



P-ISSN: 2349-8528

E-ISSN: 2321-4902

IJCS 2018; 6(1): 1584-1589

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Received: 18-11-2017

Accepted: 19-12-2017

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Determination of salinity tolerance in lentil (*Lens culinaris* M.) seedlings using salt tolerance index

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Abstract

The present investigation was conducted to determine the salt tolerance of 10 genotypes of lentil namely RLG-5, RLG-195, RLG-234, RLG-250, RLG-254, RLG-256, RLG-258, SAPNA, DPL-58 and L-4076 under different salinity levels (0.0, 20, 40 and 60 mM NaCl) during germination and seedling stage. 15 seeds of each genotype were sown in sterilized petridishes on autoclaved germination papers and irrigated daily with 3 ml of test solutions after draining out the previous day solutions at $24 \pm 2^\circ\text{C}$ in the culture room and the set was maintained in dark for the first two days followed by exposure to light achieved by tube lights and incandescent bulbs. The experimental design was RBD with three replications. The experiment was terminated after 8th day of sowing in petridishes and average germination percentage, shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, total dry weight and salt tolerance index were recorded. The salt tolerance index (STI) of the genotypes, expressed as the ratio of dry matter yield produced under the NaCl treatments compared to the control treatment, was found to be a reliable criterion for ranking genotypes for their tolerance to NaCl. Genotypes L-4076, RLG-254, RLG-234 and RLG-258 were found tolerant.

Keywords: lentil, salinity, salt tolerance index (STI), seedling, NaCl

1. Introduction

Soil salinity is a major abiotic stress in plant production worldwide. This has led to research into salt tolerance with the aim of improving crop plants or soil reclamation [1]. However, soil reclamation is a very expensive process, and hence the cultivation of tolerant species and varieties is the most practical solution when the salinity is low. It is well known that there are significant genotypic differences with respect to salt tolerance between and within plant species [2]. Lentil (*Lens culinaris* M.) is one of the most important grain legume nitrogen fixing crop and it is mainly cultivated in semi-arid regions of the world particularly in the Indian sub-continent and the dry areas of Middle East [3]. Due to increasing salinity problems India and in many other countries around the world, breeding for salinity needs more attention. Besides genetic resources, the use of efficient selection criteria would help breeders. However, it is difficult to say that the breeders have efficient selection criteria and tools for improvement of salt tolerant varieties. Rather than a long-term breeding program, the determination of more tolerant varieties to grow in saline soils may be a short-term solution [4]. Soil salinity is a condition in which the soluble salt content of the soil reaches a level harmful to crops through the reduced osmotic potential of the soil solution and the toxicity of specific ions. These soluble salts may be from those present in the original soil profile or transported to the profile by irrigation water containing an unusual high concentration [5]. All these factors manifest themselves by morphological, physiological and metabolic modifications in plant such as decrease in seed germination, decrease in shoot and root length, alterations in the integrity of cell membranes, changes in different enzymatic activities and photosynthesis. According to FAO studies 7% of the arable land affected by salinity. Seed germination is a complicated process and is sensitive to salt stress [6]. Salinity influence seed germination by reducing the osmotic potential and toxicity of specific ions such as sodium and chlorine, as well as reducing essential nutrients such as calcium and potassium. Salinity reduces the ability of plants to take up water, leading to metabolic effect that reduces plant growth. The deleterious consequences of high salt concentrations in the external solution of plant cells are hyper-osmotic shock and ionic imbalance. Although, salt stress affects all growth stages of a plant but seed germination and seedling growth stages are known to be more sensitive for most of the plant species.

A study on variability available in the material is the pre-requisite for initiating a varietal development programme. Therefore, the present study was initiated to determine the salinity tolerance of some lentil genotypes during germination and early seedling stage.

2. Materials and Methods

The laboratory experiment was carried out at Department of Plant Breeding and Genetics, Sri Karan Narendra Agriculture University, Jobner-303329, Rajasthan, India in December, 2016 where the temperature was 24 ± 2 °C. The seeds of ten genotypes of lentil namely, RLG-5, RLG-195, RLG-234, RLG-250, RLG-254, RLG-256, RLG-258, SAPNA, DPL-58 and L-4076 were used for evaluation. Prior to germination, the seeds were surface sterilized with 0.1% mercuric chloride for 1 minute and washed 3 times under running tap water followed by washing with double distilled water.

2.1 Treatments and experimental design: Four salinity levels viz., 0.0, 20, 40 and 60 mM NaCl were prepared by dissolving 0.0, 292.2, 584.4 and 876.6 mg of NaCl salt in 250 ml of double distilled water, respectively and used in experiment and designated as S₀, S₁, S₂ and S₃, respectively. The experiment with 10 genotypes and 4 salinity levels was laid out in randomized block design with three replications. The number of petridishes needed for this experiment was 120 (10×4×3).

2.2 Procedure for germination: The 15 seeds of each genotype was germinated in sterilized (165°C for 4 hours in hot air oven) petridishes of 9 cm diameter layered with autoclaved (15 psi and 121°C for 20 minutes) germination papers and then moistened with 3 ml of test solutions daily after removing previous day solution. The set was maintained in dark for first two days. The germination was monitored on 7th day from the day of seed planting.

2.3 Observations recorded: Observations were recorded on 8th day of planting on different characters and parameters. Five seedlings were randomly selected from each petridish to record the data on shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight and total dry weight. The data on shoot dry weight and root dry weight was recorded after drying in hot air oven for 48 hours at 65 °C. The methods used for recording observations are described below:

2.3.1 Germination percentage: A seed was considered as germinated at the emergence of both root and shoot up to 2 mm length [7]. The germination was recorded on 7th day after planting and germination percentage was determined by using the following formula [8]:

$$\text{Germination Percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

2.3.2 Shoot and root length: On 8th day the shoot and root length of germinated seeds was recorded. For this, five seedlings were randomly selected and divided into two parts viz., shoot and root and length was measured using measuring scale in centimeter and averaged. The hypocotyl length was included in shoot length.

2.3.3 Shoot and root fresh weight: The fresh weight of shoots and roots from the five seedlings which were selected already from each replication and in each treatment was measured in milligram by using a sensitive electronic balance and averaged.

2.3.4 Shoot, root and total dry weight: The shoots and roots which were taken for fresh weight were kept into paper bags. The name of genotypes and levels of salinity were written on paper bags by marker for further identification. After taking fresh weight these were kept in oven at 65°C for 48 hours for drying. After drying, the dried shoots and roots were weighed by sensitive electronic balance in milligram and average was recorded. The total dry weight was obtained by adding dry weight of shoot and root.

2.3.5 Salt tolerance index: Salt tolerance index from the five seedlings which were selected already from each replication and each treatment was calculated by the following formula [9]:

$$\text{STI} = \frac{\text{TDW at one of the salinity level}}{\text{TDW at control}} \times 100$$

Where, STI= Salt tolerance index and TDW= Total (root + shoot) dry weight

2.4 Statistical analysis

The data obtained from this study were subjected to analysis of variance following standard statistical methods [10] and significant differences among the mean values were compared by least significant difference (LSD) test (P<0.05).

3. Experimental Results

3.1 Analysis of variance

The pooled analysis of variance indicated that the genotypes, salinity levels and genotype x salinity interaction exhibited significant mean sum of squares for all the characters except germination percentage due to genotypes. This indicated differential response of genotypes to salinity levels for all the characters.

Table 1 The Pooled ANOVA for various traits (Mean sum of squares)

Characters	Source of variation with degree of freedom				
	Genotypes (9)	Salinity levels (3)	Replication/ Salinity (8)	Genotype x Salinity (27)	Error (72)
Germination (%)	28.853	1358.087**	28.489	35.767**	16.997
Shoot length (cm)	1.64**	21.163**	0.216	0.437**	0.172
Root length (cm)	3.767**	72.767**	0.501	2.173**	0.38
Shoot fresh weight (mg)	212.933**	1726.973**	10.974	44.461**	13.155
Root fresh weight (mg)	168.908**	1864.03**	11.1	49.739**	12.195
Shoot dry weight (mg)	1.583**	12.293**	0.339	0.507*	0.26
Root dry weight (mg)	0.892**	2.71**	0.016	0.111**	0.049
Total dry weight (mg)	4.232**	26.537**	0.251	0.646*	0.355

* and ** represent significant at 5% and 1% level of significance, respectively

3.2 Effect of salinity on mean performance

The mean values at different salinity levels for various genotypes and characters are presented in Table 3.2. Perusal of table revealed that the mean values of all the characters varied along the salinity gradient. The values were maximum in the control (S₀) and were minimum at the highest salinity level (S₃) for majority of characters.

3.2.1 Germination percentage: The magnitude of germination percentage decreased with increase in salt

concentration. It was highest in S₀ (96.22 %) then decreased progressively in S₁ (93.11 %), S₂ (89.33 %) and S₃ (80.67 %). It ranged from 91.11 % (SAPNA) to 100 % (L-4076) in S₀, 88.89 % (RLG-234) to 97.78 % (L-4076) in S₁, 84.44 % (RLG-195) to 95.55 % (RLG-250) in S₂, and 75.55 % (L-4076) to 86.67 % (RLG-254) in S₃ salinity level. The range was wider in S₂ and S₃ as compared to S₀ and S₁ salinity level indicted that higher salinity adversely affected germination.

Table 2: The mean values of genotypes for different characters over different salinity levels

Genotypes	Germination percentage				Shoot Length (cm)				Root length (cm)			
	S ₀	S ₁	S ₂	S ₃	S ₀	S ₁	S ₂	S ₃	S ₀	S ₁	S ₂	S ₃
RLG - 195	95.55	97.78	84.44	80.00	7.15	6.21	6.21	5.28	6.63	4.69	4.63	7.26
RLG - 254	97.78	91.11	91.11	86.67	7.42	6.51	5.30	5.71	7.85	5.33	4.04	6.36
RLG - 5	95.55	91.11	91.11	84.45	7.53	5.88	5.77	5.98	7.92	4.99	4.59	7.97
SAPNA	91.11	95.55	84.45	75.55	8.07	5.88	6.30	5.58	8.93	4.41	4.85	6.40
RLG - 256	97.78	95.55	88.89	77.78	7.11	6.50	6.61	6.21	5.79	4.31	3.93	4.33
RLG - 250	95.56	91.11	95.55	84.45	8.09	5.60	6.19	5.27	8.14	3.61	4.86	5.45
DPL - 58	95.55	91.11	84.45	82.22	6.55	5.51	4.57	4.50	9.04	3.77	4.79	6.31
RLG - 258	97.78	91.11	91.11	77.78	7.75	6.45	5.73	5.55	8.05	5.01	3.88	4.87
L - 4076	100.00	97.78	91.11	75.55	7.07	5.63	5.59	5.37	6.36	4.51	4.63	6.10
RLG - 234	95.55	88.89	91.11	82.22	6.97	6.42	5.43	5.16	9.43	3.91	6.39	6.35
Overall mean	96.22	93.11	89.33	80.67	7.37	6.06	5.77	5.46	7.81	4.45	4.66	6.14
C.D.	6.68	7.33	7.06	7.20	0.85	0.75	0.67	0.54	1.13	0.91	1.03	1.14
C.V. (%)	4.05	4.59	4.61	5.21	6.75	7.22	6.73	5.72	8.40	11.95	12.95	10.83
Genotypes	Shoot fresh weight (mg)				Root fresh weight (mg)				Shoot dry weight (mg)			
RLG - 195	59.91	51.77	45.15	37.91	48.34	36.63	39.85	36.10	5.87	5.45	4.50	4.19
RLG - 254	58.95	49.55	38.81	45.54	38.14	36.35	27.81	31.04	5.45	4.99	4.32	4.51
RLG - 5	53.95	44.36	39.51	42.69	46.05	31.47	28.68	27.57	6.02	4.82	4.38	4.93
SAPNA	67.71	45.95	52.20	43.17	54.41	36.95	33.80	30.85	6.91	5.01	5.87	4.92
RLG - 256	53.09	42.93	45.45	43.21	42.13	30.41	27.88	24.61	5.45	4.56	4.79	4.24
RLG - 250	61.65	42.32	41.82	39.65	39.04	29.23	29.05	30.80	5.71	4.75	4.51	4.49
DPL - 58	62.26	44.34	41.73	52.69	48.28	37.35	33.55	39.40	7.55	5.25	4.37	4.91
RLG - 258	60.92	45.67	42.96	43.34	47.91	31.03	30.62	20.97	6.04	5.21	5.02	4.64
L - 4076	51.07	45.43	39.58	39.61	48.17	29.47	33.62	32.23	5.37	4.74	4.58	4.61
RLG - 234	69.52	60.60	49.53	54.50	62.37	38.13	31.69	31.22	6.24	6.11	5.04	5.32
Overall mean	59.90	47.29	43.67	44.23	47.48	33.70	31.66	30.48	6.06	5.09	4.74	4.68
C.D.	9.51	5.21	3.87	4.72	5.55	6.54	6.46	5.31	1.23	0.81	0.69	0.65
C.V. (%)	9.26	6.42	5.17	6.22	6.81	11.32	11.89	10.16	11.80	9.27	8.49	8.16
Genotypes	Root dry weight (mg)				Total dry weight (mg)				Salt tolerant index (%)			
RLG - 195	3.35	2.80	2.63	2.21	9.22	8.25	7.13	6.41	100	89.70	77.49	69.64
RLG - 254	2.37	2.33	2.25	1.76	7.82	7.32	6.57	6.27	100	94.17	84.42	80.24
RLG - 5	2.71	2.37	2.00	2.09	8.73	7.19	6.38	7.02	100	83.92	73.88	81.67
SAPNA	2.88	2.75	2.35	2.43	9.79	7.77	8.22	7.35	100	79.37	84.00	74.89
RLG - 256	3.02	2.37	2.23	2.31	8.47	6.93	7.01	6.55	100	81.90	82.83	77.35
RLG - 250	2.64	2.43	1.80	2.13	8.35	7.19	6.31	6.61	100	86.10	75.56	79.24
DPL - 58	3.37	2.72	2.47	3.07	10.93	7.97	6.85	7.97	100	73.16	63.28	74.14
RLG - 258	3.02	2.59	2.75	2.41	9.06	7.79	7.77	7.05	100	86.82	87.17	78.64
L - 4076	2.83	2.37	2.39	2.23	8.20	7.11	6.97	6.83	100	87.42	84.94	83.63
RLG - 234	3.64	2.81	2.63	2.79	9.88	8.92	7.67	8.11	100	90.38	77.62	82.16
Overall mean	2.98	2.55	2.35	2.34	9.05	7.64	7.09	7.02	100	85.29	79.12	78.16
C.D.	0.34	0.26	0.43	0.46	1.47	0.77	0.86	0.82	-	15.75	13.84	13.60
C.V. (%)	6.61	5.94	10.77	11.44	9.46	5.91	7.11	6.84	-	10.77	10.20	10.14

3.2.2 Shoot length (cm): The shoot length was also decreased with increasing level of salinity. It was highest in S₀ (7.37 cm) then decreased progressively in S₁ (6.06 cm), S₂ (5.77 cm) and S₃ (5.46 cm). It ranged from 6.55 cm (DPL-58) to 8.09 cm (RLG-250) in S₀, 5.51 cm (DPL-58) to 6.51 cm (RLG-254) in S₁, 4.57 cm (DPL-58) to 6.61 cm (RLG-256) in S₂, and 4.50 cm (DPL-58) to 6.21 cm (RLG-256) in S₃ salinity level.

3.2.3: Root length (cm): The root length was highest in S₀ (7.81 cm). It was increased in S₂ (4.66 cm) and S₃ (6.14 cm) compared to S₁ (4.45 cm). It ranged from 5.79 cm (RLG-256)

to 9.43 cm (RLG-234) in S₀, 3.61 cm (RLG-250) to 5.33 cm (RLG-254) in S₁, 3.88 cm (RLG-256) to 6.39 cm (RLG-234) in S₂, and 4.33 cm (RLG-256) to 7.97 cm (RLG-5) in S₃ salinity level.

3.2.4: Shoot fresh weight (mg): The shoot fresh weight also decreased with increasing level of salinity except in S₃. The magnitude of this parameter was highest in S₀ (59.90 mg), then decreased in S₁ (47.29 mg) and S₂ (43.67 mg) and then further increased in S₃ (44.23 mg). It ranged from 51.07 mg (L-4076) to 69.52 mg (RLG-234) in S₀, 42.32 mg (RLG-250)

to 60.60 mg (RLG-234) in S₁, 38.81 mg (RLG-254) to 52.20 mg (SAPNA) in S₂, and 37.91 mg (RLG-195) to 54.50 mg (RLG-234) in S₃ salinity level.

3.2.5: Root fresh weight (mg): In case of root fresh weight, there was also a decreasing trend with increasing salinity level. It was maximum in S₀ (47.48 mg) followed by S₁ (33.70 mg), S₂ (31.66 mg) and S₃ (30.48 mg). It ranged from 38.14 mg (RLG-254) to 62.37 mg (RLG-234) in S₀, 29.23 mg (RLG-250) to 38.13 mg (RLG-234) in S₁, 27.81 mg (RLG-254) to 39.85 mg (RLG-195) in S₂, and 20.97 mg (RLG-258) to 39.40 mg (DPL-58) in S₃.

3.2.6: Shoot dry weight (mg): The shoot dry weight was also observed to be decreased with increase in salinity levels. It was maximum in S₀ (6.06 mg) followed by S₁ (5.09 cm), S₂ (4.74 cm) and S₃ (4.68 mg), though the magnitude in S₂ and S₃ was almost same. It ranged from 5.37 mg (L-4076) to 7.55 mg (DPL-58) in S₀, 4.56 mg (RLG-256) to 6.11 mg (RLG-234) in S₁, 4.32 mg (RLG-254) to 5.87 mg (SAPNA) in S₂, and 4.19 mg (RLG-195) to 5.32 mg (RLG-234) in S₃ salinity level.

3.2.7 Root dry weight (mg): The root dry weight also exhibited a decreasing trend with increase in salinity level. It was maximum in S₀ (2.98 mg) and then decreased in S₁ (2.55

mg), S₂ (2.35 mg) and S₃ (2.34 mg), though the magnitude was at par in S₂ and S₃. It ranged from 2.37 mg (RLG-254) to 3.64 mg (RLG-234) in S₀, 2.33 mg (RLG-254) to 2.81 mg (RLG-234) in S₁, 1.80 mg (RLG-250) to 2.75 mg (RLG-258) in S₂, and 1.76 mg (RLG-254) to 3.07 mg (DPL-58) in S₃ salinity level. The range was wider in S₃ and shorter in S₁.

3.2.8 Total dry weight (mg): The total (shoot + root) dry weight was maximum in S₀ (9.05 mg) and then decreased in S₁ (7.64 mg), S₂ (7.09 mg) and S₃ (7.02 mg), though the magnitude was at par in S₂ and S₃. It ranged from 7.82 mg (RLG-254) to 10.93 mg (DPL-58) in S₀, 6.93 mg (RLG-256) to 8.92 mg (RLG-234) in S₁, 6.31 mg (RLG-250) to 8.22 mg (SAPNA) in S₂, and 6.27 mg (RLG-254) to 8.11 mg (RLG-234) in S₃ salinity level.

3.2.9 Salt tolerance index (%): The salt tolerance index was maximum in S₀ (100%) and then decreased in S₁ (85.29%), S₂ (79.12%) and S₃ (78.16%), though the magnitude was at par in S₂ and S₃. It ranged from 73.16% (DPL-58) to 94.17% (RLG-254) in S₁, 63.28% (DPL-58) to 87.17% (RLG-258) in S₂, and 69.64% (RLG-195) to 83.63% (L-4076) in S₃ salinity level. The genotypes were ranked on the basis salt tolerance index (STI) of three salinity levels viz., S₁, S₂ and S₃ and position of rank for each genotype was obtained which is presented in Table 3.

Table 3: Ranking of genotypes based upon salt tolerance index (STI)

Genotypes	Salinity levels and rank of genotypes							Rank Total	Overall Rank
	S ₀	S ₁		S ₂		S ₃			
	STI	STI	Rank	STI	Rank	STI	Rank		
RLG - 195	100	89.7	3	77.49	7	69.64	10	20	7
RLG - 254	100	94.17	1	84.42	3	80.24	4	8	2
RLG - 5	100	83.92	7	73.88	9	81.67	3	19	5
SAPNA	100	79.37	9	84	4	74.89	8	21	9
RLG - 256	100	81.9	8	82.83	5	77.35	7	20	7
RLG - 250	100	86.1	6	75.56	8	79.24	5	19	5
DPL - 58	100	73.16	10	63.28	10	74.14	9	29	10
RLG - 258	100	86.82	5	87.17	1	78.64	6	12	4
L - 4076	100	87.42	4	84.94	2	83.63	1	7	1
RLG - 234	100	90.38	2	77.62	6	82.16	2	10	3

The table revealed that the genotypes RLG-254, RLG-234 and RLG-195 were performed better in S₁, RLG-258, L-4076 and RLG-254 in S₂ and L-4076, RLG-234 and RLG-5 in S₃. Based upon overall rank the genotypes L-4076, RLG-254 and RLG-234 were found tolerant.

4. Discussion

Lentil is an important legume which is in cultivation in various agro climatic regions of the India. Studies on screening for salinity tolerance in lentil are limited as compared to other legume crops especially for seedling traits. The findings emanating from the present investigation are discussed here in the light of available literature.

4.1 Analysis of variance: Genotype x environment interaction is a common phenomenon present in crop plant species [11]. In the present investigation the pooled analysis of variance showed that the mean squares due to salinity levels and genotypes were significant, indicating significant differences among genotypes and the effect of salinity on the genotypes. The genotype x salinity interaction was found significant for all the characters studied which indicated differential response of genotypes to salinity. Similar results

have also been reported in lentil by Azene *et al.* [12] and Tesfaye *et al.* [13].

4.2 Effect of salinity on mean performance: Comparison of mean values of different characters indicated that for most of the characters, these values decreased under saline condition particularly for germination percentage, shoot length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight and salt tolerance index. Differences were less for fresh and dry weight of shoots and roots. Similar findings were also reported by Kandil *et al.* [14] and Saroj and Soumana [15] in mung bean and Keshtiban *et al.* [16] in lentil. It might be concluded that effect of salinity on different characters were not uniform, some character were influenced more while other less. This reduction in mean performance was due to salts of different nature and concentration because increased water potential, restricted the movement of water towards the seed surface [17]. The effect of salinity on germination and seedling characteristics was widely reported in mung bean [18], in lentil, chickpea and faba bean [19], in cowpea [20], in *Pisum sativum* var. abyssinicum and *Lathyrus sativus* [21], in moth bean and mung bean [15] and in fenugreek [22]. The salinity gradient adversely affected the mean values of almost all the

characters, except root length of seedlings. The effect of higher levels namely, S_2 and S_3 was highest on all the characters in comparison to S_0 and S_1 . Interestingly the mean values of root length were higher at S_2 and S_3 than at S_1 . Such stimulatory effect of salinity has been reported earlier in lentil [23]. It may be due to toxic effects of the NaCl used as well as unbalanced nutrient uptake by the seedlings and lower water availability [24]. None of the genotypes showed uniform response under different salinity levels for any given character. Based upon rank totals the genotype L-4076 was least affected by salinity because it gained rank 1 among the genotypes.

In order to get meaningful comparison the genotypes were ranked on the basis of salt tolerance index (STI) of three salinity levels viz., S_1 , S_2 and S_3 and position of rank for each level was obtained which is presented in Table 3.3. A genotype with least rank total will be the best genotype. Comparison to rank totals indicated that L-4076 ranked first followed by RLG-254, RLG-234 and RLG-258. Thus, these were ideal genotypes which are expected to give good response over salinity levels. Direct or indirect exploitation of these genotypes through hybridization is recommended for breeding of genotypes suitable for salinity.

5. Conclusion and Recommendation

The effect of salinity on seedling traits showed specific trend although at higher salinity levels the means of various traits were reduced in comparison to S_0 and S_1 . However, variation existed; the genotypes L-4076, RLG-254, RLG-234 and RLG-258 were found to be the best suited for salinity. Direct or indirect exploitation of these genotypes through hybridization is recommended for breeding of genotypes suitable for salinity. It would be ideal if studies on effect of salinity on germination and seedling characters be done at some higher concentrations of salts to identify genotypes for salt tolerance for further breeding programmes.

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