Research Article

Screening for Salt-Tolerant Genotypes of Brassica Juncea L. Based On Growth and Physiological Attributes

Vanya Bawa*[1], Surinder Kumar Gupta[1], Manmohan Sharma[2], S. K. Rai[1], Heena Attri[1], Rubby Sandhu, and M. Iqbal Jeelani Bhat[3]

[1]Division of Plant Breeding and Genetics, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu, 180009, India
[2]School of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu, 180009, India
[3]Division of Statistics & Computer Science, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu, 180009, India

Abstract

In *Brassica juncea* species Salt stress is one of the key abiotic stress that disturbing its normal morpho-biochemical processes. Therefore, the present study was designed to screen the different genotypes under the effect of different NaCl treatments 0.42, 0.56, 0.7 g/ 100ml. Various morphological and biochemical characters are evaluated and The effect of salinity on, shoot/ root fresh and dry weight, proline and chlorophyll contents was recorded after 30 days of sowing. Variance analysis showed that all traits were significantly affected by salinity ans have shown difference in salinity tolerance percentage. The salinity tolerance percentage was calculated which was observed to be significant along with differences between genotypes in most traits were also significant. The genotype Cutlass, PGR 12585, VNIIMK 351showed good biochemical and morphological performance at all stress levels followed by Lethbridge 22A as compared to Check variety RSPR03. The genotype Donskaja 4 gave very poor performance and retard growth. Chlorophyll contents were decreased with the increase of salt concentration. While, the proline contents was increased with raising of salt stress. The genotype PGR 12585 showed maximum tolerance to salt stress at early germination stages as compare to check RSPR03 one. The present study will serve as easiest method to screen quick salt tolerant genotypes among plant species against salt stress. While, the proline contents was increased with raising of salt stress. The genotype PGR 12585 showed maximum tolerance to salt stress at early germination stages as compare to check RSPR03 one. The present study will serve as easiest method to screen quick salt tolerant genotypes among plant species against salt stress.

Keywords: Brassica juncea, germination, salt stress, salt tolerance

***Correspondence**
Author: Vanya Bawa
Email: vanyaadarsh@gmail.com

Introduction

Salinity is one of the major abiotic stress in India and Salt affected area in India is 13.3 million hectare. Andaman and Nicobar Islands, West Bengal, Orissa, Andhra Pradesh, Pondicherry, Tamil Nadu, Kerala, Maharashtra, Gujarat, Uttar Pradesh and Haryana have salinity problem as reported data by the Irrigation Department of Uttar Pradesh [11]. Excess amount of NaCl occurs as an abiotic environmental factor in many places such as salt deserts in the arid and semi-arid areas. During the last decades, apart from the natural salinity, salinization of soils due to intensive agriculture and irrigation is also becoming a major concern in agriculture. The main effect is Exposure to plants, as high salt concentrations causes ion imbalance, ion toxicity and hyper osmotic stress ultimately causing the death of plant or poor growth productivity [15]. Salinization is constraint for sustainable development of agricultural production. The majority of staple crop plants are susceptible and cannot survive under saline condition which produces the physiological and biochemical disorders which alters phenological stages of plants and leads to a series of changes in metabolic activities including enzymatic and photosynthetic activity. It was noted that soils with high salt concentrations with Na_, Cl^1_ and SO_4 _ Ca^2_, Mg^2_, as the major ionic components, which interfere with crop growth by: (i) restricting water availability causing high osmotic pressure of with respect to soil solution where by creating an inbalance between essential nutrients, specific ion toxicities, the degradation of photosynthetic pigments [10][14].

Whereas the present study is concerned with Oilseed brassica. It occupies the third place of the human food needs, and Indian mustard (*Brassica juncea* L. Czern. & Coss) due to the high oil yield and quality of oil thorough the large amounts of polyunsaturated fatty acids (PUFA) is preferable compared to other oilseeds [13]. Salinity as one of

the most common environmental stresses in arid and semi-arid areas reduced crop yield severely [9] and Arid region is listed among the agro-climatic zones of India. But to feed the huge population the bottleneck have to be eradicated using modern technological approaches for enhancing the land use availability for better cultivation practices. For optimal use of the salt affected soils, planting the salt resistant crops is one of the essential strategies. Because the saline soil improvement needs more time and it is not economical, therefore, finding tolerant genotypes needed our emphasis [11]. Before starting and performing any breeding program it is essential to identify varieties and genotypes resistant to salinity, recognition of characteristics which related to salt tolerance in plants and their genotypes will be the another important key feature [3]. The previous Salt stress researches indicated that salt tolerance is not a simple and monogenic trait [18], so in breeding programs for resistance to salt, various secondary traits that was associated with stress viz. Photosynthetic activities, chlorophyll content, plant fresh and dry matter and other related traits can examined to obtain more precise and comprehensive results to screen the germplasm [17]. However, the reports on the salt tolerance in mustard genotypes are meagre and salt stress analysis have no optimized protocol to screen salt tolerant genotypes at early germination and developemental stage. Therefore, the present study was conducted to develop a quick, novel screening system for the identification and further evaluation of *Brassica juncea* at early germination stage under artificial application of NaCl with known Electrical conductivity.

Materials and Methods

The experiment was performed in a glass house of the Department of Plant Breeding & Genetics, SKUAST-J, India, under natural conditions. A total of 9 genotypes which included 8 genotypes of *Brassica juncea* L. Czern. & Coss. and one established variety RSPR-03 as a check was tested shown in **Table 1**. The plastic pots, lined with polythene bags (to avoid contamination), were filled with sand. The pots were arranged in a complete randomized design and three replicates. The seeds were surface sterilized with 0.01% mercuric chloride solution and washed 2-3 times with double distilled water. The sterilized seeds were sown in earthen pots of 25x25 cm containing salt treatments soil amended. These pots were placed in net house under natural conditions. Thirty-day-old plants were sampled to assess the following parameters. The experiment was conducted according to simple randomised block design. The analysis of variance (ANOVA) was performed as described in **Table 2** (8).

Table 1 Brassica juncea germplasm and their source countries.

S no.	*Crop*	Germplasm name	Country/ Source
1	*Brassica juncea*	Cutlass	Canada
2	*Brassica juncea*	63-0134-68	Spain
3	*Brassica juncea*	Lethbridge 22A	Canada
4	*Brassica juncea*	PGR 12585	Pakistan
5	*Brassica juncea*	Donskaja 4	Russian Federation
6	*Brassica juncea*	VNIIMK 351	Russian Federation
7	*Brassica juncea*	Volgogradskaja 189/191	Russian Federation
8	*Brassica juncea*	J807/1/6	United Kingdom
9	*Brassica juncea*	RSPR03	India

Table 2 ANALYSIS OF VARIANCE TABLE for different traits

Sources of Variation	Germination Percentage	Proline Content	Chlorophyll content	Salt Tolerance
Cultivar	*20229.61**	1.76*	1962.63*	16400.62*
Salinity	*1141.47**	0.49*	1 10.25*	2998.58*
Cultivar x Salinity	*1205.98**	0.26*	117.92*	3135.44*
Error	**239.26**	**0.10**	**33.70**	**237.41**

Seeds Germination with Different Salt Levels

The sterilized seeds were kept on the top of filter papers inside Petri dishes. About 3-4 ml of NaCl was added at 3 different concentrations 0.42, 0.56, 0.7 g/100ml of and a control. After germination the germinated ones were sown in treated soil pots.

Fresh mass, dry mass, length per plant

Plants were removed along with soil and dipped in water to dislodge the adhering soil particles without injuring the roots. The roots were cut from plants and blotted. The blotted roots were weighed to record their fresh mass and

placed in an oven (808C for 72 h). The samples were weighed again to record their dry mass. Salt tolerance indices were calculated for fresh and dry mass of plants by dividing respective data from saline-treated plants with those of the control. The data was subjected to statistical analysis by using software Sheoran, O. P. S. T. A. T.

SPAD chlorophyll

A SPAD chlorophyll meter (Minolta) was used to assess SPAD value of chlorophyll in fresh leaves.

Proline content

The proline content in fresh leaf was determined by adopting the method of (4), extracted in sulphosalicylic acid. To the extract, an equal volume of glacial acetic acid and ninhydrin solutions were added. The sample was heated at 100 degree Celcius, to which 5 ml of toluene was added after cooling in ice bath. The absorbance of toluene layer was read at 528 nm, on a spectrophotometer.

Results and Discussions

Proline content

Proline, an amino acid, plays important role in plants under abiotic stress conditions in case of high saline soils and drought conditions. In abiotic stressful condition plant it provide protection to plants by three ways; by maintaining cell turgor or osmotic balance; stabilizing membranes thereby preventing electrolyte leakage; and normalize the reactive oxygen species (ROS) concentration. In present study the proline content was recorded at different salt concentrations in all 9 genotypes that were raised under the salt stress. The mean values of maximum proline contents 4.96, 4.545, 5.575 and 5.165 µmol g–1were recorded in genotype PGR 12585 at salinity level viz. 0.42, 0.56, 0.7 g/ 100ml and control where as mean values i.e 4.021, 3.859, 4.307 and 3.679 µmol g^{-1} of check variety RSPR03 was observed on the same level of salinity treatment respectively. The similar type increase was calculated in all other genotypes at high salt concentrations. The lowest proline content was recorded in genotype no. Donskaja 4 as its mortality rate was higher than others at all stress levels as compare to other genotypes and check RSPR03.

Chlorophyll

Plants of all the varieties that were raised from the soil amended with different levels of salinity showed a linear decrease in the values of SPAD chlorophyll and photosynthetisis. The genotype 63-0134-68 and Lethbridge 22A exhibited a minimum 37.55 and 34.50 respectively in terms of mean values decrease in response to the highest level of salinity 0.7 g/100 ml as values of SPAD chlorophyll, where as maximum genotype J807/1/6 i.e. 24.665 response was observed. Mean value of RSPR03 was 26.720 i.e check performed better overall genotypes in terms of chlorophyll content in highest salinity level i.e 0.7g/100ml. However, the lowest was recorded in genotype Zaria in treatment T2 i.e 0.5g/100ml and genotype J807/1/6 in treatment T3 i.e. 0.7g/100ml and have showed chlorophyll value 12.50 and 10.00 under severe salinity level. The check RSPR03 has shown moderate response under all treatments with average mean value 28.75. Despite the increment of chlorophyll content in some of the genotypes in this research, there are many reports that have shown the decrement of this trait in different plants under the salt stress [15], [6]. This might caused by increasing the density of chlorophyll in leaf area due to leaf area decline under salt stress.

Screening the Effects of Salt Stress on Fresh, Dry Weight of B. Juncea genotypes in terms of Salinity tolerance percentage

The salt stress negatively inhibits the fresh and dry weight of *B. juncea* sub-species. The maximum total fresh and dry weight was recorded in genotype Lethbridge 22A under highest level of salinity 0.7 g/ml. The mean values of Total fresh weights were recorded under treatment T2 with salinity level 0.56g/100ml for genotypes PGR 12585, VNIIMK 351, Volgogradskaja 189/191, J807/1/6 i.e 109.84, 145.53, 147.11, 122.53 (gms) respectively, the stability of genotypes under this treatment was observed to be optimum. Our findings showed that the genotypes 63-0134-68, Lethbridge 22A and PGR 12585 gave close to stable performance over check variety RSPR03 in all treatments as shown in **Figure 1-3**. The susceptible check shows low shoot fresh and dry weight in all salt treatments consequently low salt tolerance percentage. The Figure 1 the genotype PGR 12585 showing the highest accumulation of dry matter content and highest salinity tolerance percentage value in T 3. In Figure 2 the genotypes PGR 12585 and VNIIMK 351 have shown higher performance in terms of highest salinity tolerance percentage at the salinity concentration 0.56g/100ml whereas Cutlass and Donskaja 4 genotypes have shown lowest tolerance in terms of

salinity in T2 Figure 2. The genotypes 63-0134-68, PGR 12585, VNIIMK 351 have shown stable salt tolerance percentage in all the three salinity treatment. It is to be noted that many genotypes have performed better over the susceptible check in comparison to the controlled conditions. As the present study is concerned with all attributes which are directly or indirectly related to salt tolerance level which have shown significant difference among germplasm for the estimated traits as shown in Table 2. These results are in agreement with those of [7] shown that salinity caused a marked reduction in growth parameters similarly as in sugar beet plants

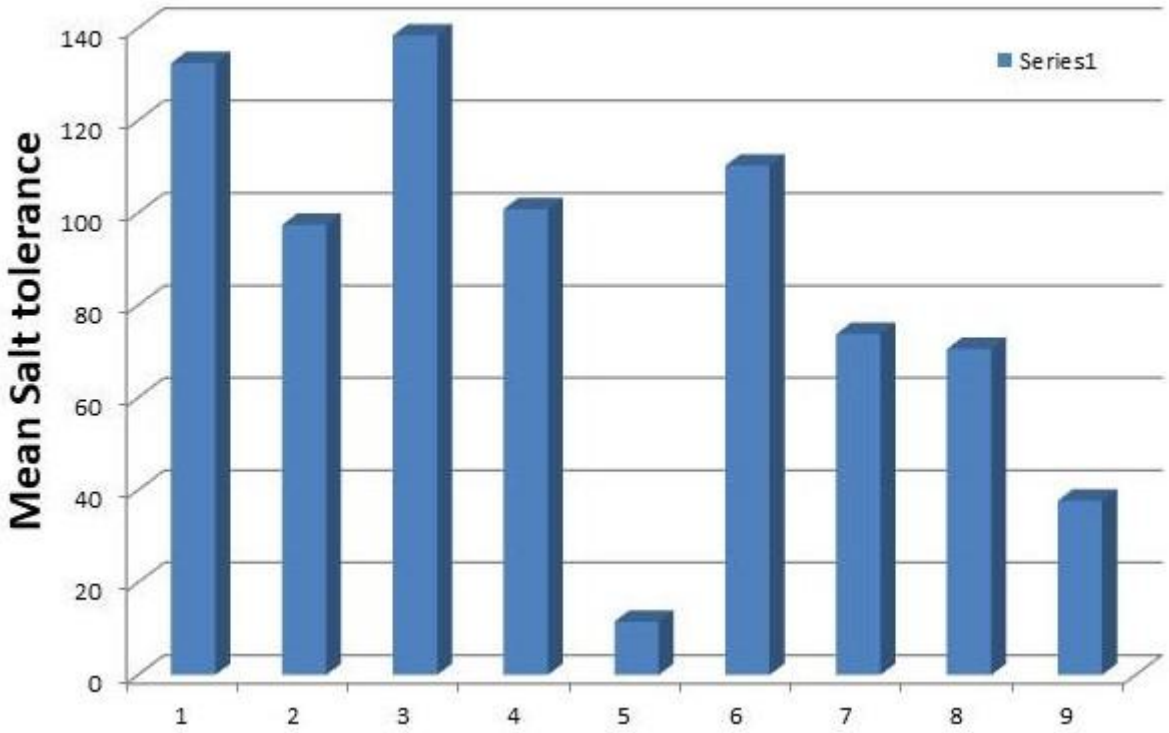

Figure 1 Treatment 1 –Salinity level 0.42 g/ 100ml, 1 to9 number of genotypes

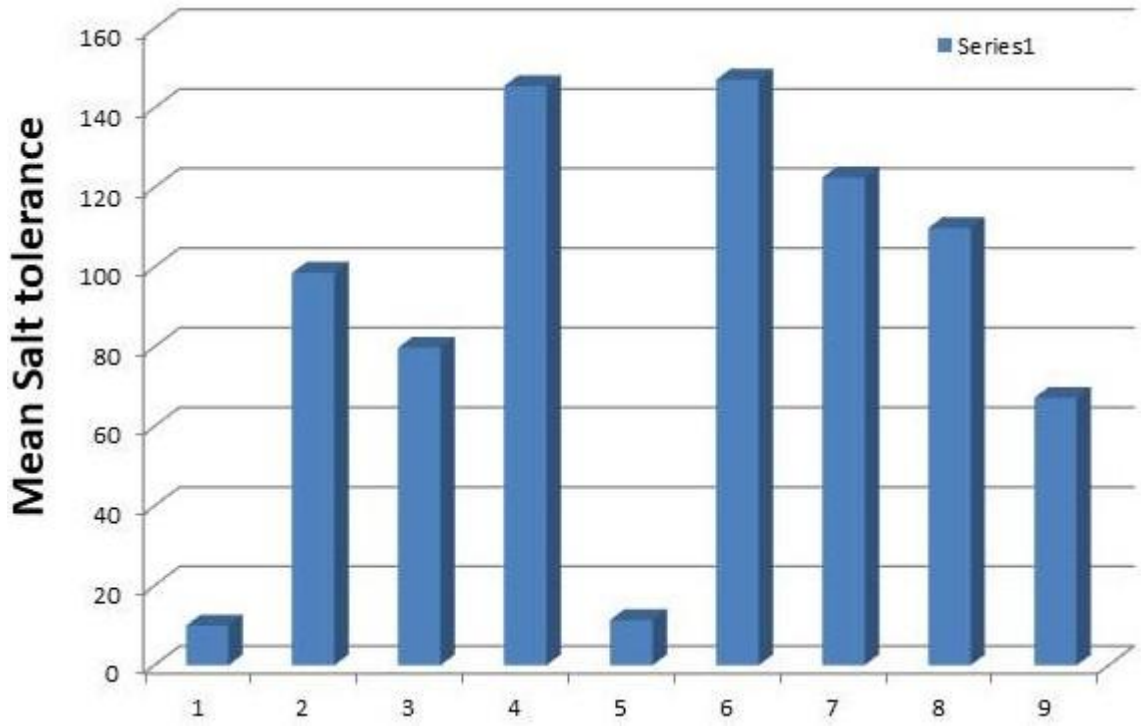

Figure 2 Treatment 2 - 0.56 g/ 100ml, 1 to9 number of genotypes

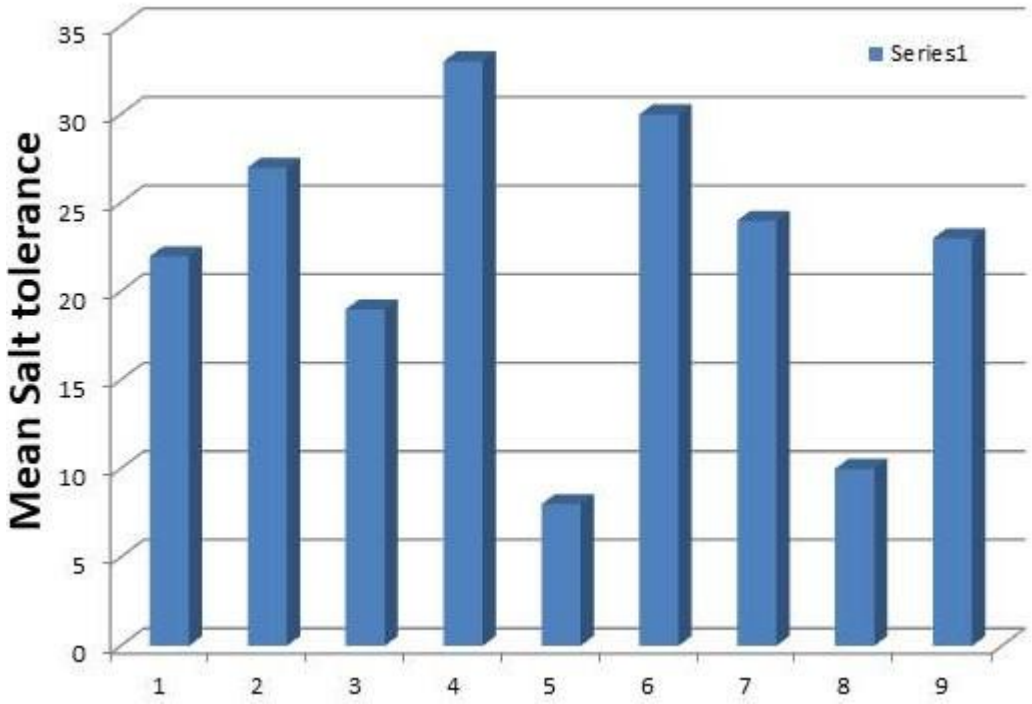

Figure 3 Treatment 3 –Salinity level 0.7 g/ 100ml. 1 to9 number of genotypes

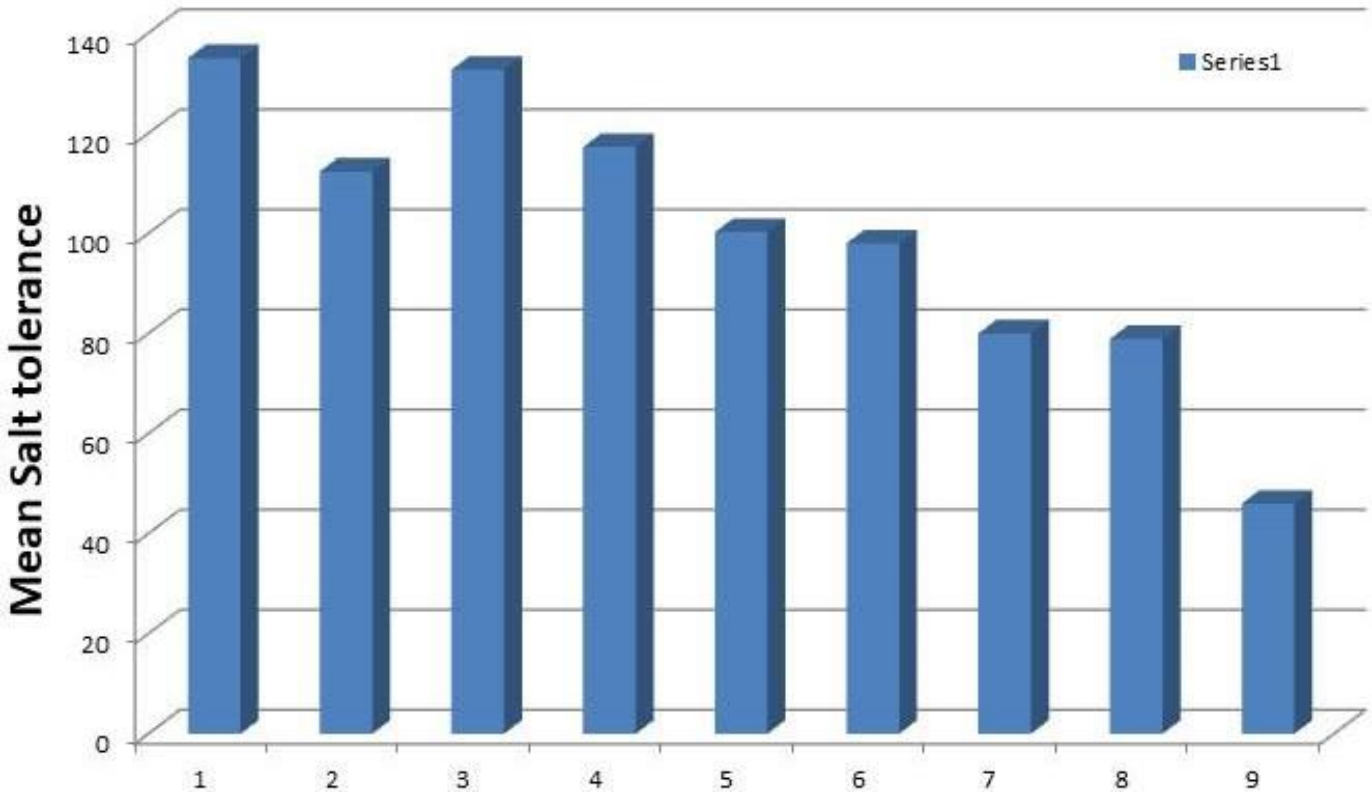

Figure 4 Treatment 4 – Control, 1 to9 number of genotypes

Conclusion

This experiment showed significant variability among genotypes, which can be used in subsequent researches and breeding for salt tolerance genotypes. Since the genotypes responded to all three treatments differently against susceptible check but screening was efficient for some genotypes in terms of growth related traits under salinity stress treatments.

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