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SCREENING OF LANDRACES OF RICE UNDER CULTIVATION IN KUMAUN HIMALAYA FOR SALINITY STRESS DURING GERMINATION AND EARLY SEEDLING GROWTH

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SUMMARY

Thirty landraces of rice (*Oryza sativa* L.) along with an introduced variety VL-206 were screened for seed germination and seedling growth under salinity stress (0.10, 0.15 and 0.20 M NaCl). Among the landraces, Saunji showed maximum germination (90%) even at the highest salinity level (0.20 M NaCl) and was considered most tolerant to salinity stress. The maximum reduction in seed germination was found in Syaudhan (36%) when exposed to 0.20 M NaCl. Early seedling growth in different landraces varied with the salinity level. Increasing the NaCl concentration from 0.10 M to 0.20 M led to a significant reduction in root and seedling (shoot) growth, irrespective of the landrace in comparison to control. The results revealed that a valuable source of rice germplasm, in the form of landraces, is still being maintained through cultivation in remotely located villages in Kumaun region of Indian Central Himalaya, which may be useful for introgression of salinity resistant genes for the development of stress tolerant varieties.

***Key words*: Germination, Kumaun Himalaya, landraces, *Oryza sativa* L., salinity stress.**

INTRODUCTION

Crop varieties differ in their response to various biotic and abiotic stresses. Salinity is a major and wide spread abiotic stress that constrains crop productivity under rainfed as well as irrigated conditions (Epstein *et al*. 1980). Excessive salt concentration decreases water potential and thus results in reduced water availability to the plant. Under such situations plants often show wilting due to “physiological drought” while water may be present. Poor germination, seedling emergence and establishment under saline conditions lead to poor crop stand and productivity. An increase in salinity level has been found to reduce seed germination in different genotypes of wheat, barley and pearl millet (Srivastava 1998).

Rice is generally a salt sensitive crop (Chowdhury *et al*. 1995), however, there are varieties which show variation in salt tolerance (De Datta 1972). In plants that survive high salinity, growth and yield are substantially reduced (Flower and Yeo 1981). Although the physiological basis for salt resistance is still not fully understood (Chowdhury *et al*. 1995), there has always been a need for developing rice varieties that are tolerant to salinity. The International Rice Research Institute (IRRI) has adopted a screening method by exposing glasshouse grown plants to high salt levels.

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Salt stress is a major constraint to cereal production worldwide. In Asia alone, 21.5 million ha are affected, of which 12 million ha are saline and 9.5 million ha are alkaline/sodic (Lafitte *et al*. 2004). Although salt sensitive, rice is the only cereal that has been recommended as a desalinization crop because of its ability to grow under flooded conditions, and because the standing water in rice fields can help salt to leach from the topsoil to a level low enough for subsequent crops (Bhumbla and Abrol 1978). Furthermore, considerable variation in tolerance to salt has been observed in rice (Akbar *et al*. 1972, Flowers and Yeo 1981). It is comparatively tolerant to salt stress during germination, active tillering, and towards maturity. In India, large tracts of land (11 million hectares out of approx. 329 million hectares) are becoming unsuitable for cultivation due to high salt level (Grover 1999). An attempt has, therefore, been made in the present study to screen thirty landraces of rice for salt tolerance, still under cultivation in the Kumaun region of Indian Central Himalaya.

MATERIALS AND METHODS

Seeds of thirty landraces of rice (*Oryza sativa* L.) were collected during the months of March – April 1998 from 13 villages located in the Lesser Himalayan region of Kumaun, Uttaranchal (see Agnihotri *et al*. 2003 for details). VL-206 (a recommended high yielding variety for rainfed uplands of the Himalayan region) was obtained from the Vivekanand Parvatiya Krishi Anusandhan Sansthan, Almora for comparison.

Seeds of 30 landraces of rice after six months of storage at room temperature ($30 \pm 5^{o}C$) following harvest were allowed to germinate in a plant growth chamber (Model: 3500 G; Saveer Biotech Ltd, New Delhi) at $25^{o}C \pm 5^{o}C$ under dark, following the recommendations of the International Seed Testing Association (Anonymous 1996). Twenty-five seeds (in triplicate) of each landrace and that of VL-206 were placed in a petridish (100x17mm) on a single layer of Whatman No. 1 filter paper moistened with distilled water (control) or NaCl solutions (0.10, 0.15, 0.20 M) representing different salinity levels (Heenan *et al*. 1988). The petridishes were inspected daily and the seeds were considered germinated when the radicle length reached 5 mm or more. Germinated seeds were counted and discarded; although the experiment was continued till 15 days but no further germination after 11 days was recorded.

In order to screen different landraces, 15 seedlings (7 days old and grown in petridishes, moistened with water at room temperature; $30 \pm 5^{o}C$) were transferred to 100 ml jars containing nutrient medium (control) and nutrient medium supplemented with different levels of NaCl, each in triplicate. The NaCl solutions of different molarity (0.10, 0.15 and 0.20M) were prepared in the nutrient medium (Evans and Nason 1953) to represent different salinity levels. The seedlings were so placed that the lower half of each seedling was immersed in the solution. These were kept at room temperature. Initial length and fresh weight of seedlings were measured. Fifteen days later the length of root and seedlings (shoot) was measured (Heenan *et al*. 1988).

RESULTS

Significant differences in per cent seed germination, at various salinity levels were observed amongst the landraces. Irrespective of the landrace, per cent germination progressively decreased with increase in the salinity level (Table 1, Fig. 1). Among the 30 landraces, Saunji showed best performance (>90 % germination) across all salinity levels. The reduction in seed germination was severe in a few landraces, and values below 50% were recorded in five of the thirty landraces tested at 0.20 M NaCl (critical salinity level), with minimum (36 %) germination in Syaudhan. Thirteen landraces recorded less than 80 % germination even at 0.10 M NaCl. At 0.15 M and 0.20 M NaCl, nine and only one of the landraces, respectively showed 80 % and above germination. Value of less than 60 % were recorded for one, three and nine landraces at 0.10 M, 0.15 and 0.20 M salt concentrations, respectively. Below 40 % germination was observed at 0.15 M and 0.20 M salt solutions in 3.33 % and 10 %, landraces, respectively. The cumulative germination % (trend on per day basis) along with the standard deviation at different salinity levels for representative landraces is depicted in Fig. 1, which indicates that some landraces are tolerant even upto the highest salinity level (critical level) used in the present study.

Table 1. Effect of salinity (NaCl) stress on seed germination in various landraces of rice.

Landraces	Germination (%) at different salinity levels			
	0 (Control)	0.10 M	0.15 M	0.20 M
Rainfed				
Anjani	97.33 ±1.88	96.00 ±5.65	90.66 ±4.98	64.00 ±5.65
Bauran	97.33 ±3.77	78.66 ±12.36	77.33 ±6.11	70.66 ±8.21
Bauriya	96.00 ±11.31	90.66 ±10.49	72.00 ±6.53	53.33 ±9.97
Bindudhan	100.00 ±0.00	94.66 ±4.98	78.66 ±4.98	69.33 ±4.98
Chhatuli	65.33 ±4.98	62.66 ±12.36	60.00 ±3.26	55.33 ±10.49
Chhotiya	90.66 ±3.77	76.00 ±3.26	70.66 ±19.73	61.33 ±12.36
Dalbadal	100.00 ±0.00	97.33 ±1.88	82.66 ±3.77	76.00 ±6.53
Danbasmati	93.33 ±1.88	88.00 ±6.53	72.00 ±8.64	37.33 ±4.98
Dehradoonibasmati	58.66 ±9.97	56.00 ±11.77	44.00 ±3.26	42.66 ±12.36
Dudhikapkoti	98.66 ±1.88	92.00 ±8.64	85.33 ±4.98	68.00 ±6.53
Dutiyau	96.00 ±3.26	84.00 ±3.26	72.00 ±8.64	72.00 ±3.26
Jhungia	94.66 ±4.98	82.66 ±3.77	76.00 ±3.26	68.00 ±3.26
Kaladur	80.00 ±11.77	60.00 ±9.79	57.33 ±8.21	38.66 ±7.59
Kaun-kaun	94.66 ±4.98	80.00 ±8.00	80.00 ±6.53	68.00 ±6.53
Khurinandhani	90.66 10.49	77.33 ±3.77	68.00 ±3.26	53.33 ±9.79
Kururidhan	85.33 ±7.54	77.33 ±16.75	76.00 ±6.53	74.66 ±10.49
Laldhan	96.00 ±3.26	90.66 ±4.98	78.66 ±1.88	69.33 ±1.88
Nandhani	96.00 ±5.65	90.66 ±7.54	84.00 ±3.26	60.00 ±9.79
Nauli	94.66 ±4.98	80.00 ±2.00	80.00 ±6.53	68.00 ±6.53
Patoli	97.33 ±1.88	72.00 ±4.00	70.16 ±19.73	65.33 ±13.19
Sabhawati	89.33 ±6.79	78.66 ±1.88	76.00 ±3.26	73.33 ±16.11
Sailani	98.66 ±1.88	94.66 ±3.77	78.66 ±6.79	78.66 ±17.98
Santoli	98.66 ±1.88	94.66 ±4.98	81.33 ±10.49	73.33 ±4.98
Saunji	100.00 ±0.00	100.00 ±0.00	97.33 ±3.77	92.00 ±3.26
Saurajubawan	84.00 ±8.64	70.66 ±10.06	70.66 ±13.59	49.33 ±19.13
Syaudhan	66.66 ±6.79	65.33 ±10.49	36.00 ±5.65	36.00 ±11.77
Taichin	98.66 ±1.88	80.00 ±5.65	70.66 ±8.21	64.00 ±11.77
Tilansi	98.66 ±1.88	92.00 ±8.64	85.33 ±4.98	73.33 ±4.98
Irrigated				
Kantolia	73.33 ±6.79	65.33 ±12.36	65.33 ±9.97	53.33 ±13.59
Thapchini	98.66 ±1.88	77.33 ±3.77	72.00 ±5.65	65.33 ±3.77
Introduced variety				
VL-206	97.77 ±1.88	94.66 ±4.98	90.66 ±4.98	65.33 ±4.98

Values are an average mean of 3 replications ± standard deviation following 11 days of exposure to different NaCl solutions.

Anova summary table

Source of variation	SS	df	MS	F	P-value	F crit
Between Groups	14564.44	30	485.4814	2.753912	0.00011***	1.581862
Within Groups	16394.77	93	176.2879			
Total	30959.22	123				

*** Significant at $P < 0.001$

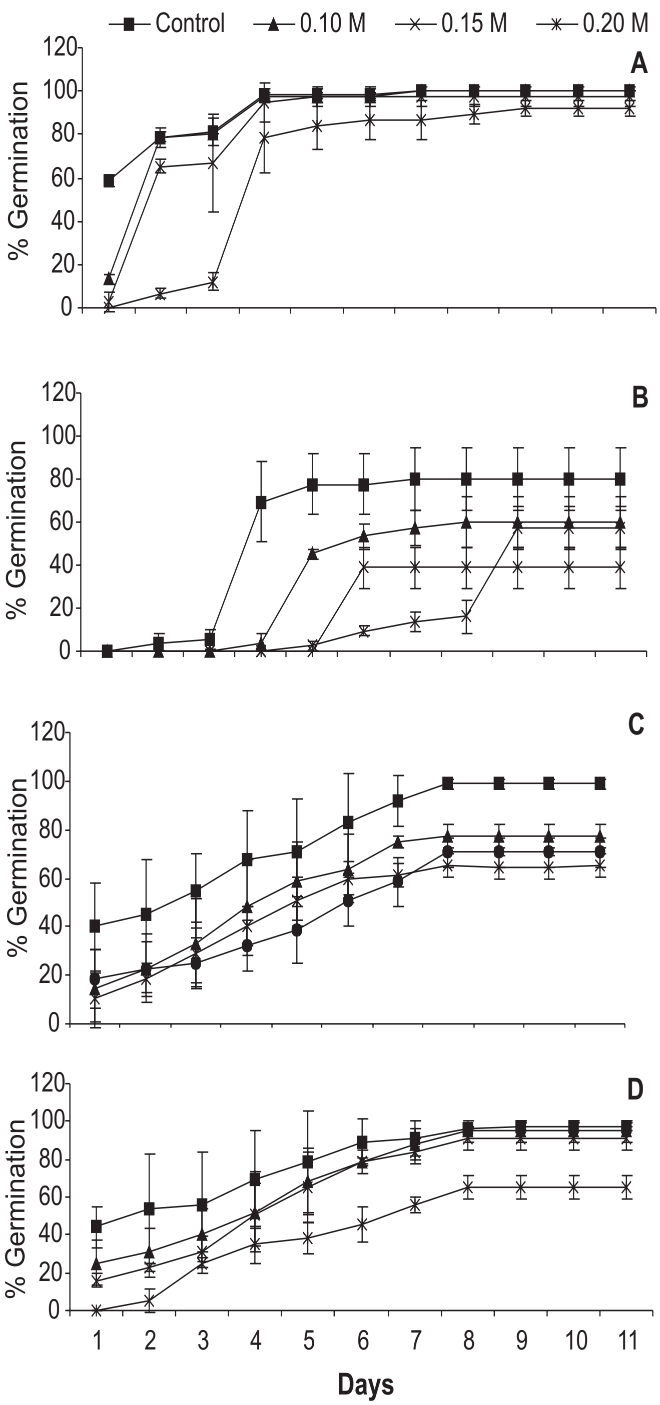

Fig. 1. Representative figures showing per cent seed germination in landraces of rice in response to NaCl stress at 25 ± 5 °C of (A) Saunji; least affected (B) Kaladur; highly affected (C) Thapchini; irrigated and (D) VL – 206; introduced variety

Increasing the NaCl concentration from 0.10 M to 0.20 M led to a high reduction in root and seedling growth in all landraces and VL-206 in comparison to control (Table 2). In control seedlings, the difference in root length over the initial values (at the start of various treatments) ranged from 0.09 cm (Bindudhan) to 1.26 cm (Anjani), while the difference in seedling length ranged from 1.0 cm (Kantolia) to 5.90 cm (Syaudhan). At 0.10 M salt solution the increment in root length ranged from 0.04 cm (Tilansi) to 1.43 cm (Thapchini), while the increment in seedling length ranged from 0.26 cm (Thapchini) to 2.33 cm (Sabhawati). When the salinity level was 0.15 M the root length increment ranged from 0.01 cm (Anjani) to 1.23 cm (Nauli), while the increment in seedling growth ranged from 0.07 cm (Thapchini) to 1.84 cm (Syaudhan). At 0.20 M NaCl concentration, further reduction in root length increase occurred and was from 0.08 cm (Taichin) to 0.75 cm (Patoli). A similar trend was also recorded for seedling growth, which ranged from 0.07 (Kaun-kaun) to 0.97 cm (Danbasmati) (Table 2).

Significant variation existed among various landraces in terms of early seedling growth when subjected to NaCl salinity (Table 2). Increase in the salinity level led to high reduction in the length of roots and seedlings, irrespective of the landraces. The greatest reduction in seedling length was observed when the concentration was increased from 0.10 to 0.20 M.

DISCUSSION

Recent advances in plant genomics have provided molecular tools that allow scientists to dissect the biochemical and molecular mechanism of stress tolerance or resistance. These can be utilized to develop new rice varieties that show varied level of protection against the impact of abiotic stresses. Since, many of the major abiotic stresses arise as a result of common biochemical phenomena, efforts to improve plant tolerance to one abiotic stress have potential to confer tolerance to other abiotic stresses. Currently efforts have been focused on modifying higher plants with stress tolerance-conferring genes (Cheikh *et al*. 2000). A number of transgenic crop plants tolerant to abiotic stresses have been developed, including transgenic rice with tolerance against salt stress

Table 2. Effect of salinity (NaCl) stress on seedling growth of different landraces of rice.

Landraces	Root length (cm) (% increase over initial value in parenthesis)				Shoot length (cm) (% increase over initial value in parenthesis)			
	Control	0.10 M NaCl	0.15 M NaCl	0.20 M NaCl	Control	0.10 M NaCl	0.15 M NaCl	0.20 M NaCl
Rainfed								
Anjani	1.26 (29.7)	0.49 (16.6)	0.01 (0.3)	0.62 (21.4)	3.80 (38.5)	1.94 (25.6)	1.33 (19.2)	0.54 (8.4)
Bauran	0.26 (5.1)	0.44 (10.6)	0.40 (10.5)	0.35 (7.9)	3.17 (35.3)	1.40 (20.7)	0.58 (9.5)	0.42 (6.0)
Bauriya	0.37 (8.5)	0.42 (8.4)	0.26 (6.5)	0.43 (9.4)	2.54 (25.5)	0.81 (10.2)	0.84 (10.5)	0.34 (4.4)
Bindudhan	0.09 (1.9)	0.44 (8.4)	0.28 (5.6)	0.15 (2.7)	2.49 (26.9)	1.14 (16.5)	0.95 (12.5)	0.26 (3.3)
Chhatuli	0.26 (5.1)	0.52 (9.2)	0.58 (13.7)	0.31 (6.5)	3.44 (38.2)	1.02 (12.8)	0.66 (9.4)	0.24 (3.6)
Chhotiya	0.44 (7.2)	0.59 (12.2)	0.48 (11.9)	0.22 (4.5)	4.08 (45.0)	1.07 (17.9)	1.01 (19.2)	0.68 (11.8)
Dalbadal	0.48 (8.9)	0.36 (8.0)	0.61 (8.9)	0.70 (11.4)	3.62 (41.7)	1.15 (19.3)	0.78 (13.5)	0.38 (7.0)
Danbasmati	0.55 (8.9)	0.57 (11.4)	0.39 (8.1)	0.35 (5.4)	3.35 (30.7)	1.10 (13.3)	0.52 (8.2)	0.97 (13.7)
Dehradoonibasmati	0.17 (4.3)	0.09 (2.3)	0.35 (10.6)	0.62 (13.9)	4.29 (49.5)	0.92 (18.9)	1.69 (32.1)	0.11 (2.6)
Dudhikapkoti	0.40 (6.4)	0.82 (12.3)	0.78 (11.9)	0.36 (5.9)	3.75 (33.9)	0.78 (9.2)	0.60 (7.5)	0.25 (3.4)
Dutiyau	0.62 (9.4)	0.24 (3.3)	0.56 (8.1)	0.53 (7.1)	3.47 (35.6)	1.76 (21.3)	1.25 (16.1)	0.42 (5.9)
Jhungia	0.47 (7.4)	0.21 (4.0)	0.45 (7.4)	0.29 (4.9)	3.42 (35.4)	1.55 (22.1)	1.35 (19.6)	0.48 (7.1)
Kaladur	0.69 (18.4)	0.57 (15.2)	0.31 (8.4)	0.31 (8.5)	1.13 (16.4)	0.87 (13.7)	1.12 (16.8)	0.59 (10.3)
Kaun-kaun	0.90 (13.8)	0.77 (13.3)	0.16 (2.5)	0.37 (5.7)	3.62 (32.0)	1.41 (15.2)	0.68 (8.4)	0.07 (0.9)
Khurinandhani	0.52 (11.3)	0.76 (15.8)	0.41 (9.8)	0.41 (10.0)	3.99 (41.4)	0.83 (12.3)	0.82 (13.0)	0.19 (2.9)
Kururidhan	0.15 (2.0)	0.14 (2.0)	0.34 (5.0)	0.26 (4.2)	5.34 (48.2)	1.74 (22.4)	0.34 (5.4)	0.44 (7.1)
Laldhan	0.58 (10.3)	0.29 (5.3)	0.15 (2.6)	0.47 (6.8)	4.43 (42.6)	0.81 (12.6)	0.83 (13.8)	0.24 (4.0)
Nandhani	0.86 (15.9)	0.43 (7.6)	0.37 (7.4)	0.37 (5.5)	3.96 (35.7)	0.83 (11.2)	0.32 (4.3)	0.28 (3.6)
Nauli	0.80 (11.9)	0.68 (9.9)	1.23 (18.1)	0.28 (4.0)	4.14 (43.1)	1.67 (21.6)	1.38 (18.6)	0.24 (3.9)
Patoli	0.41 (6.4)	0.56 (8.6)	0.47 (7.9)	0.75 (12.4)	3.22 (34.1)	1.17 (17.6)	0.96 (14.1)	0.40 (6.9)
Sabhawati	0.45 (9.8)	0.80 (2.3)	0.24 (5.2)	0.67 (14.1)	3.56 (31.9)	2.33 (29.5)	1.18 (13.6)	0.35 (5.0)
Sailani	0.51 (8.2)	0.65 (10.3)	0.22 (3.8)	0.71 (12.0)	2.80 (27.2)	1.29 (14.6)	0.55 (7.6)	0.08 (1.2)
Santoli	0.80 (13.9)	0.32 (5.7)	0.26 (6.1)	0.28 (6.5)	1.41 (17.8)	0.62 (8.4)	0.17 (2.4)	0.16 (2.7)
Saunji	0.24 (6.0)	0.48 (12.1)	0.44 (11.4)	0.50 (12.4)	3.14 (30.9)	0.80 (11.0)	1.39 (19.1)	0.09 (1.3)
Saurajubawati	0.57 (9.4)	0.29 (6.6)	0.19 (3.8)	0.65 (11.6)	1.10 (15.0)	0.66 (10.2)	0.58 (8.8)	0.55 (9.8)
Syaudhan	0.33 (7.3)	0.22 (5.6)	0.26 (6.1)	0.36 (10.4)	5.90 (55.0)	1.14 (16.8)	1.84 (27.2)	0.42 (9.1)
Taichin	0.18 (4.7)	0.29 (7.0)	0.06 (1.5)	0.08 (2.2)	1.71 (21.9)	0.74 (11.0)	0.67 (10.9)	0.68 (11.8)
Tilansi	0.66 (9.8)	0.04 (0.7)	0.87 (13.7)	0.39 (8.2)	4.45 (45.1)	1.25 (17.4)	0.93 (13.8)	0.28 (4.3)
Irrigated								
Kantolia	0.30 (8.1)	0.89 (16.3)	0.04 (1.1)	0.44 (11.3)	1.00 (15.7)	0.45 (7.9)	0.32 (6.0)	0.23 (4.4)
Thapchini	1.16 (17.0)	1.43 (20.5)	0.38 (6.5)	0.43 (6.1)	2.01 (22.6)	0.26 (4.1)	0.07 (0.9)	0.12 (1.7)
Introduced variety								
VL206	0.80 (12.9)	0.41 (8.4)	0.25 (3.9)	0.66 (12.4)	1.92 (24.2)	0.78 (11.2)	0.13 (2.1)	0.19 (3.1)

Values are an average of 3 replications 15 days after initiation of the salinity stress.

Anova summary table

Source of variation	SS	df	MS	F	P-value	F crit
Rows	12.3379	30	0.411263	1.65325	0.022705**	1.513648
Columns	204.5523	7	29.22175	117.4694	4.89E-69	2.053383
Error	52.2397	210	0.24876			
Total	269.1299	247				

**Significant at P<0.05

(Tayal *et al*. 2004). Upto a certain level of salinity, suppression of germination is brought about by osmotic effect (Narale *et al*. 1969 and Ayers 1953). In this study, at 0.15 and 0.20 M (critical level) a greater reduction in germination was observed, which may be due to the specific effect of salts in the nutrient medium, possibly as a result of toxic ions. Here "critical level" refers to the salinity level at which drastic inhibition in germination occurred. Elevated salinity slows down water uptake by seeds, thereby inhibiting their germination and root elongation (Baalbaki *et al*. 1999). NaCl inhibited imbibition, germination and root elongation at a low osmotic potential. NaCl is likely to inhibit the activities of some enzymes that play critical role in seed germination (Katembe *et al*. 1998).

Increasing level of salinity decreased germination and seedling growth and the effect varied with the landrace (Table 1 and 2). The application of a direct screening method under field conditions is difficult (consumes more time, energy and space than screening seedlings in solution culture), hence laboratory based screening is employed. It is well known that rice is sensitive to salt during early seedling and reproductive stages. The physiological basis of salt tolerance during early seedling stage may include high seedling vigour, salt exclusion at the root level, compartmentation of ions in structural and older tissues, high tissue tolerance, responsive stomata that close within minutes after exposure to salt stress but partially reopen after a period of acclimation and up regulation of antioxidant systems, particularly the ascorbate/glutathione pathway of oxidative stress tolerance (Lafitte *et al*. 2004).

The present investigation demonstrates that a valuable source of rice germplasm is still being maintained through cultivation in remotely located villages in Kumaun Himalaya. There is an urgent need of evaluation of these traditionally maintained genetic resources for use in the introgression of salinity resistant genes for development of stress tolerant varieties. Based on the salinity resistant characteristics of rice landraces, it may be suggested that the landraces, namely Saunji & Danbasmati can be introduced for development of salt resistant varieties.

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