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In Vitro Screening of Introgressed Brassica Genotype against Callus Induction Ability under Salt Tress Condition

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Abstract

The experiment was conducted at the Biotechnology Laboratory at the Department of Biotechnology, Bangladesh Agricultural University, Mymensingh, during the period from January to May 2007 with the objectives of *in-vitro* screening of thirty *Brassica* genotypes of Bangladesh for NaCl tolerance at seedling stage and to modify the general tissue culture protocols of *Brassica* for satisfying the present saline media situation for appropriate testing of the performance of genotypes through germination of seeds. Seeds of these thirty genotypes were germinated in Murashige and Skoog (MS) media containing 0, 0.3%, 0.6% and 0.9% NaCl. The genotypes and the different salinity levels exhibited significant variation in respect to all the characters studied. The regression coefficient estimates indicated variable degree of increase or decrease in performance of the individual genotypes as influenced by different salinity. Germination percentage, number of germinated seedling, seedling height and weight and root length were highest in control and lowest at 0.9% (14.1EC) salinity level. Fresh root weight and shoot length minimum at 0.9% salinity levels. Considering all the characters the genotypes G₂₆ which was one of the selection of the cross between *Brassica napus* × *Brassica juncea*, G₂₅ one of the line of cross between *Brassica napus* × *Brassica juncea*, G₁₆ one of the line of cross between *Brassica napus* × *Brassica juncea*, G₁₇ and G₂₄ were found to be the more saline tolerant genotypes than the others. Again the genotypes GH one of the line of cross between *Brassica napus* × *Brassica juncea*, G₁₀ one of the line of cross between *Brassica napus* × *Brassica juncea*, G₂₀ one of the line of cross between *Brassica napus* × *Brassica juncea*, G₁₂, G₁₄ and G₁₅ were more susceptible than others. G₂₂ was not germinated in the saline and control condition.

Keywords: *In Vitro*; Screening; *Brassica*; Callus; Salt stress

Introduction

Brassica represented by rapeseed and mustard which plays an important role in the World for the production of vegetable oil. The genus *Brassica* belongs to the family Cruciferae and has generally been divided into three groups namely (a) the mustard (b) the rapeseed and (c) the cole. The component species of rapeseed are *Brassica napus* and *Brassica campestris* L. while mustard group includes *Brassica juncea* Czern and Coss, *Brassica nigra* Koch and *Brassica carniata* Braun [1]. Among these species varieties of *B. rapa*, *B. juncea* and *B. napus* are commercially grown in Bangladesh. Bangladesh is facing a huge shortage of edible oils. On Recommended Dietary Allowance (RDA) basis, Bangladesh requires 0.29 million tons of oil equivalent to 0.8 million tons of oil seeds for nourishing her people. At present the oil seed production is about 27 million tons, which covers only 43% of the domestic need [2]. In view of insufficient production, a huge amount of foreign exchange involving over Tk. 3595 crores is being spent every year for importing edible oils and oil seeds [3]. In recent years there have been declining trends in both acreage and production of oil crops in Bangladesh. This reduction in acreage is due to more area coverage by cereals and vegetables of winter season in order to meet the country's food demand, and the decrease in total production has been due to reduction in acreage, although the yield has increased in recent years.

In Bangladesh more than 30% of the net cultivable area is in the coastal belts. Out of 2.85 million hectares of the coastal and off-shore landmass, about 0.83 million hectares are affected by different degrees of salinity [4]. However, this area has further increase till recently agricultural land use patterns of this area showed mostly single cropping with rice during summer season.

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Table 1: The materials used in the experiment have been shown below.

Selected genotype of 5 th generation	F ₅ line numbers	Introgressed product of
G ₁	2.9	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₂	4.1	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₃	4.7	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₄	5.2	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₅	5.3	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₆	5.4	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₇	5.6	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₈	5.9	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₉	5.1	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₁₀	6.5	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₁₁	6.6	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₁₂	6.7	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₁₃	6.8	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₁₄	6.9	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₁₅	6.1	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₁₆	9.7	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₁₇	9.6	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₁₈	9.8	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₁₉	10.6	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₂₀	11.1	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₂₁	11.7	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₂₂	12.9	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₂₃	13.7	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₂₄	13.1	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₂₅	14.1	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₂₆	14.7	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₂₇	16.6	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₂₈	16.5	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₂₉	16.1	<i>Brassica napus</i> × <i>Brassica juncea</i>
G ₃₀	19.6	<i>Brassica napus</i> × <i>Brassica juncea</i>

Rice is the major crop cultivated in salt affected areas of the country in Boro season. After harvesting Aman rice, a vast area of land in the coastal belt remains either unused or covered by some minor crops at marginal level of production practices. In salt free areas of coastal belt, the farmers usually prefer to grow wheat, different kinds of pulses and vegetables after harvesting Aman rice. In the coastal belt of Bangladesh, mustard and rapeseed are usually not cultivated mostly due to non-availability of varieties adaptable to saline conditions. However, a few exploratory experiments were conducted in relation to salt tolerance of mustard and rapeseed in Bangladesh. Therefore, there is a need for studying the level of salinity tolerance of the developed varieties of mustard and rapeseed. Steps should also be taken to develop varieties of mustard and rapeseed suitable for growing in the coastal belt of Bangladesh so that these areas may be brought under cultivation of rapeseed and mustard.

Salinity tolerance test in various crops have been carried out by different scientists in different ways. In the laboratory condition, it has been carried out in sand culture using petridishes [5], soil culture using pots [6], water culture as hydroponics [7] and directly

Table 2: The composition and concentrations used for this media are given below.

Macro nutrients	Amount per liter (mg)
KNO ₃	1900
NH ₄ NO ₃	1650
MgSO ₄ .7H ₂ O	370
CaCl ₂ .2H ₂ O	440
KH ₂ PO ₄	170
Micro nutrients	Amount per liter (mg)
MnSO ₄ .4H ₂ O	22.3
H ₃ BO ₃	6.2
ZnSO ₄ .7H ₂ O	8.6
Na ₂ MoO ₄ .2H ₂ O	0.25
CuSO ₄ .5H ₂ O	0.025
CoCl ₂ .6H ₂ O	0.025
KI	0.83
Iron source	Amount per liter (mg)
FeSO ₄ .7H ₂ O	-27.8
Na ₂ EDTA.2H ₂ O	37.3
Vitamins	Amount per liter (mg)
Nicotinic acid	0.5
Pyridoxine HC1	0.5
Thiamine HC1	0.1
Glycine	2
Myo inositol	100mg
Sucrose	30000mg

in the field of the saline zone of Bangladesh [8]. Although salinity tolerance tests are being carried out directly in the saline soils, but it is difficult to maintain the exact level of salinity. Because with the advancement of time and plant growth there would be constant change in the macroclimatic conditions i.e. loss of moisture from soil which increases the salt concentration at the root level of the crop being grown. In studying the genetics of salinity, the possibility of studying under controlled condition of laboratory appeared to be better than field condition particularly when the seedlings stage is considered important. In order to study the impact of salinity in seed and seedling stage, seeds can be grown under laboratory condition using glass vials containing [9] media treated with different concentrations of salt (NaCl). Through this method a large number of plants can be screened and obtained within short time. Again, to variation in a genotype tissue culture may be used as an aid as tissue culture techniques have already offered the advantage of fast multiplication rate of plants than conventional propagation method. Many plant breeding programmes of today are taking advantage of this propagation method for generating new variations as well as screening different genetic materials under constant condition of growth media. Therefore an experiment was conducted for *in-vitro*

Table 3:

Growth regulators (Phytohormones)	Solvents
BAP	O.INNaOH
AgNO ₃	Distilled water
2,4-D	INNaOH

screening of thirty rapeseed and mustard genotypes of Bangladesh for NaCl tolerance at seedling stage and to modify the general tissue culture protocols of *Brassica* for satisfying the present saline media situation for appropriate testing of the performance of genotypes through germination of seeds.

Materials and Methods

The experiment was conducted at the Biotechnology Laboratory at the Department of Biotechnology, Bangladesh Agricultural University, Mymensingh, during the period from January to May 2007 with the objectives of *in-vitro* screening of thirty *Brassica* genotypes of Bangladesh for NaCl tolerance at seedling stage and to modify the general tissue culture protocols of *Brassica* for satisfying the present saline media situation for appropriate testing of the performance of genotypes through germination of seeds. The experiment was conducted with 30 selected genotypes from the introgressed products of *Brassica napus* and *Brassica juncea* as shown in Table 1.

MS media containing 0%, 0.3%, 0.6% and 0.9% NaCl were used in the experiment [9]. The composition of the MS media is shown in Table 2.

Preparation of stock solution

The first step in the preparation of the media was the preparation of stock solutions. As different ingredients were required in different concentrations, separate stock solutions for macro nutrients, Vitamins etc. were prepared.

a) Stock Solution of macro nutrients: Stock solution of macro-nutrients was prepared upto 10 times the concentration of the final media in 1000ml. of distilled water. Ten times the weight of the salts required per liter of the media were weighed properly and dissolved in about 750ml. of distilled water and then made upto 1000ml by further addition of distilled water. The stock solution was labeled and stored in a refrigerator at 4°C.

b) Stock solution of micro nutrients: The stock solution of micro-nutrients was made upto 100 times the final strength of necessary constituents of the media in 1000ml. of distilled water as described for the stock solution of macro nutrients. The stock solution was labeled and stored in a refrigerator at 4°C.

c) Stock solution of iron: The stock solution of iron was made upto 10 times the final strength of necessary constituents of the media in 1000ml. of distilled water as described for the stock solution of macro nutrients. The solution was labeled and stored in a refrigerator at 4°C.

d) Stock solution of vitamins: Vitamin solutions were prepared 100 times the concentration of their final strength and stored at - 20°C in 10ml. aliquots.

e) Preparation of salt solution (10% NaCl solution): To prepare the salt solution, 10gm of laboratory grade NaCl was placed in a beaker and then it was dissolved in 50ml. of distilled water. The solution was then washed off with distilled water and collected in a 100ml. measuring cylinder and made upto 100ml. by further addition of distilled water.

f) Hormonal stock solutions: Stock solution of hormones was prepared separately at 100ppm by dissolving the desired quantity of ingredients in appropriate solvent and made the required final volume with distilled water and stored in a refrigerator at 4°C for later use.

The following growth regulators (phytohormone supplements) were used in the present investigation

- a) Auxin: 2, 4-D (2,4-Dichlorodiphenoxy acetic acid).
- b) Cytokinins: 6-benzyl amino purine (BAP) Silver nitrate (AgNO₃) (as cytokinin).

The growth regulators were dissolved in appropriate solvent as shown against each of them

To prepare stock solution of any of these hormones, 25mg of each of the hormone powder was taken on a clean watch glass and dissolved in 1ml of the particular solvent. Then the mixture was taken in a 250ml measuring cylinder and the volume was made up to 250ml by the further addition of distilled water. After that the solution was poured into a clean plastic or glass container and stored at 4°C temperature and used for a maximum period of 2 weeks.

Step followed for the preparation of culture media for experiment

I. The required Volume of each stock solution (100ml macro nutrients, 100ml iron source, 10ml. micro nutrients, 10ml. vitamin) were poured into a beaker and mixed.

II. About 200ml distilled water was added to this.

III. 100mg of myo inositol was added directly to the solution and dissolved well.

IV. 30gm of sucrose was added to this solution and gently agitated to dissolve completely.

V. The solution was poured into a 500ml measuring cylinder and the volume was made upto 500ml.

VI. This solution was poured into 4 beakers taking 125ml of the solution in each beaker. Required Volume of NaCl solution was directly added to the solutions in the beakers, with a View to getting 0.3%, 0.6% and 0.9% salinity in the media. (0.3%=4.6ds m⁻¹, 0.6%=9.4ds m⁻¹ 0.9%=14.1dsm⁻¹). In a beaker there was no NaCl solution and it was considered as control.

VII. The 4 solutions were poured separately into 500ml measuring cylinder and each was made 250ml by adding distilled water. Each of the solutions was poured into 500ml conical flask and mixed well.

VIII. The pH of the media was adjusted to 5.8 with a digital pH meter with the help of 0.1 N NaOH or 0.1 N HCl as necessary.

IX. After adjusting the pH, 8gm of agar was divided into 4 groups and in each solution 2gm of agar was added. Each mixture was then gently heated in a hot plate magnetic stirrer till complete dissolution of agar took place. Care was taken so that the solution did not get boil while melting agar.

X. About 20ml of warm media was dispensed into small glass jars or vials and were closed with black plastic caps and marked to indicate specific treatment.

Sterilization: In *in-vitro* techniques, aseptic condition is a prerequisite. So all instruments, glassware and culture media were sterilized.

Sterilization of culture media: The culture vessels containing the media were autoclaved with 1.16kg/cm² of pressure at 121°C for 20 minutes. After autoclaving the culture vessels containing the culture media were allowed to cool after sterilization.

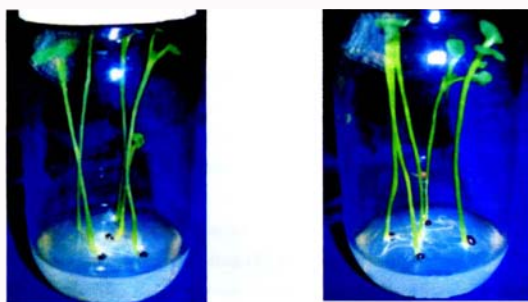


Plate 1: *In vitro* germinated G₂₆, selected line of cross *Brassica napus* × *Brassica juncea* in control and at 0.3% salinity levels.

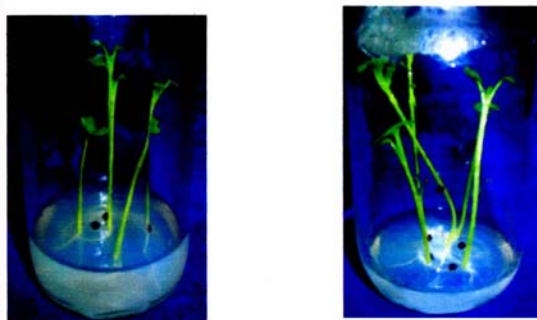


Plate 2: *In vitro* germinated G₂₆, selected line of cross *Brassica napus* × *Brassica juncea* at 0.6% and 0.9% salinity levels.

Plate 1: *In Vitro* germinated G₂₆, selected line of cross *Brassica napus* × *Brassica juncea* in control and at 0.3% salinity levels.

Plate 2: *In Vitro* germinated G₂₆, selected line of cross *Brassica napus* × *Brassica juncea* in control and at 0.6% salinity levels.

Sterilization of glass wares and instruments: Beakers, pipettes, metal instruments such as forceps, scalpels, needles, spatulas and aluminum foils were sterilized by autoclave at 121°C under 1.16kg/cm² pressure.

Sterilization of culture room and transfer area: The culture room was initially cleaned by gently washing all the floors and walls with detergent. This was followed by carefully wiping them with 70% ethyl alcohol. The process of sterilization was repeated at regular intervals. Generally laminar airflow cabinet was sterilized by switching on the cabinet with UV light and wiping the working surface with 70% ethyl alcohol.

Sterilization of explants (seed): Mature seeds of thirty genotype from the introgressed products of *B. napus* and *B. juncea* were washed in running tap water surface sterilized with 70% ethyl alcohol with 3 washing in sterilized distilled water. The seeds were dipped into 0.1% HgCl₂ solution for 3 minutes. This was followed by washing in sterilized distilled water for 3 times. Then the seeds were dipped into 15% chlorax solution along with 1-2 drops Tween-20 for 3 minutes. This was followed by washing in sterilized distilled water for 3-5 times 3-4 minutes each. Finally the seeds were kept under sterilized distilled water.

Culture method: Sixteen glass vials were used for each genotypes. These vials were arranged under four treatments and four replications. The vials were marked for treatments, replications and genotypes. Five seeds were placed in each vial. After 7 days of seed placement all the seedlings of each vial were removed for further observation and recording data. Generally laminar airflow cabinet was sterilized by switching on the cabinet with UV light and wiping the working surface with 70% ethyl alcohol.

Table 4: Germination percentage of 30 rapeseed and mustard genotypes grown under different salinity levels when raised in MS media for seven day.

Genotypes	Germination (%) against salinity levels				Mean	Regression coefficient (b)
	0	0.3	0.6	0.9		
G ₁	50	20	30	15	28.76h	-1.5833
G ₂	90	80	85	75	82.5ab	-0.6667
G ₃	85	50	65	20	55eef	-3
G ₄	30	35	20	0	21.26hij	-1.75
G ₅	30	15	30	10	21.26hij	-0.75
G ₆	10	20	10	5	11.26ijk	-0.4167
G ₇	30	10	25	25	22.5hij	0
G ₈	45	20	20	10	23.76hi	-1.75
G ₉	30	40	25	15	27.5h	-1
G ₁₀	10	0	0	0	2.5k	-0.5
G ₁₁	5	0	0	0	1.25k	-0.25
G ₁₂	25	5	0	20	12.5ijk	-0.3333
G ₁₃	15	5	5	10	8.76jk	-0.25
G ₁₄	5	5	5	5	5k	0
G ₁₅	10	5	5	0	5k	-0.5
G ₁₆	90	55	95	65	76.26bc	-0.5833
G ₁₇	70	80	75	50	68.76bcde	-1.0833
G ₁₈	10	55	80	20	56.26def	0.9167
G ₁₉	55	45	50	30	45fg	-1.1667
G ₂₀	10	5	5	5	6.26k	-0.25
G ₂₁	20	15	55	40	32.5hg	1.6667
G ₂₂	0	0	0	0	Ok	0
G ₂₃	65	65	70	70	67.5cde	0.3333
G ₂₄	80	65	65	70	70bcd	-0.5
G ₂₅	85	50	65	65	66.26cde	-0.75
G ₂₆	95	90	95	95	93.76a	0.0833
G ₂₇	45	60	80	45	57.5def	0.3333
G ₂₈	60	45	55	30	47.5f	-1.3333
G ₂₉	60	50	50	35	48.76f	-1.25
G ₃₀	85	70	65	50	67.5cde	-1.8333
Mean	45.34	35.34	41	29.34		

Sterilization of explants (seed): Mature seeds of thirty genotype from the Introgressed products of *B. napus* and *B. juncea* were washed in running tap water surface sterilized with 70% ethyl alcohol with 3 washing in sterilized distilled water. The seeds were dipped into 0.1% HgCl₂ solution for 3 minutes. This was followed by washing in sterilized distilled water for 3 times. Then the seeds were dipped into 15% chlorax solution along with 1-2 drops Tween-20 for 3 minutes. This was followed by washing in sterilized distilled water for 3-5 times 3-4 minutes each. Finally the seeds were kept under sterilized distilled water.

Culture method: Sixteen glass vials were used for each genotypes. These vials were arranged under four treatments and four replications. The vials were marked for treatments, replications and genotypes. Five seeds were placed in each vial. After 7 days of seed placement all the seedlings of each vial were removed for further observation and recording data.

Table 5: Number of germinated seedling of 30 rapeseed and mustard genotypes grown under different salinity levels when raised in MS media for 7th day.

Genotypes	Number of seedling germinated against salinity levels at 7 th day				Mean	Regression coefficient (b)
	0	0.3	0.6	0.9		
G ₁	3.725	1.282	1.283	0.817	1.777gh	-2.9077
G ₂	5.833	4.549	4.783	3.966	4.783ab	-1.789
G ₃	5.641	2.683	3.499	1.05	3.218def	-4.319
G ₄	1.867	1.866	1.166	0	1.225MJ	-2.1003
G ₅	1.516	0.583	1.398	0.583	1.020hijkl	-0.6613
G ₆	0.466	1.05	0.583	0.35	0.612ijklm	-0.2717
G ₇	1.75	0.467	1.167	1.516	1.225hij	-0.0007
G ₈	2.683	0.816	0.7	0.583	1.196hijk	-2.1387
G ₉	1.05	2.333	1.283	0.7	1.341hi	-0.7
G ₁₀	0.116	0	0.233	0	0.087m	-0.0383
G ₁₁	0.35	0	0.233	0	0.146m	-0.2723
G ₁₂	0.933	0.233	0	1.05	0.554ijklm	0.0393
G ₁₃	0.7	0.35	0	0.467	0.379jklm	-0.3497
G ₁₄	0.233	0.35	0.233	0.233	0.2621m	-0.039
G ₁₅	0.583	0.35	0.233	0	0.2921m	-0.622
G ₁₆	5.95	2.916	5.716	3.383	4.491bc	-1.6337
G ₁₇	4.083	4.433	4.083	2.683	3.820cde	-1.5167
G ₁₈	3.966	3.149	4.433	2.683	3.558de	-0.855
G ₁₉	3.616	2.566	2.683	1.516	2.595f	-2.061
G ₂₀	0.583	0.233	0.233	0.35	0.350klm	-0.233
G ₂₁	0.7	0.817	2.916	1.866	1.575h	1.8657
G ₂₂	0	0	0	0	0.000m	0
G ₂₃	3.5	3.383	3.966	3.966	3.704cde	0.6603
G ₂₄	4.666	3.733	3.966	3.85	4.054bcd	-0.7383
G ₂₅	4.899	3.15	3.85	3.616	3.879cde	-1.0497
G ₂₆	5.133	5.483	5.116	5.599	5.333a	0.3437
G ₂₇	2.567	2.8	4.316	2.566	3.062ef	0.5043
G ₂₈	3.266	1.983	2.566	1.866	2.42 lfg	-1.2057
G ₂₉	3.149	1.983	2.683	2.216	2.508fg	-0.6997
G ₃₀	4.335	4.316	3.732	3.033	3.854cde	-1.4967
Mean	2.595a	1.929c	2.235b	1.684c		

Ten dayold seedlings were ready for the source of explants and thus provided contamination free explants. The aseptically germinated seedlings were carefully rescued and placed on a sterile petridish. The shoots tips were separated from the seedlings with a sterile scalpel. Six explants were inoculated into each culture vessel. Twelve glass vials were used for each genotype. These vials were arranged under four treatments and 3 replications. The vials were marked for treatments, replications and genotypes.

Precautions to ensure aseptic condition

All inoculation and aseptic manipulations were carried out in a laminar airflow cabinet. The cabinet was switched on for half an hour before use and cleaned with 70% ethyl alcohol to reduce the chance of contamination. The instruments like scalpel, forceps, needles etc. were dipping in 70% ethyl alcohol followed by flaming and cooling inside the inoculation chamber before use. Hands were also made sterile inside the laminar airflow cabinet by rubbing 70% ethyl alcohol with cotton. Aseptic conditions were followed during each and every

operation to avoid contamination. The culture vessels with inoculated seed were incubated both in dark and light in a temperature controlled growth room ($23\pm 2^{\circ}\text{C}$) under 12 hour photo period with a light intensity of $120\mu\text{mol}/\text{m}^2/\text{sec}$. Day to day observation were carried out to note the response.

The data for the character under present study were statistically analyzed wherever applicable. The experiments were conducted in growth room and arranged in Randomized complete Block Design (RCBD). The analysis of variance for different characters was performed by F test [10] and means were compared by the Duncan's Multiple Range Test (DMRT).

Regression coefficient for each character was calculated using the mean values of different treatments for each genotype of genotypic effect as depending and treatment effect as independent variables following the method as cited by [11]. The formula was used as follows:

Table 6: Fresh seedling height of 30 rapeseed and mustard genotypes grown under different salinity levels when raised in MS media for 7th day.

Genotypes	Seedling height (cm) against salinity levels				Mean	Regression coefficient (b)
	0	0.3	0.6	0.9		
G ₁	8.443	5.75	1.813	3.375	4.845ghijk	-6.3803
G ₂	9.5	6.563	8.041	3.069	6.793defg	-5.9383
G ₃	6.362	6.75	7.042	3.175	5.832efghi	-3.0897
G ₄	1.95	6.75	1.563	0	2.566klmn	-3.679
G ₅	5.913	1.688	2.813	1.75	3.041jklm	-3.788
G ₆	3.2	0.85	2.6	0.25	1.7251mno	-2.3667
G ₇	10.025	5.75	4.5	3.685	5.990efghi	-6.7567
G ₈	5.55	2.8	4.55	2.656	3.889ijkl	-2.3107
G ₉	2.938	7.883	2.5	1.413	3.683ijklm	-3.3193
G ₁₀	1.8	0	0	0	0.450no	-1.8
G ₁₁	1.75	0	0	0	0.438no	-1.75
G ₁₂	2.3	3.5	0	2.918	2.1791mno	-0.5487
G ₁₃	6.4	2.5	3	3.75	3.912ijkl	-2.4833
G ₁₄	4.875	2	2.5	1.625	2.750jklmn	-3.0833
G ₁₅	7.6	2.25	1.125	0	2.744jklmn	-7.975
G ₁₆	14.124	9.95	8.45	6.64	9.791 abc	-7.984
G ₁₇	13.725	10.421	7.375	4.71	9.058abcd	-10.0303
G ₁₈	11.412	6.813	8.833	6.298	8.339abcde	-4.4407
G ₁₉	7.43	0.5	4.6	2.808	5.084fghij	-3.2553
G ₂₀	2.625	1.05	0.875	1	1.387mno	-1.6833
G ₂₁	2.312	4.125	8.133	2.221	4.198hijkl	1.245
G ₂₂	0	0	0	0	0	0
G ₂₃	5.669	7.935	5.55	6.598	6.438efgh	0.134
G ₂₄	9.577	7.229	8.246	4.614	7.417cdef	-4.624
G ₂₅	15.194	6.631	13.707	4.35	9.97 lab	-8.4853
G ₂₆	11.694	11.233	11.6	7.569	10.524a	-4.0027
G ₂₇	8.525	7.813	11.1	3.191	7.657bcde	-4.2383
G ₂₈	8.573	10.237	7.646	4.563	7.755bcde	-4.8737
G ₂₉	11.793	4.462	9.521	5.55	7.832bcde	-4.5567
G ₃₀	11.3	4.35	8.717	3.054	6.855defg	-6.7903
Mean	7.085a	5.093b	5.213b	3.028c		

$$b = \frac{\sum xy - \frac{(\sum x)(\sum y)}{n}}{\sum x^2 - \frac{(\sum x)^2}{n}}$$

where,

b= Regression coefficient

X= values of independent variable (Salinity dose)

Y= values of dependent variable (genotypic mean)

n= Total number of observation

Results and Discussion

The analysis of variance for the characters like germination percentage, number of seedling germinated, seedling height, fresh seedling weight, root length, fresh root weight and shoot length at the 7th day. According to the table the variations among the genotypes (Factor A), the salinity levels (Factor B) and the interaction between

the salinity levels and the genotypes of (A×B) were highly significant for all the characters.

Brassica genotypes varied significantly for germination percentage, all the characters studied. The results related to this character have been shown in Table 3. Germination percentage decreased with the increase of salinity level. The highest value 45.34% was found in control and the lowest 29.34% at 0.9% salinity level. Among the genotypic means, the highest germination percentage (93.76%) was found in G₂₆ which is one of the selection of the cross between *Brassica napus* × *Brassica juncea* and the lowest (1.25%) was in gh, one of the line of cross *Brassica napus* × *Brassica juncea* and G₂₂ was not germinated both control and saline condition (Plate 1 and 2). On the basis of regression coefficient values the best performance was found in G₂₆ of cross *Brassica napus* × *Brassica juncea* and the worst in G₃ of cross *Brassica napus* × *Brassica juncea*. Decreased germination percentage with increased salinity levels in rapeseed and mustard [12-14]. Similar result was also observed by [15] and [16] in

Table 7: Fresh seedling weight of 30 rapeseed and mustard genotypes grown under different salinity levels when raised in MS media for 7th day.

Genotypes	Seedling weight (mg) against salinity levels				Mean	Regression coefficient (b)
	0	0.3	0.6	0.9		
G ₁	41.67	22.7	9.25	21.75	23.84fghi	-24.4033
G ₂	84.09	39.44	57.12	39.75	55.10bcd	-38.4467
G ₃	35.03	33.64	36	24.25	32.23efg	-9.9933
G ₄	17.17	15.58	22.75	0	13.87hij	-14.78
G ₅	30.63	11.88	30.75	10.25	20.88ghi	-14.09
G ₆	32	14.68	24	7.75	19.61ghi	-21.1433
G ₇	31	37	29.33	38.6	33.98efg	5.0433
G ₈	21.14	23.13	39.38	28.5	28.03fgh	12.7767
G ₉	21.63	43.58	48	16.5	32.43efg	-3.6567
G ₁₀	7.5	0	0	0	1.88J	-7.5
G ₁₁	7.75	0	0	0	1.94J	-7.75
G ₁₂	19.88	12.75	0	17.33	12.49MJ	-6.8
G ₁₃	7.88	20	16.5	12.63	14.25hij	3.5833
G ₁₄	3.63	18.25	17.25	12.25	12.84hij	8.2867
G ₁₅	6.38	10.75	10.75	0	6.97ij	-6.38
G ₁₆	75.79	42.92	56.34	37.76	53.20bcd	-33.5567
G ₁₇	72.75	44.83	39.87	31.85	47.33cde	-42.5533
G ₁₈	64.63	45.19	54.17	40.03	51.00bcd	-21.6067
G ₁₉	53.33	47.83	39.7	19.13	40.00def	-36.91
G ₂₀	9	7.75	8.75	10.5	9.001J	1.8333
G ₂₁	24.88	27.88	51.67	23.33	31.94efg	6.38
G ₂₂	0	0	0	0	0.00j	0
G ₂₃	53.44	62.04	51.75	68.06	58.82abc	11.19
G ₂₄	52.95	59.04	59.38	49.28	55.16bcd	-3.5567
G ₂₅	121.1	70.41	66.77	31.9	72.54a	-90.4133
G ₂₆	75.59	91.26	51.8	42.88	65.38ab	-45.8633
G ₂₇	65.08	62.33	58.47	36.19	55.52bcd	-30.1767
G ₂₈	54.83	77	50.56	30.25	53.16bcd	-33.3933
G ₂₉	120.1	49.75	13.9	54.25	59.5 labc	-77.8
G ₃₀	105.8	49.44	15.53	55	56.46abcd	-62.1033
Mean	43.89a	34.70b	31.99b	25.33c		

rapeseed and mustard.

Number of germinated seedling was significant for the differences due to genotype, salinity level and interaction. The results related to this character have been shown in Table 4. It was observed that increase in salinity levels significantly delayed the number of germinated seedling (Table 4). The maximum number of seedling (2.595) was found when there was no salinity level and the minimum number of seedling (1.684) at 0.9% salinity levels. The maximum number of seedling (5.333) were found in G₂₆ one of the line of cross *Brassica napus* × *Brassica juncea* and the minimum number of seedling (0.087) in G₁₀ one of the line of cross *Brassica napus* × *Brassica juncea*. On the basis of regression coefficient values the best performance was found in the G₂₆, one of the line of cross *Brassica napus* × *Brassica juncea* and the worst in G₃₀, one of the line of cross *Brassica napus* × *Brassica juncea*. Delay in germination in rapeseed with increasing salinity [17]. Similar results were found by [5,18].

Seedling height showed significant difference among the genotypes, salinity levels and their interactions. According to the data presented in Table 5 it was observed that in the higher salinity levels, seedlings height decreased gradually. The highest seedling height (7.085cm) was observed in control and the lowest (3.028cm) at 0.9% salinity level (Table 5). Among the genotypes the highest value (10.524cm) was found in the G₂₆, one of the selection of cross *Brassica napus* × *Brassica juncea* and the lowest (0.438cm) was found in G₁₁, one of the selection of cross *Brassica napus* × *Brassica juncea* (Plate 1 and 2). On the basis of regression coefficient values the best performance was found 2 in G₂₃, one of the selection of cross *Brassica napus* × *Brassica juncea* and the worst in G₁₇, one of the selection of cross *Brassica napus* × *Brassica juncea*. Salt stress condition reduced the plant height in *Brassica* [13,19]. Similar result was also found by [18] in soybean.

The results of fresh seedling weight have been shown in Table 6. The fresh seedling weight was significant for the difference due

Table 8: Root length of 30 rapeseed and mustard genotypes grown under different salinity levels when raised in MS media for 7th day.

Genotypes	Root length (cm) against salinity levels				Mean	Regression coefficient (b)
	0	0.3	0.6	0.9		
G ₁	4.66	3	1.39	1.88	2.73defgh	-3.3167
G ₂	5.88	3.61	4.06	1.93	3.87abcde	-3.8
G ₃	3.53	2.36	4	1.09	2.75defgh	-1.8933
G ₄	1.22	3.96	1.7	0	1.72hijkl	-1.9733
G ₅	2.9	3.41	1.65	0.35	2.08ghij	-3.1367
G ₆	1.05	0.75	2.5	0.08	1.09jklmn	-0.3867
G ₇	3.97	3	1.17	2.28	2.61efghi	-2.3
G ₈	2.83	1.65	2.15	1.25	1.97ghijk	-1.4133
G ₉	2.1	3.02	2.38	0.51	2.00ghijk	-1.8033
G ₁₀	0.93	0	0	0	0.23mm	-0.93
G ₁₁	0.19	0	0	0	0.05n	-0.19
G ₁₂	0.56	0.88	0	1.08	0.631mn	0.2267
G ₁₃	2.85	1.13	1.5	0.93	1.60hijkl	-1.7967
G ₁₄	2.72	0.81	1	0.8	1.33ijklm	-1.8567
G ₁₅	2.75	0.31	0.95	0	1.33ijklm	-2.5367
G ₁₆	7.72	4.56	3.95	2.79	4.76ab	-5.1333
G ₁₇	9.49	4.79	4.14	1.57	5.00a	-8.1367
G ₁₈	6.22	4.08	4.25	3.42	4.49abc	-2.7433
G ₁₉	3.36	2.21	2.7	1.4	2.42fghi	-1.7967
G ₂₀	1.42	0.25	1	0.38	0.761kmn	-0.79
G ₂₁	1.9	2.31	5.25	1.79	2.81defgh	0.87
G ₂₂	0	0	0	0	0.00n	0
G ₂₃	1.47	4.74	4.25	2.51	3.24cdefg	0.8767
G ₂₄	3.97	3.48	4.25	3.09	3.70bcdef	-0.6233
G ₂₅	7.12	4.49	5.52	2.81	4.99a	-3.9667
G ₂₆	6.89	4.18	4.93	3.88	4.97a	-2.76
G ₂₇	4.8	4.37	3.83	1.44	3.61bcdef	-3.54
G ₂₈	5.04	5.49	3.45	2.15	4.03abcd	-3.57
G ₂₉	5.14	3.76	3	2.11	3.51bcdef	-3.2833
G ₃₀	7.44	2.23	3.52	0.95	3.54bcdef	-6.06
Mean	3.67a	2.63b	2.62b	1.42c		

to genotypes, salinity levels and their interactions. The weights were found to decrease gradually with the increase of salinity levels. The highest seedling weight (43.89mg) was observed in control and the lowest (25.33mg) at 0.9% salinity level (Table 6). Among the genotypic means, the highest value (72.54mg) was found in G₂₅, one of the selection of cross *Brassica napus* × *Brassica juncea* and the lowest (1.88mg) was in G₁₀, one of the selection of cross *Brassica napus* × *Brassica juncea*. On the basis of regression coefficient values, the best performance was found in G₂₀, one of the selections of cross *Brassica napus* × *Brassica juncea* and worst in G₂₅, one of the selection of cross *Brassica napus* × *Brassica juncea*. Reduction in seedling growth due to salinity in sunflower [20].

At the 7th day of seed sowing the root length was determined and the results have been shown in Table 7. The difference due to genotypes, salinity levels and their interactions were significant. Considering the salinity levels the highest root length (3.67cm) was observed in control and the lowest (1.42cm) at 0.9% salinity levels.

The highest value (5.00cm) was found was in G₁₂, one of the selection of cross *Brassica napus* × *Brassica juncea* and the lowest (0.05cm) was in G₁₁, one of the selection of cross *Brassica napus* × *Brassica juncea*. On the basis of regression coefficient values the best performance was found in G_n, one of the selection of cross *Brassica napus* × *Brassica juncea* and the worst in G_n, one of the selection of cross *Brassica napus* × *Brassica juncea*. Reduction in radicle length in the salt stress condition in *Brassica* genotypes [13]. In sunflower [20] also found reduction in root length with increase of salinity levels. Reuction in root lengths in the salt stress condition in *Brassica* genotype [21].

The fresh root weight was determined in mg at 7th day of seed sowing and the results have been shown in Table 8. The root weight differed significantly for the genotypes, salinity levels and interactions. The highest root weight (12.97mg) was observed at 0.3% salinity levels and the lowest (7.91mg) at 0.9% salinity levels (Table 8). Among the genotypic means, the highest value (25.32mg) was found in G₂₃, one of the selection of cross *Brassica napus* × *Brassica juncea*

Table 9: Fresh root weight of 30 rapeseed and mustard genotypes grown under different salinity levels when raised in MS media for 7th days.

Genotypes	Root weight (ing) against salinity levels				Mean	Regression coefficient (b)
	0	0.3	0.6	0.9		
G ₁	15.248	7.25	3.212	12.75	9.615fgh	-41.3467
G ₂	26.26	14.75	19.01	11.13	17.79bcd	-137,100
G ₃	10.32	21.08	9.33	8.38	12.28defg	-5.8567
G ₄	5.83	15.58	8	0	7.35ghij	-8.3567
G ₅	8.75	10.75	16	4.25	9.94fgh	-2.75
G ₆	10.5	3	15	2.75	7.81ghij	-3.75
G ₇	7.63	6.75	8.5	11.39	8.57ghi	4.3433
G ₈	3.75	9.5	14.13	6.25	8.41ghi	4.0433
G ₉	5.63	17.17	19	6	11.95defg	0.98
G ₁₀	46.88	0	0	0	11.72k	-46.88
G ₁₁	31.25	0	0	0	7.81k	-31.25
G ₁₂	2.13	0.25	0	5.33	1.93jk	3.1167
G ₁₃	25	4	4.5	4.38	9.47hijk	-20.4533
G ₁₄	31.25	6.75	5.75	2	11.44hijk	-29.5833
G ₁₅	4.5	2.25	6.25	0	3.25ijk	-3.1667
G ₁₆	13.14	28.5	15.08	15.07	17.94bcd	-2.5433
G ₁₇	15.94	23.85	10.73	10.92	15.36cdef	-9.3933
G ₁₈	20.63	14.94	25.1	9.13	17.45bcde	-8.1133
G ₁₉	12.03	13.92	13.95	6.54	11.61efg	-5.48
G ₂₀	9	2.25	4.5	4.25	5.00hijk	-4
G ₂₁	9.38	9.75	19.33	9.67	12.03defg	3.4833
G ₂₂	0	0	0	0	0.00k	0
G ₂₃	18.54	47.08	19.55	16.13	25.32bcd	-11.5867
G ₂₄	16.59	29.48	34.63	15.34	24.01 a	0.4667
G ₂₅	36.41	22.29	12	6.05	19.19abc	-33.79
G ₂₆	20.19	35.67	23.4	13.39	23.16ab	-10.89
G ₂₇	21.5	18.77	21.77	10.54	18.14bcd	-9.96
G ₂₈	18.29	20	12.88	9.63	15.20cdef	-11.0333
G ₂₉	522.1	16.69	13.9	18.75	17.87bcd	-4.28
G ₃₀	521.1	16.75	15.53	17.25	17.65bcde	-4.2567
Mean	12.48a	12.97a	12.37a	7.91b		

and the lowest value (1.93mg) was found in V₁₂, one of the selection of cross *Brassica napus* × *Brassica juncea*. On the basis of regression coefficient values the best performance was found in G₂₄, one of the selection of cross *Brassica napus* × *Brassica juncea* and the worst in G₁₀, one of the selection of cross *Brassica napus* × *Brassica juncea*. Reduction in root growth due to salinity levels in sunflower [20].

At the 7th day of seed sowing the shoot length was determined and the results have been shown in Table 9. The difference due to genotypes, salinity levels and interaction were found significant. Considering the salinity levels, the highest shoot length (2.83cm) was observed at 0.6% salinity levels and the lowest shoot length (1.68cm) was at 0.9% salinity levels. Among the genotypes the highest shoot length (5.11cm) was found in G₁₇, one of the selection of cross *Brassica napus* × *Brassica juncea* and the lowest (0.22cm) were found in both G₁₀, one of the selections of cross *Brassica napus* × *Brassica juncea* and G₁₁, one of the selection of cross *Brassica napus* × *Brassica juncea*. On the basis of respectively coefficient values the best performance was

found in G₆, one of the selection of cross *Brassica napus* × *Brassica juncea* and the worst in G₁₀, one of the selection of cross *Brassica napus* × *Brassica juncea*. Reduction of shoot lengths in the salt stress condition in *Brassica* genotype [21].

Ranking the characters

The genotypes were tested on the cumulative performance of characters due to the different levels of salinity. The characters such as germination percentage, days to germination, seedling height, fresh seedling weight, root length, fresh root weight and shoot length were used. The genotypes showed different degree of response due to levels of salinity including control treatment for different characters. In order to find out the overall performance of a genotype ranking was done. This was done using the value of regression coefficient "b", which is the measure of rate of increase or decrease due to increase levels of treatment starting from control upto 0.9% salinity. Average rank as well as genotypes position has also been found out. Similar ranking procedure was followed by [22] in screening rapeseed and

Table 10: Shoot length of 30 rapeseed and mustard genotypes grown under different salinity levels when raised in MS media for 7th day.

Genotypes	Shoot length (cm) against salinity levels				Mean	Regression coefficient (b)
	0	0.3	0.6	0.9		
G ₁	3.32	2.5	0.59	1.75	2.04efgh	-2.2067
G ₂	2.79	1.89	4.25	1.91	2.71cde	-0.0933
G ₃	1.83	2.38	4.12	1.16	2.37def	-0.09
G ₄	1.01	3.24	2.17	0	1.61efghi	-1.3667
G ₅	2.15	2.06	2.17	0.65	1.76efghi	-1.4633
G ₆	0.49	1.42	3.5	0.17	1.40fghij	0.3733
G ₇	2.25	3	2.25	1.9	2.35def	-0.6
G ₈	2.34	1.81	2.38	1	1.88efghi	-1.15
G ₉	1.56	3.27	2.45	1.02	2.08efgh	-0.8133
G ₁₀	0.88	0	0	0	0.22jk	-0.88
G ₁₁	0.88	0	0	0	0.22jk	-0.88
G ₁₂	0.72	1.8	0	0.92	0.86hijk	-0.4
G ₁₃	1.5	1.75	1.5	1.2	1.49efghi	-0.3833
G ₁₄	1.13	0.75	1.5	0.88	1.06ghijk	0
G ₁₅	1.75	1.25	0.93	0	0.98ghijk	-1.8567
G ₁₆	4.77	3.84	5.18	2.66	4.1 lab	-1.6633
G ₁₇	6.89	5.66	3.93	3.94	5.1la	-3.5267
G ₁₈	4.12	3.56	4.33	3.06	3.77bc	-0.8033
G ₁₉	3.55	2.62	2.56	1.15	2.47def	-2.42
G ₂₀	1.2	0.63	0.5	0.63	0.74ijk	-0.6133
G ₂₁	1.44	1.69	3.67	1.69	2.12defg	0.91
G ₂₂	0	0	0	0	0.00k	0
G ₂₃	3.09	4.62	5.55	3.66	4.23ab	0.88
G ₂₄	3.13	3.76	4.85	3.54	3.82bc	0.7733
G ₂₅	5.36	3.89	5.25	2.39	4.22ab	-2.5167
G ₂₆	5.63	4.92	2.7	3.89	4.28ab	-2.48
G ₂₇	3.8	3.06	4.17	2.17	3.30bcd	-1.26
G ₂₈	4.17	5.43	4.16	2.83	4.14ab	-1.7633
G ₂₉	5.43	2.67	6.05	3.63	4.44ab	-0.6733
G ₃₀	5.24	3.54	4.07	2.67	3.88b	-2.3933
Mean	2.75a	2.57a	2.83a	1.68b		

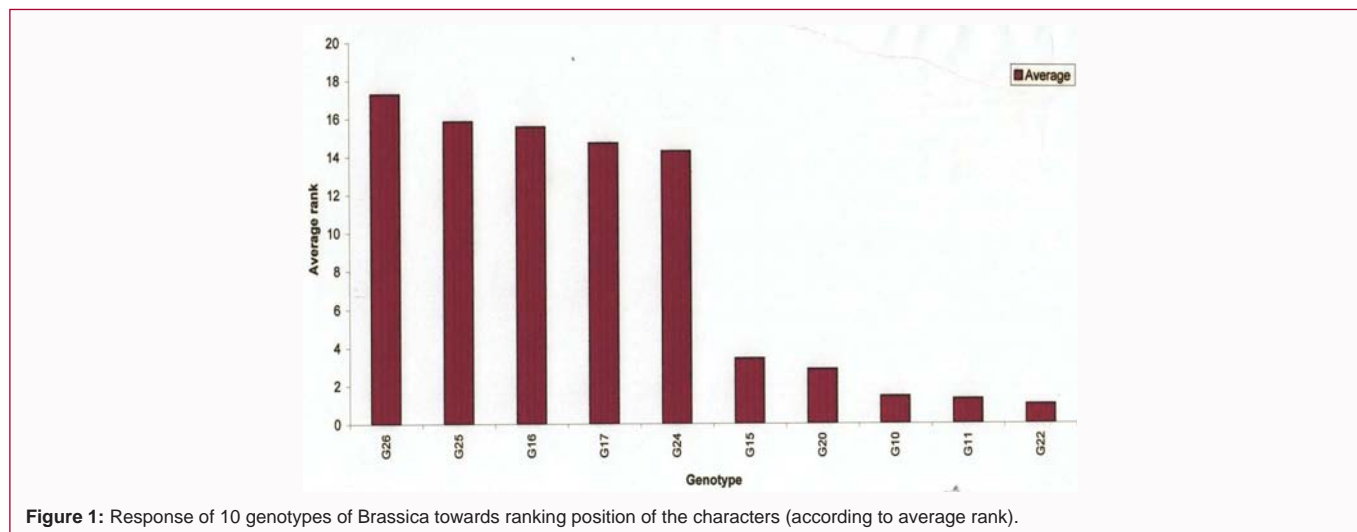


Figure 1: Response of 10 genotypes of Brassica towards ranking position of the characters (according to average rank).

Table 11: Rank position of the genotypes according to cumulative values of characters.

Genotypes	No. of 1 Days of germinated seedling		Seedling height	Fresh seedling weight	Fresh root weight	Root length	Shoot length	Average rank	Genotype position
G ₁	6	11	11	5	7	12	8	8.571	16
G ₂	16	20	15	10	12	15	11	14.143	6
G ₃	10	15	13	7	9	12	10	10.857	13
G ₄	4	8	5	3	5	7	7	5.571	21
G ₅	4	6	7	4	7	9	7	6.286	20
G ₆	3	5	4	4	5	5	6	4.571	23
G ₇	4	8	13	7	6	11	10	8.429	17
G ₈	5	1	9	6	6	8	7	6.857	19
G ₉	6	9	8	7	9	8	8	7.857	18
G ₁₀	1	1	2	1	1	2	2	1.429	27
G ₁₁	1	1	2	1	1	1	2	1.286	28
G ₁₂	3	5	4	3	2	3	4	3.429	25
G ₁₃	2	4	9	3	4	7	7	5.143	22
G ₁₄	1	2	6	3	4	6	5	3.857	24
G ₁₅	1	2	6	2	3	5	5	3.429	25
G ₁₆	15	19	20	10	12	18	15	15.571	3
G ₁₇	13	17	19	9	10	19	16	14.714	4
G ₁₈	11	16	18	10	11	17	13	13.714	7
G ₁₉	8	13	12	8	8	10	10	9.857	14
G ₂₀	1	3	3	2	4	4	3	2.857	26
G ₂₁	7	10	10	7	9	12	9	9.143	15
G ₂₂	1	1	1	1	1	1	1	1	29
G ₂₃	12	17	14	12	12	13	15	13.571	8
G ₂₄	14	18	16	10	15	14	13	14.286	5
G ₂₅	12	17	21	14	13	19	15	15.857	2
G ₂₆	17	21	22	13	14	19	15	17.286	1
G ₂₇	11	14	17	10	12	14	12	12.857	11
G ₂₈	9	12	17	10	10	16	15	12.714	12
G ₂₉	9	12	17	12	12	14	15	13	10
G ₃₀	12	17	15	11	11	14	14	13.429	9

mustard varieties for salt tolerance. The results have been shown in Table 10. Considering all the characters the genotypes G₂₆, one of the selection of cross *Brassica napus* × *Brassica juncea*, G₂₅, one of the selection of cross *Brassica napus* × *Brassica juncea*, G₁₆, one of the selection of cross *Brassica napus* × *Brassica juncea*, G₁₇, one of the selection of cross *Brassica napus* × *Brassica juncea* and G₂₄, one of the selection of cross *Brassica napus* × *Brassica juncea* were found to be the more saline tolerant genotypes than the other. Again the genotypes G₁₁, one of the selection of cross *Brassica napus* × *Brassica juncea*, G₁₀, one of the selection of cross *Brassica napus* × *Brassica juncea*, G₂₀, one of the selection of cross *Brassica napus* × *Brassica juncea*, G₂₂, G₁₂ and G₁₅ were more susceptible than others. G₂₂ was not germinated in control condition and at salinity levels (Figure 1 and Plate 1 & 2).

Conclusion

Among the genotypes, the highest germination percentage was found in the variety G₂₆, one of the selection of cross *Brassica napus* × *Brassica juncea*. Number of germinated seedling was reduced with

the increase of salinity level. The highest number of seedling was found in control. Among the genotypes, G₂₆, one of the selection of cross *Brassica napus* × *Brassica juncea* recorded the highest number of seedling, one of the selection of cross *Brassica napus* × *Brassica juncea*. At the 7th day of seed sowing, considering genotypes, the highest seedling height was in the variety G₂₆, one of the selection of cross *Brassica napus* × *Brassica juncea* and the lowest was in the variety gh, one of the selection of cross *Brassica napus* × *Brassica juncea*. Considering salinity level, the highest seedling weight was in control and the lowest was at 0.9%. Considering genotypes the highest value was found in G₂₅, one of the selection of cross *Brassica napus* × *Brassica juncea* and the lowest in G₁₀, one of the selection of cross *Brassica napus* × *Brassica juncea*. Among the genotypes the highest shoot length was found in G₁₀, one of the selection of cross *Brassica napus* × *Brassica juncea* and the lowest in both G₁₀ and G₁₁, one of the selection of cross *Brassica napus* × *Brassica juncea*. Considering the above characters G₂₆, G₂₅, G₁₆, G₁₇ and G₂₄ have more salt tolerant than other tested in the study.

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