSm⁻¹ and 14 dSm⁻¹ respectively) shown as dark blue color in Fig. 8.

Photosynthetic rate ($\mu\text{Mm}^{-2}\text{s}^{-1}$) differed in all genotypes with different salinity levels at vegetative and flowering

stages as presented in Table 1. The photosynthetic rate decreased with increasing levels of salinity. At vegetative stage, the photosynthetic rate in control plant leaf was found $13.5 \mu\text{Mm}^{-2}\text{s}^{-1}$ which decreased to $11.9 \mu\text{Mm}^{-2}\text{s}^{-1}$ with 8 dSm^{-1} , $10.7 \mu\text{Mm}^{-2}\text{s}^{-1}$ with 10 dSm^{-1} and $10.2 \mu\text{Mm}^{-2}\text{s}^{-1}$ with 12 dSm^{-1} when considered on mean basis irrespective of genotypes (Table 1). Among different genotypes, minimum photosynthetic rate was observed in RH 8812 ($10.2 \mu\text{Mm}^{-2}\text{s}^{-1}$) followed by genotype RH 1520 ($11.3 \mu\text{Mm}^{-2}\text{s}^{-1}$). Highest photosynthetic rate was observed in genotype RH 725 ($12.7 \mu\text{Mm}^{-2}\text{s}^{-1}$) followed by genotype RH 1512 ($12.3 \mu\text{Mm}^{-2}\text{s}^{-1}$) when considered on the mean basis irrespective of salinity levels. Highest percent reduction in photosynthetic rate was observed in genotype RH 1520 (26.4 %) followed by genotype RH 8812 (32.2%) whereas, genotype RH 725 showed that minimum reduction (19.9 %) followed by genotype RH 1512 (19.2 %). Among the interactions maximum photosynthetic rate was observed in genotype RH 725 ($14.8 \mu\text{Mm}^{-2}\text{s}^{-1}$) in control whereas minimum photosynthetic rate was observed in RH 8812 ($8.3 \mu\text{Mm}^{-2}\text{s}^{-1}$) at 12 dSm^{-1} salinity level as shown in Table 1.

At flowering stage, similar trend of photosynthetic rate observed. Maximum photosynthetic rate was observed in genotype RH 725 ($14.9 \mu\text{Mm}^{-2}\text{s}^{-1}$) followed by genotype RH 1512 ($14.1 \mu\text{Mm}^{-2}\text{s}^{-1}$) whereas, minimum photosynthetic rate was observed in genotype RH 8812 ($9.6 \mu\text{Mm}^{-2}\text{s}^{-1}$) followed by genotype RH 1520 ($11.8 \mu\text{Mm}^{-2}\text{s}^{-1}$) when considered on mean basis irrespective of salinity levels. Maximum photosynthetic rate was observed in control ($14.8 \mu\text{Mm}^{-2}\text{s}^{-1}$) which decreased to $13.5 \mu\text{Mm}^{-2}\text{s}^{-1}$ with 8 dSm^{-1} , $11.7 \mu\text{Mm}^{-2}\text{s}^{-1}$ with 10 dSm^{-1} and $10.5 \mu\text{Mm}^{-2}\text{s}^{-1}$ with 12 dSm^{-1} when considered on mean basis irrespective of genotypes. Maximum percent reduction in photosynthetic rate was observed in genotype RH 8812 (33.7 %) followed by genotype RH 1520 (30.4 %) and minimum in genotype RH 725 (17.6 %) followed by genotype RH 1512 (18.8 %) with 12 dSm^{-1} . Among the interactions maximum photosynthetic rate was observed in genotype RH 725 ($16.4 \mu\text{Mm}^{-2}\text{s}^{-1}$) in control and minimum in genotype RH 8812 ($8.96 \mu\text{Mm}^{-2}\text{s}^{-1}$) with 12 dSm^{-1} salinity level (Table 1). Photosynthetic rate decreased with the gradual increase in salinity level at both stages (vegetative and flowering). The reason behind the decrease in photosynthetic rate is, the salinity stress degrades the chlorophyll molecules and also inhibit the synthesis of chlorophyll molecules by inhibiting the enzymes involved in chlorophyll synthesis. Salinity induces the ions accumulation inside the leaves; which results in the stomatal closure so less CO_2 enters and ultimately reduces the photosynthetic rate. Na^+ ions

Table 1: Effect of different levels of salinity on photosynthetic rate ($\mu\text{M m}^{-2}\text{s}^{-1}$) in different genotypes of Indian mustard

Genotype (G)	Salinity level (S)				Mean	% Reduction at 12 dSm ⁻¹ over to control
	Control	8 dSm ⁻¹	10 dSm ⁻¹	12 dSm ⁻¹		
Vegetative Stage						
RH 725	14.88	13.62	12.47	11.92	12.66	19.8
RH 1512	13.67	12.64	11.75	11.04	12.28	19.2
RH 1520	13.14	11.86	10.35	9.67	11.26	26.3
RH 8812	12.32	10.68	9.28	8.35	10.16	32.2
Mean	13.50	11.87	10.73	10.25		
CD at 5%	G=0.04, S=0.04, G×S=0.09					
Flowering Stage						
RH 725	16.36	15.50	14.35	13.47	14.92	17.6
RH 1512	15.87	14.97	13.96	12.88	14.07	18.8
RH 1520	14.34	12.84	10.24	9.97	11.85	30.4
RH 8812	13.52	11.77	9.96	8.96	9.63	33.7
Mean	14.77	13.52	11.68	10.50		
CD at 5%	G=0.01, S=0.01, G×S=0.02					

accumulate in the leaves of plants under salinity which affects the integrity and function of photosynthetic membranes (stomal and grannal thylakoid membranes). The salinity stress reduces the Rubisco activity for CO₂ assimilation. Similar decreasing trend in photosynthetic rate have been recorded by Meloni *et al.* (2003) in cotton.

Effects of different salinity levels on transpiration rate ($\text{mMm}^{-2}\text{s}^{-1}$) in different genotypes at vegetative and flowering stages as presented in Table 2. At vegetative stage, the transpiration rate decreased with increasing

levels of salinity and it was $4.67 \text{ mMm}^{-2}\text{s}^{-1}$ in leaf of control plant which decreased to $4.05 \text{ mMm}^{-2}\text{s}^{-1}$ with 8 dSm⁻¹, $3.79 \text{ mMm}^{-2}\text{s}^{-1}$ with 10 dSm⁻¹, and $3.35 \text{ mMm}^{-2}\text{s}^{-1}$ with 12 dSm⁻¹ when considered on mean basis irrespective of genotypes. Among different genotypes, minimum transpiration rate was observed in genotype RH 8812 ($3.79 \text{ mMm}^{-2}\text{s}^{-1}$) whereas highest transpiration rate was observed in genotype RH 725 ($4.03 \text{ mMm}^{-2}\text{s}^{-1}$) followed by genotype RH 1512 ($3.84 \text{ mMm}^{-2}\text{s}^{-1}$) when considered on the mean basis irrespective of salinity levels. Transpiration rate differed in different genotypes in

Table 2: Effect of different levels of salinity on transpiration rate ($\text{mMm}^{-2}\text{s}^{-1}$) in different genotypes of Indian mustard

Genotype (G)	Salinity level (S)				Mean	% Reduction at 12 dSm ⁻¹ over to control
	Control	8 dSm ⁻¹	10 dSm ⁻¹	12 dSm ⁻¹		
Vegetative Stage						
RH 725	4.92	4.12	3.97	3.12	4.03	36.5
RH 1512	4.90	3.98	3.47	3.01	3.84	38.4
RH 1520	4.85	4.24	3.99	3.85	4.21	20.7
RH 8812	4.03	3.86	3.74	3.54	3.79	12.0
Mean	4.67	4.05	3.79	3.36		
CD at 5%	G=0.01, S=0.01, G×S=0.02					
Flowering Stage						
RH 725	4.86	3.99	3.87	3.063	3.95	36.9
RH 1512	4.85	3.96	3.34	2.987	3.78	38.4
RH 1520	4.78	4.05	3.89	3.897	4.11	18.4
RH 8812	3.95	3.75	3.61	3.500	3.70	11.4
Mean	4.61	3.94	3.68	3.307		
CD at 5%	G=0.01, S=0.01, G×S=0.03					

control condition so, percent reduction was calculated with respect to highest level of salinity and it was observed the maximum percent reduction in transpiration rate was observed in genotype RH 1512 (38.4 %) followed by genotype RH 725 (36.5 %) whereas, genotype RH 8812 showed the minimum reduction (12.0 %) followed by genotype RH 1520 (20.7 %). Among the interactions maximum transpiration rate was observed in genotype RH 725 (4.9 mMm⁻²s⁻¹) in control whereas minimum transpiration rate was observed in genotype RH 8812 (3.54 mMm⁻²s⁻¹) with 12 dSm⁻¹ salinity level (Table 2).

At flowering stage, similar trend of transpiration rate was observed. Maximum transpiration rate was observed in genotype RH 1520 (4.10 mMm⁻²s⁻¹) followed by genotype RH 1512 (3.78 mMm⁻²s⁻¹) whereas, minimum transpiration rate was observed in genotype RH 8812 (3.69 mMm⁻²s⁻¹) followed by genotype RH 725 (3.94 mMm⁻²s⁻¹) when considered on mean basis irrespective of salinity levels. Maximum transpiration rate was observed in control (4.61 mMm⁻²s⁻¹) which decreased to 3.94 mMm⁻²s⁻¹ with 8 dSm⁻¹, 3.67 mMm⁻²s⁻¹ with 10 dSm⁻¹ and 3.30 mMm⁻²s⁻¹ with 12 dSm⁻¹ when considered on mean basis irrespective of genotypes. Minimum percent reduction in transpiration rate was observed in genotype RH 8812 (11.5 %) followed by genotype RH 1520 (18.5 %) and maximum in genotype RH 725 (37.0 %) followed by genotype RH 1512 (38.4 %) with 12 dSm⁻¹. Among the interactions maximum transpiration rate was observed in genotype RH 725 (4.86 mMm⁻²s⁻¹) in control and minimum in genotype RH 8812 (3.69 mMm⁻²s⁻¹) with 12 dSm⁻¹ salinity level. The transpiration rate decreased with the increasing level of salinity. In this study, the highest transpiration rate was observed in control plants while lowest transpiration rate was recorded with higher level of salinity (12 dSm⁻¹). The reason behind the decrease in transpiration rate with increasing salinity levels is that, the salinity reduces the hydraulic conductivity of roots which decreased the rate of flow of water from root to shoot and due to lowering in water content in leaf, which results in stomata closure to

maintain the water status of plants. Similar results of decreasing transpiration rate with salinity have been observed by Neto *et al.* (2004) in maize genotypes, O Leary (1969) in wheat and Prisco (1980) in legumes.

The 1000-seeds weight significantly decreased in all genotype with gradual increase in salinity (Table 3). The maximum 1000-seeds weight was observed in genotype RH 1512 (4.24 g) followed by genotype RH 725 (3.86 g) and minimum was observed in genotype RH 1520 (2.99 g) followed by genotype RH 8812 (3.34 g), when considered on the mean basis irrespective of salinity levels. The maximum 1000-seeds weight was observed as 4.31 g in control and decrease to 3.70 g with 8 dSm⁻¹, 3.42 g with 10 dSm⁻¹, and 3.00 g with 12 dSm⁻¹ considered on mean basis irrespective of genotypes. Minimum percent reduction in weight of 1000-seeds was observed in genotype RH 1512 (12.9 %) followed by genotype RH 725 which got percent reduction of 13.2 % whereas, maximum percent reduction was recorded in genotype RH 1520 (51.4 %) with 12 dSm⁻¹. This was followed by genotype RH 8812 (44.5 %) with 12 dSm⁻¹. Decrease in 1000-seed weight may be due to less oil content and stored starch. Similarly, Suman *et al.* (2016) recorded that no. of pods/plant, no. of seeds/pod and 1000 seed weight reduced under salinity in senna.

Salinity is a severe issue that has an impact on crop growth and yield. Salinity inhibits plant growth, which may be due to the presence of salts in the soil solution, which reduces the plant's ability to uptake of water, resulting in a reduction in growth rate; additionally, if excessive amounts of salt enter the plant through the transpiration stream, there will be injury to cells in the transpiring leaves, resulting in further growth reductions (Greenway and Munns, 1980). Salinity stress makes *B. juncea* extremely vulnerable.

Plant biomass significantly decreased in all genotype with gradual increase in salinity levels. Plant biomass differed

Table 3: Effect of different levels of salinity on 1000-seeds weight (g) in different genotypes of Indian mustard

Genotype (G)	Salinity level (S)					% Reduction at 12 dSm ⁻¹ over to control
	Control	8 dSm ⁻¹	10 dSm ⁻¹	12 dSm ⁻¹	Mean	
RH 725	4.15	3.95	3.74	3.6	3.86	13.2
RH 1512	4.56	4.26	4.17	3.97	4.24	12.9
RH 1520	3.99	3.19	2.85	1.94	2.99	51.4
RH 8812	4.53	3.4	2.92	2.51	3.34	44.5
Mean	4.31	3.7	3.42	3.00		

CD at 5% G=0.14, S=0.14, G×S=0.29

Table 4: Effect of different levels of salinity on plant biomass (g) in different genotypes of Indian mustard

Genotype (G)	Salinity level (S)				Mean	% Reduction at 12 dSm ⁻¹ over to control
	Control	8 dSm ⁻¹	10 dSm ⁻¹	12 dSm ⁻¹		
RH 725	10.0	8.7	8.3	7.9	8.7	20.9
RH 1512	10.4	8.7	8.2	8.0	8.7	23.1
RH 1520	10.2	8.3	7.6	6.6	8.3	35.5
RH 8812	10.3	8.1	7.4	6.7	8.1	34.8
Mean	10.2	8.5	7.9	7.4		

CD at 5% G=0.15, S=0.15, G×S=0.29

in different genotypes as presented in Table 4 indicates maximum plant biomass was observed in genotype RH 1512 (8.7 g) followed by genotype RH 725 (8.7 g) and minimum plant biomass was observed in genotype RH 8812 (8.1 g) followed by genotype RH 1520 (8.3 g), when considered on the mean basis irrespective of salinity levels. Maximum plant biomass was observed in genotype RH 725 (10.2 g) in control and decrease to 8.5 g with 8 dSm⁻¹, 7.9 g with 10 dSm⁻¹, and 7.4 g with 14 dSm⁻¹ considered on mean basis irrespective of genotypes. Minimum percent reduction in plant biomass was observed in genotype RH 725 (20.9 %) followed by genotype RH 1512 (23.1 %) whereas, maximum percent reduction was recorded in genotype RH 1520 (35.5 %) with 12 dSm⁻¹. This was followed by genotype RH 1520 (34.5 %) with 12 dSm⁻¹. Plant biomass differed in different genotypes under salinity as indicated in table-19 and plant biomass decreased with the increasing level of salinity. The reason for decrease in plant biomass under salinity may be because reduction in photosynthetic rate, transpiration rate, respiration and relative water content. Parida and Das (2005) have shown that salinity can reduce total plant biomass.

Conclusion

The impact of salinity on seed germination and growth characteristics in Indian mustard genotypes led to the conclusion that salinity had a significant impact on all of the measured features. The results of this study show that with increasing salinity, germination, dry weight per seedling, seedling length, seedling vigor-I and seedling vigor-II decreased in all genotypes, which is consistent with previous reports that show that these traits provide protection during stressful conditions. These organic solutes and ionic balances could be used to assess salt tolerance in Indian mustard genotypes as physiological parameters. Overall, RH 725 and RH 1512 were found to be salinity tolerant genotypes, while RH 1520 and RH 8812 were found to be salinity sensitive genotypes.

Acknowledgement

I would like to convey my heartfelt gratitude to the institution, which provided me with the necessary resources, as well as to Dr. Sunita Sheok and Dr. JK Sandooja, who assisted me in completing this study.

References

- Agnihotri RK, Palni LMS and Pandey DK. 2006. Screening of landraces of rice under cultivation in *Kumaun Himalayan* for salinity stress during germination and early seedling growth. *Indian J Plant Physiol* **11**: 262-272.
- Ahmed SRH, Sharief AE and Kandil AA. 2012. Germination and seedling growth of some chickpea cultivars (*Cicer arietinum* L.) under salinity stress. *J Basic Appl Sci* **8**:561-571.
- Asha and Dhingra HR. 2007. Salinity mediated changes in yield and nutritive value of chickpea seeds. *Indian J Plant Physiol* **12**(3): 271-275.
- Ashebir Y, Sharma HR, Alemu K and Kebede G. 2013. Latrine use among rural households in northern Ethiopia: A case study in Hawzien district, Tigray. *Int J Environ Sci* **70**: 629-636.
- Ashkan A and Jalal M. 2013. Effects of salinity stress on seed germination and seedling vigor indices of two halophytic plant species. *Int J Agri Crop Sci* **5**(22): 2669-2676.
- Bawa V, Gupta SK, Sharma M, Rai SK, Attri H, Sandhu R and Bhat MIJ. 2019. Screening for salt-tolerant genotypes of *B. juncea* based on growth and physiological attributes. *Chem Sci Rev Lett* **8**: 148-154.
- EL-Hendawy SE, Hu Y, Sakagami JI and Schmidhalter U. 2005. Growth, ion content, gas exchange and water relations of wheat genotypes differing in salt tolerances. *Aust J Agri Res* **56**: 123-134.
- El-Shaieny AH. 2015. Seed germination percentage and early seedling establishment of five *Vigna*

- unguiculata* genotypes under salt stress. *Europ J Exp Biol* **5**: 22–32.
- Greenway H and Munns R. 1980. Mechanisms of salt tolerance in non-halophytes. *Ann Rev Plant Physiol* **30**: 149-190.
- Hooks T, Niu G and Ganjegunte G. 2019. Seedling emergence and seedling growth of mustard and rapeseed genotypes under salt stress. *Agrosyst Geosci Environ* **2**:190062.
- Hu Y, Fricke W and Schmidhalter U. 2005. Salinity and the growth of non-halophytic grass leaves: the role of mineral nutrient distribution. *Functional Plant Biol* **32**: 973–985.
- Kandil AA, Shareif AE and Gad MA. 2016. Effect of salinity on germination and seeding parameters of forage cowpea seed. *Res J Seed Sci* **10**: 17-26.
- Kaya M, Kaya G, Kaya MD, Atak M, Saglam S, Khawar KM and Cintel CY. 2008. Interaction between seed size and NaCl on germination and early seedling growth of some Turkish cultivars of chickpea (*Cicer arietinum*). *J Zhejiang Univ Sci* **9**: 371–377.
- Keiffer CH and Ungar IA. 1997. The effect of extended exposure to hypersaline conditions of the germination of five inland halophyte species. *Am J Bot* **84**:104-111.
- Kumar G, Purty RS, Sharma MP, Singla-Pareek SL and Pareek A. 2009. Physiological responses among *Brassica* species under salinity stress show strong correlation with transcript abundance for SOS pathway-related genes. *J Plant Physiol* **166**: 507–520.
- Lobata ASK, Filho BGS, Costa RCL, Vidigal MCG, Moraes EC, Neto CFO, Rodrigues VLF, Cruz FJR, Ferreira AS, Pita JD and Barreto AGT. 2009. Morphological, physiological and biochemical responses during germination of the cowpea (*Vigna unguiculata*) Cv. Pitiuba seeds under stress. *World J of Agri Sci* **5**: 590-596.
- Meloni, DA, Oliva MA, Martinez CA and Cambraia J. 2003. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environ Exp Bot* **49**: 69-76.
- Mojarad MA, Hassandokht MR, Abdoss V, Tabatabae SA and Larijani K. 2014. Effects of different salinity levels on germination and seedling growth of turnip (*B. rapa*). *Bull Env Pharmacol Life Sci* **3**: 31-33.
- Moud AM and Maghsoudi K. 2008. Salt stress effects on respiration and growth of germinated seeds of different wheat (*T. aestivum*.) cultivars. *World J Agric Sci* **4**: 351–358.
- Mtilimbanya KY, Jakhar ML, Ram M, Ramkunwar, Ahmad S, Chahar and Jat HR. 2020. Assessment of variability parameters for germination and seedling traits in mustard (*B. juncea*) under salinity stress condition. *J. Pharmacogn. Phytochem.* **9**(3): 2151-2154
- Munns R and Tester M. 2008. Mechanisms of salinity tolerance. *Ann Rev Plant Biol* **59**: 651-681.
- Nabipour A, Hemayati SS, Shirzadi MH and Moosavi B. 2009. Study of effects of soluble and soil manganese sulfate on spring canola in Jiroft. *Iran Pl Ecophysiol* **3**: 123-127.
- Neto ADA, Prisco JT, Enéas-Filho J, Lacerda CF, Silva JV, Costa PHA and Gomes-Filho E. 2004. Effects of salt stress on plant growth, stomatal response and solute accumulation of different maize genotypes. *Braz J Plant Physiol* **16**:31-38.
- Nikolai O, Ulbasyn J, Huifan Z and Sergei V. 2016. Effect of salinity on seed germination, growth and ion content in dimorphic seeds of *Salicornia europaea*. *Pl Diversity* **38**: 183-189.
- O Leary JW. 1969. The effect of salinity on the permeability of roots to water. *Israel J Bot* **18**: 1-9.
- Pandey M and Penna S. 2017. Time course of physiological, biochemical, and gene expression changes under short-term salt stress in *B. juncea*. *Crop J* **5**: 219–230
- Pandey SK, Srivastava KK, Negi S, Khan NA and Singh RK. 2020. Variability, trait relationship and path analysis for seed yield and seed quality parameters in Indian mustard (*B. juncea*) *J Oilseed Brassica* **11**: 69-76.
- Parida AK, and Das AB. 2005. Salt tolerance and salinity effects on plants: a review. *Ecotoxicol Environ Saf* **60**: 324-349.
- Pervaiz Z, Afzal M, Xi S, Xiaoe Y and Ancheng L. 2002. Physiological parameters of salt tolerance in wheat. *Asian J Plant Sci* **1**: 478–81.
- Prisco JT. 1980. Alguns aspectos da fisiologia do “stress” salino. *Rev Bras Bo.* **3**: 85-94.
- Rao DLN, Giller KE, Yeo A and Flowers TJ. 2002. The effects of salinity and sodicity upon nodulation and nitrogen fixation in chickpea (*C. arietinum*). *Ann Bot* **89**: 563-570.
- Saeid Hokmalipour 2015. Effect of salinity and temperature on seed germination and seed vigor index of chicory (*Chichorium intyus*), cumin (*Cuminum cyminium*) and fennel (*Foeniculum vulgare*). *Indian J of Sci and Tech* **8**: 35.
- Shareif AE, Kandil AA and Gad MA. 2016. Effect of salinity on germination and seeding parameters of forage cowpea seed. *Res J Seed Sci* **10**: 17-26.

- Sharief AE, Kandil AA, Abido WAE Ibrahim MM. (2012). Effect of salinity on seed germination and seedling characters of some forage sorghum cultivars. *Int J of Agric Sci* **4**: 306-311.
- Sharma P, Sardana V and Banga SS. 2013. Salt tolerance of Indian mustard (*B. juncea*) at germination and early seedling growth. *Env Exp Biol* **11**: 39–46.
- Singh D, Arya RK, Chandra N, Niwas R and Salisbury P. 2010. Genetic diversity studies in relation to seed yield and its component traits in Indian mustard (*B. juncea*). *J Oilseed Brassica* **1**: 19- 22.
- Suman B, Savita D, Poonam K and Manohar L. 2016. Yield attributes and biochemical constituents of al constituents of medicinal plant senna (*Cassia angustifolia*) as affected by salinity. *The Bioscan* **11**: 737-740.
- Zamani Z, Nezami MT, Habibi D and Khorshidi MB. 2010. Effect of quantitative and qualitative performance of four canola cultivars (*B. napus*) to salinity conditions. *Adv Env Biol* **4**: 422-427.