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Screening of Salt Tolerance in Different Varieties of *Hordeum vulgare*

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Abstract: Soil salinity is the major abiotic stress in plant agriculture strongly influencing productivity worldwide and it delimit the crop yield. Several abiotic stresses are united by the fact that at least part of their detrimental effect on plant performance is caused by disruption of plant water status. This can occur through decreased availability of water in the environment during drought, altered ion content and water uptake caused by salinity or cellular dehydration caused by formation of extracellular ice during freezing stress. Barley is used as food and in production of beverage all over the world. In the present study, screening of few barley varieties for their tolerance against salt stresses and then be able to find the best tolerant variety. In the experimental set up, Hoagland medium is used for hydroponic studies in which seeds were grown on different salt concentration to find out their salt tolerance. Out of five varieties of *Hordeum vulgare* BH 924 and RD 2508 are the good salt tolerant varieties in comparison to others.

Key words: *Hordeum vulgare* · Hydroponics · Hoagland medium · Salinity · Varieties · Abiotic stress

INTRODUCTION

The growth and development of the plant are processes that under the control of the environment temperature, moisture, radiation, nutrients and gases can either enhance or check the growth and development of the plant. These factors may act as stress leading to injury and in extreme cases the death of the plant [1]. Soil salinity is a major constraint to food production because it limits crop yield and restricts use of land previously uncultivated. Higher salt concentration expressed in atmospheres was a greater factor in determining the amount of growth reduction and Growth reduction was in according to osmotic concentration of substrate [2], salts have injurious effects on plant growth and excessive concentrations kill growing plants [3]. Agricultural productivity is severely affected by soil salinity and the damaging effect of salt accumulation in agricultural soils has become an important environmental concern [4]. Salinity effects are more conspicuous in arid and semiarid regions, where limited rainfall, high evapo-transpiration and high temperature associated with poor water and soil management contributes to the salinity problem and is also of great importance to the agricultural production in these regions [5]. In India, out of 9.38 million ha of salt-affected soils, 3.88 million ha are alkali soil and 5.5 million ha (including coastal lands) are saline soils [6]. Among various types of biotic and abiotic stresses, Salinity stress negatively impacts agricultural yield throughout the world affecting production whether it is for subsistence or economic gain [7]. The plant response to salinity consists of numerous processes that must function in coordination to alleviate both cellular hyper-osmolarity and ion disequilibrium. In addition, crop plants must be capable of satisfactory biomass production in the saline environment and yield stability. Lots of salt lakes are present in India as well as in the world, Sambhar Lake of Rajasthan (India) is one of the examples of it, where a few number of crops can grow. Tolerance and yield stability are complex genetic traits that are difficult to establish in crops since salt stress may occur as a catastrophic episode, be imposed continuously or intermittently, or become gradually more severe and at any stage during development. Natural boundaries imposed by soil salinity also limit the caloric and the nutritional potential of agricultural production. These constraints are most acute in areas of the world where food distribution is problematic because of insufficient infrastructure or

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political instability. Water and soil management practices have facilitated agricultural production on soils marginalized by salinity but additional gain by these approaches seems problematic [8].

Excess amount of salt in the soil adversely affects plant growth and development. Nearly 20% of the world's cultivated area and nearly half of the world's irrigated lands are affected by salinity. Processes such as seed germination, seedling growth, vegetative growth, flowering and fruit set are adversely affected by high salt concentration, ultimately causing diminished economic yield and also quality of produce. [9]. The dominance of salt water across the surface of the earth has lead to the widespread occurrence of salt-affected soils. Salt-tolerant plants (halophytes) have evolved to grow of these soils, with halophytes and less tolerant plants showing a wide range of adaptations [10].

Barley is a cereal grain derived from the annual grass *Hordeum vulgare*. It serves as a major animal fodder, base malt for beer and certain distilled beverages and as a component of various health foods and medicines. It is used in soups and stews and in barley bread of various cultures, from Scotland to Africa. It belongs to family Poaceae [11]. Barley is selected as a model to study due to its use as food by a large population of the world and could show a promising tolerant against biotic and abiotic stresses. The present Study has been done to evaluate the salt tolerance of Barley's different varieties and will help us to solve many problems of agriculture related to salinity and stresses.

MATERIALS AND METHOD

Seeds of five different varieties of *Hordeum vulgare* BH 924, RD 2052, RD 2508, RD 2053 and RD 2660 were kindly provided by Durgapura, Jaipur. They were then surface sterilized with 70% ethanol and 0.1% Mercuric Chloride.

Viability Test: Sterilized petri plates were used for the viability test of all the varieties of *Hordeum vulgare.* 5 seeds of all the varieties were placed in the petri plates in wet cotton base. Light and dark condition is provided as per need of the seed to germinate [12].

Growth Media: Hoagland medium was used as the plant nutrition medium as the experiment was based on hydroponics. Hoagland medium was prepared using stocks of MgSO4. 7H20, Ca(NO3)2. 4H2O, KH2PO4, KNO3, micronutrients and Fe- EDTA in requisite amounts and the pH was set to 5.8. Hoagland medium is more salty than the normal water [13].

Experimental Group: Experiment divided into 5 groups

Group I:- *Hordeum vulgare* BH 924 variety seeds
Group II:- *Hordeum vulgare* RD 2052variety seeds
Group III:- *Hordeum vulgare* RD 2508 variety seeds
Group IV:- *Hordeum vulgare* RD 2508variety seeds
Group V:- *Hordeum vulgare* RD 2660 variety seeds

All group divided in 5 sub-groups seeds were grown in different mili-molar (mM) concentrations of NaCl, ranging from 0mM to 400mM concentrations i.e. 0 mM, 100mM, 200mM, 300mM and 400mM. Fields affected from salt excess have abundance of NaCl So NaCl is used in this study as Salt stressing agent. Where 0 mM concentration of Nacl is control in the experiment. Whole experiment was carried out for 3 weeks and weekly observations were taken and each experiment repeated five times.

The seeds were placed in round plastic boxes at the temperature of 26°C and in a photoperiod of 14 hours. The seeds were placed on thin foam sheets of the width of mm range and small openings were made, for the roots to penetrate easily into the medium (Fig. 5). The plastic boxes were covered with black paper to avoid light, so that the roots could easily move down due to their geotropic behavior and the shoots may flourish towards light due their phototropic behavior.

Since the experiment is based on hydroponics, great care was taken to prevent excess loss of water through evaporation and so the medium was continuously replaced after every 4-5 days. NaCl Solution was also continuously replaced by freshly prepared NaCl solution so as to maintain the equivalent mM concentration of the salt throughout the experiment. Root and Shoot length measurements were taken by measuring the length of shoots and roots by measuring scale and viability is recorded by observation of rooting in seeds.

RESULTS

All these verities are good growing in natural field condition and shows almost 100% viability and in viability test all the five varieties were found to be 100% viable, with completely elongated and healthy shoots and roots. This shows that the seeds are healthy and viable in laboratory condition.

Table 1: Viability and Root-Shoot length After 1 week at 0 mM concentration

Variety	No. of Seeds germinated %	No. of Rooted seeds %	No. of seeds in which shooting starts %	Average root length	Average Shoot length
RD 2660	4/5 (80%)	4/5(80%)	3/5(60%)	2.3 cms	1 cms
RD 2052	5/5(100%)	5/5(100%)	5/5(100%)	3.4 cms	12.3 cms
RD 2503	4/5(80%)	4/5(80%)	3/5(60%)	2.3 cms	1.5 cms
RD 2508	5/5(100%)	5/5(100%)	5/5(100%)	4.5 cm	8.3 cms
BH 924	5/5(100%)	5/5(100%)	5/5(100%)	4 cms	11 cms

Table 2: Viability and Root-Shoot length After 2 week at 0 mM concentration

Variety	No. of Seeds germinated %	No. of Rooted seeds %	No. of seeds in which shooting starts %	Average root length	Average Shoot length
RD 2660	4/5 (80%)	4/5(80%)	3/5(60%)	4.5 cms	1.9 cms
RD 2052	5/5(100%)	5/5(100%)	5/5(100%)	5.7 cms	14.5 cms
RD 2503	4/5(80%)	4/5(80%)	3/5(60%)	4.4 cms	2.1 cms
RD 2508	5/5(100%)	5/5(100%)	5/5(100%)	6.1 cm	9.4 cms
BH 924	5/5(100%)	5/5(100%)	5/5(100%)	5.5 cms	11.9 cms

Table 3: Viability and Root-Shoot length After 3 week at 0 mM concentration

Variety	No. of Seeds germinated %	No. of Rooted seeds %	No. of seeds in which shooting starts %	Average root length	Average Shoot length
RD 2660	4/5 (80%)	4/5(80%)	3/5(60%)	5.1 cms	2.4 cms
RD 2052	5/5(100%)	5/5(100%)	5/5(100%)	6.1 cms	15.2 cms
RD 2503	4/5(80%)	4/5(80%)	4/5(80%)	4.9 cms	2.9 cms
RD 2508	5/5(100%)	5/5(100%)	5/5(100%)	6.7 cm	10.1 cms
BH 924	5/5(100%)	5/5(100%)	5/5(100%)	5.9 cms	12.6 cms

Table 4: Viability and Root-Shoot length After 1 week at 100 mM concentration

Variety	No. of Seeds germinated %	No. of Rooted seeds %	No. of seeds in which shooting starts %	Average root length	Average Shoot length
RD 2660	2/5(40%)	2/5(40%)	0/5(0%)	0.6 cms	--------------
RD 2052	5/5(100%)	5/5(100%)	5/5(100%)	3 cms	4.5 cms
RD 2503	2/5(40%)	2/5(40%)	0/5(0%)	0.9 cms	--------------
RD 2508	5/5(100%)	5/5(100%)	5/5(100%)	3.5 cms	4.5 cms
BH 924	5/5(100%)	5/5(100%)	5/5(100%)	3 cms	4.5 cms

Table 5: Viability and Root-Shoot length After 2 week at 100 mM concentration

Variety	No. of Seeds germinated %	No. of Rooted seeds %	No. of seeds in which shooting starts %	Average root length	Average Shoot length
RD 2660	2/5(40%)	2/5(40%)	1/5(20%)	1.1 cms	1 cms
RD 2052	5/5(100%)	5/5(100%)	5/5(100%)	3.9 cms	5.4 cms
RD 2503	2/5(40%)	2/5(40%)	0/5(0%)	1.2 cms	--------------
RD 2508	5/5(100%)	5/5(100%)	5/5(100%)	3.9 cms	5.1 cms
BH 924	5/5(100%)	5/5(100%)	5/5(100%)	3. 3cms	5.7 cms

Table 6: Viability and Root-Shoot length After 3 week at 100 mM concentration

Variety	No. of Seeds germinated %	No. of Rooted seeds %	No. of seeds in which shooting starts %	Average root length	Average Shoot length
RD 2660	3/5(60%)	3/5(60%)	1/5(20%)	1.6 cms	1.9 cms
RD 2052	5/5(100%)	5/5(100%)	5/5(100%)	4.4 cms	6.3 cms
RD 2503	3/5(60%)	3/5(60%)	1/5(20%)	1.6 cms	1.2 cms
RD 2508	5/5(100%)	5/5(100%)	5/5(100%)	4.4 cms	5.9 cms
BH 924	5/5(100%)	5/5(100%)	5/5(100%)	4.2 cms	6.4 cms

Table 7: Viability and Root-Shoot length After 1 week at 200 mM concentration

Variety	No. of Seeds germinated %	No. of Rooted seeds %	No. of seeds in which shooting starts %	Average root length	Average Shoot length
RD 2660	1/5(20%)	1/5(20%)	0/5(0%)	0.4 cms	--------------
RD 2052	4/5(80%)	4/5(80%)	0/5(0%)	1.5 cms	-------------
RD 2503	1/5(20%)	1/5(20%)	0/5(0%)	0.4 cms	--------------
RD 2508	3/5(60%)	3/5(60%)	1/5(20%)	2.5 cms	2 cms
BH 924	5/5(100%)	5/5(100%)	0/5(0%)	1.7 cms	---------------

Table 8: Viability and Root-Shoot length After 2 week at 200 mM concentration

Variety	No. of Seeds germinated %	No. of Rooted seeds %	No. of seeds in which shooting starts %	Average root length	Average Shoot length
RD 2660	1/5(20%)	1/5(20%)	0/5(0%)	0.7 cms	--------------
RD 2052	4/5(80%)	4/5(80%)	1/5(20%)	1.9 cms	1.1 cms
RD 2503	1/5(20%)	1/5(20%)	0/5(0%)	0.6 cms	--------------
RD 2508	3/5(60%)	3/5(60%)	1/5(20%)	2.9 cms	2.8 cms
BH 924	5/5(100%)	5/5(100%)	1/5(20%)	2.2 cms	1.2 cms

Table 9: Viability and Root-Shoot length After 3 week at 200 mM concentration

Variety	No. of Seeds germinated %	No. of Rooted seeds %	No. of seeds in which shooting starts %	Average root length	Average Shoot length
RD 2660	1/5(20%)	1/5(20%)	0/5(0%)	1.2 cms	--------------
RD 2052	4/5(80%)	4/5(80%)	1/5(20%)	2.1 cms	1.9 cms
RD 2503	1/5(20%)	1/5(20%)	0/5(0%)	0.9 cms	--------------
RD 2508	3/5(60%)	3/5(60%)	2/5(40%)	3.4 cms	3.2 cms
BH 924	5/5(100%)	5/5(100%)	2/5(40%)	2.7 cms	1.9 cms

Table 10: Viability and Root-Shoot length After 1 week at 300 mM concentration

Variety	No. of Seeds germinated %	No. of Rooted seeds %	No. of seeds in which shooting starts %	Average root length	Average Shoot length
RD 2660	0/5(0%)	0/5(0%)	0/5(0%)	------------	--------------
RD 2052	1/5(20%)	1/5(20%)	0/5(0%)	1 cms	--------------
RD 2503	0/5(0%)	0/5(0%)	0/5(0%)	------------	--------------
RD 2508	3/5(60%)	3/5(60%)	0/5(0%)	1.5 cms	---------------
BH 924	2/5(40%)	2/5(40%)	0/5(0%)	1.2 cms	-----------------

Table 11: Viability and Root-Shoot length After 2 week at 300 mM concentration

Variety	No. of Seeds germinated %	No. of Rooted seeds %	No. of seeds in which shooting starts %	Average root length	Average Shoot length
RD 2660	0/5(0%)	0/5(0%)	0/5(0%)	------------	--------------
RD 2052	1/5(20%)	1/5(20%)	0/5(0%)	1.3 cms	--------------
RD 2503	0/5(0%)	0/5(0%)	0/5(0%)	------------	--------------
RD 2508	3/5(60%)	3/5(60%)	1/5(20%)	1.9 cms	1.1 cms
BH 924	2/5(40%)	2/5(40%)	1/5(20%)	1.8 cms	1 cms

Table 12: Viability and Root-Shoot length After 3 week at300 mM concentration

Variety	No. of Seeds germinated %	No. of Rooted seeds %	No. of seeds in which shooting starts %	Average root length	Average Shoot length
RD 2660	0/5(0%)	0/5(0%)	0/5(0%)	------------	--------------
RD 2052	1/5(20%)	1/5(20%)	0/5(0%)	1.5 cms	--------------
RD 2503	0/5(0%)	0/5(0%)	0/5(0%)	------------	--------------
RD 2508	3/5(60%)	3/5(60%)	1/5(20%)	2.1 cms	1.6 cms
BH 924	2/5(40%)	2/5(40%)	1/5(20%)	2.1 cms	1.4 cms

Table 13: Viability and Root-Shoot length After 1 week at 400 mM concentration

Variety	No. of Seeds germinated %	No. of Rooted seeds %	No. of seeds in which shooting starts %	Average root length	Average Shoot length
RD 2660	0/5(0%)	0/5(0%)	0/5(0%)	------------	--------------
RD 2052	0/5(0%)	0/5(0%)	0/5(0%)	------------	--------------
RD 2503	0/5(0%)	0/5(0%)	0/5(0%)	------------	--------------
RD 2508	0/5(0%)	0/5(0%)	0/5(0%)	------------	--------------
BH 924	0/5(0%)	0/5(0%)	0/5(0%)	------------	--------------

Table 14: Viability and Root-Shoot length After 2 week at 400 mM concentration

Variety	No. of Seeds germinated %	No. of Rooted seeds %	No. of seeds in which shooting starts %	Average root length	Average Shoot length
RD 2660	0/5(0%)	0/5(0%)	0/5(0%)	------------	--------------
RD 2052	0/5(0%)	0/5(0%)	0/5(0%)	------------	--------------
RD 2503	0/5(0%)	0/5(0%)	0/5(0%)	------------	--------------
RD 2508	0/5(0%)	0/5(0%)	0/5(0%)	------------	--------------
BH 924	0/5(0%)	0/5(0%)	0/5(0%)	------------	--------------

Table 15: Viability and Root-Shoot length After 3 week at 400 mM concentration

Variety	No. of Seeds germinated %	No. of Rooted seeds %	No. of seeds in which shooting starts %	Average root length	Average Shoot length
RD 2660	0/5(0%)	0/5(0%)	0/5(0%)	------------	--------------
RD 2052	0/5(0%)	0/5(0%)	0/5(0%)	------------	--------------
RD 2503	0/5(0%)	0/5(0%)	0/5(0%)	------------	--------------
RD 2508	0/5(0%)	0/5(0%)	0/5(0%)	------------	--------------
BH 924	0/5(0%)	0/5(0%)	0/5(0%)	------------	--------------

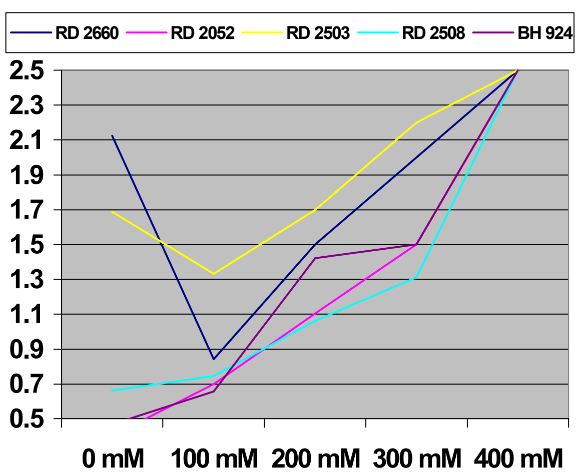

Fig. 1: Root to Shoot length ratio of all varieties at different concentration after Full Period of observations.

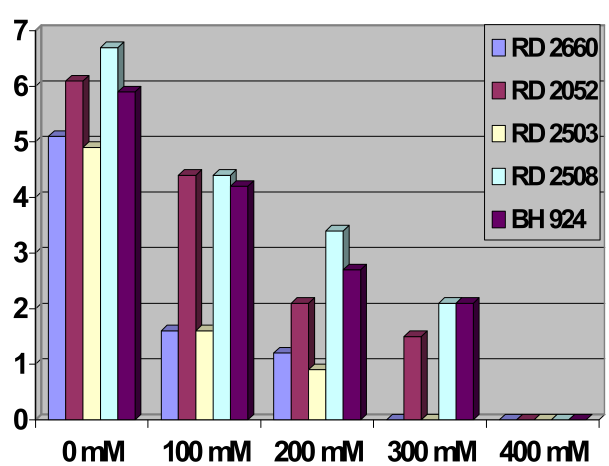

Fig. 2: Root length of all varieties at different concentration after Full Period of observations.

Salinity causes significant reduction in root and shoots length in Hordeum Vulgare (Tables 1-15). Increase in salinity causes reduction in growth and viability of seeds. Increase in salinity from 0 mM/L to 400 mM/L causes a great variance in root length, shoot length of plants and viability of seeds.

The ratio of root to shoot length increases as salinity increases (Fig. 1). When salinity increases it inhibits shoot length more than root length. Roots would appear to be the most vulnerable part of the plant as they are directly exposed to salt or to drying soil, but nevertheless they are surprisingly robust.

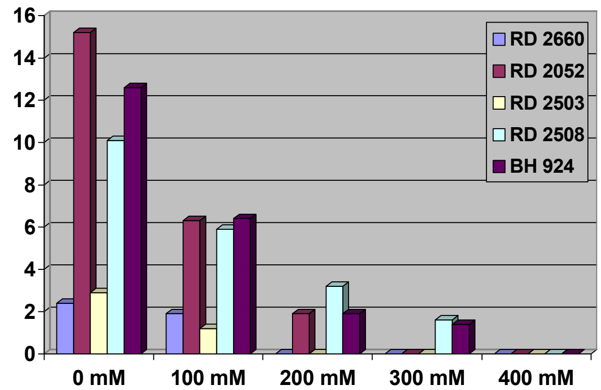

Fig. 3: Shoot length of all varieties at different concentration after Full Period of observations.

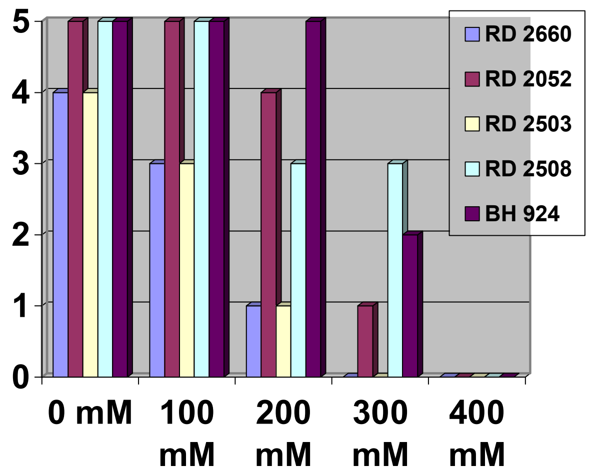

Fig. 4: Viability of All the varieties at different concentration after Full Period of observations.

The results shows RD 2660 and RD 2503 are unable to show a promising salt tolerance. Even they are not promising in the liquid salts and nutrient condition of Hoagland medium. As the concentration of Nacl increases in the medium both the varieties are fail to grow normally. The viability of both variety decrease up to 50% and root-shoot length also decrease at 100mM in comparison to control up to 2 weeks and after 3 weeks 60% plants were growing (Fig 1, 2, 3). At the salinity 200 mM, viability of plant only 25% and at greater concentration i.e. 300 and 400mM, viability of plants is 0% (Fig. 4).

RD 2052 is showing salt tolerance 100% up to 100 mM but falls 20% when salt concentration increases to 200 mM. The root length and shoot length is normal till 100 mM but there is a fall at 200 mM. Only 20% seeds of RD 2052 are showing rooting but shooting was not observe at 300 mM and they failed to grow at 400 mM.

Fig. 5: Hoagland media with different salt concentrations RD 2052 variety growing First and last observations.

RD 2058 shows approximately same results to RD 2052, the only difference at 200 mM and 300 mM where at 200 mM it showing more shooting than RD 2052 and at 300 mM 60% rooting with 20% shooting were observed (Tables 1-12) (Fig. 5).

BH 924 is 100% viable up to 200 mM but viability decease 60% when concentration reached to 300 mM (tables 1-12, fig.4). All the seeds were rooted but only 40% seeds were lead to shooting at 200 mM concentration (tables 7-9). At the 300 mM concentration, shooting remain up to 20% (tables 10-12). BH 924 is most viable variety till 200 mM concentration but at the 300 mM concentration, RD 2508 is more viable than BH 924(tables 10-12, fig 4)).Growth of germinated seeds are more in RD 2508 in comparison to BH 924 at all the concentration levels(fig 2,3). Viability is more in BH 924 at 200 mM concentration but the growth of germinated seeds are more in RD 2508 at 200 mM concentration (fig 1-4).

DISCUSSION

All verities grow normally in general natural condition but in salty water condition only BH 924 and RD 2508 are growing so it may be possible in some genetic difference [14].

A negative relationship between percentage seed germination and concentration of salt was obtained it may be possible due to change in activity of many enzyme in germinating seeds.es [15]. In these all verities two BH 924 and RD 2508 have high root length this indicate both are good for dry and salty habit [16].

The results indicates that in these varieties RD 2052, RD 2508 and BH 924 are salt tolerant but BH 924 is the highly salt tolerable variety up to 200 mM (fig 2,3)and RD 2508 is most salt tolerant variety at 300 mM concentration(fig 1-3). These results indicate that at concentration more than 200 mM RD 2508 is preferable but at lower concentration BH 924 is preferable variety. All the varieties fail to show viability at 400 mM which indicates that 400 mM concentration of Nacl is the lethal concentration for the barley. The metabolic pathway conformation could help us to prove these results.

CONCLUSION

The results of Salt Screening Test conclude that BH 924 is the best salt tolerant variety up to 200 mM and RD 2508 is best for more than 200 mM concentration.

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