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Genotypic variation for root and shoot length in response to salt stress during germination stage in parental lines of hybrid rice (*Oryza sativa* L.)

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ABSTRACT : An experiment was conducted at Indian Institute of Rice Research, Hyderabad, to assess the response of 34 hybrid rice parental lines to 120mM Nacl salt stress at germination stage. All the genotypes significantly responded to salt stress and most of the genotypes showed tolerance to threshold salt level and the results clearly depicted that germination stage is moderately tolerant to salinity. Root and shoot length of all cultivars were significantly affected by salt stress, The genotypes BK49-76, BK36-167, INDAM300-007, AjayaR, FL-478 and DRRH-2 manifested maximum tolerance where as genotypes BCW 56, IR 58025B, APMS6B showed susceptibility on par with IR28 at germination stage. The most of the genotypes were tolerant to salinity at seed germination which is an indicator for raising nursery in salt affected soils.

Key Words: Germination percentage, Imbibition rate, Shoot length, Salt stress, Root length

Rice (*Oryza sativa* L.) is the staple food for more than 2700 million people around the world. The overall production of rice globally is estimated to be around 575 million tonnes and the average productivity is about 3.83 tonnes/ha. Abiotic stress in rice alone contributes about 50 percent of the total yield loss and basically stress in terms of salinity, drought and extreme temperatures are the major barriers to limit rice crop production. In India and Bangladesh elevated levels of salt concentration in the soil is the major constraint in rice production (Mohammadi-Nejad *et al*., 2008). About 20 per cent of the world's cultivated area (800 M ha) and nearly half of the world's irrigated lands are affected by salinity (Zhu *et al*., 2001 and Maser *et al*., 2002). Razzaque *et al*. (2009) have found an adverse effect of salt on plant height, root, shoot and dry matter of seven rice genotypes. Salinity adverse effect on seed germination could be due either to osmotic effect or to ion toxicity (Huang and Redmann, 1995).

Salinity tolerance is a complex trait and phenotypic responses of plants to salinity stress are highly affected by the environment (Gregorio and Senadhira *et al*., 1993; Koyama *et al*., 2001; Flowers, 2004). Gregorio *et al*. (1997) emphasized that salinity symptoms were noteworthy on the first and second leaves and were visualized by leaf rolling, formation of new leaf, brownish and whitish leaf tip, drying of leaves, reduction in root growth, stunted plant height and stem thickness leading to complete cessation of growth and mortality. Extreme salt stress conditions cause severe damage to plants, while moderate to low salt stress affects the plant growth rate along with growth and yield parameters like low tillering, stunting, spikelet sterility, less florets per panicle, low 1000-grain weight and leaf scorching etc. Heenan *et al*. (1988) and Lutts and Geurrier (1995) reported that rice is extremely sensitive to salinity during germination, young seedling and early developmental stages for most commonly used rice varieties. Seed germination, seedling emergence, and their survival are particularly sensitive to substrate salinity (Mariko *et al*., 1992; Baldwin *et al*., 1996). High levels of soil salinity can significantly inhibit seed germination and seedling growth, due to the combined effects of high osmotic potential and specific ion toxicity. Inhibition of seed germination, shoot and root elongation was noticed as a result of sodium chloride treatments in rice and cabbage (Jamil and Rha, 2007). According to the classification of crop tolerance to salinity, the rice crop is within the sensitive division from 0 to 8 dsm^{-1} (Maas and Hoffman, 1986). The susceptibility of rice to salinity stress varies with growth stages.

Salinity impacts seed germination, seedling survival, number of leaves, shoot weight, plant height, length and surface area of roots (Mohammed *et al*., 2006 and Meloni *et al*., 2008). Germination and seedling characteristics are the most viable criteria used for selecting salt tolerance in plants due to the fact that the final plant stand of a crop primarily depends on seedling characteristics. Germination per-centage, germination rate and seedling growth are most commonly used criterias for genotype selection (Bybordi and Tabatabaei, 2009). The present study was undertaken to evaluate the response of 34 hybrid rice parental lines to 120mM salt stress at germination stage.

Materials and Methods

Plant materials

Thirty-four rice genotypes namely 21 restorer lines, two maintainer lines, six check entries (three tolerant checks namely FL- 478, Pokkali and CSR 30; one sensitive check IR 28 and two varietal checks known for heat tolerance are Nagina 22 and Bala) and five hybrid checks in early, medium early and medium duration group KRH-2, DRRH-2, DRRH-3, PA-6444, INDAM300-007 were screened for salinity tolerance at the germination stage (Table-1).

Screening methodology

Un-imbibed seeds of the 34 rice genotypes were incubated at 50°C for 5 days to break any residual seed dormancy.

Parameters screened

Germination test: Germination is the emergence of seedling which was done by using petridish method. In each petri-dish containing germination paper or blotting paper which is used as a substrate, 25 seeds of rice were placed. Salt solutions with different EC's were used for treatment of respective seeds and distilled water was used in control. For emergence, the petri-dishes were placed at 25°C for 7 days in laboratory conditions. The petri-dishes were kept under observation everyday and watered with respective solutions whenever required.

Data collection: Data were collected and calculated on germination percentage (%), seedling vigour index, seedling growth rate (mg/day), shoot length (cm), root length (cm).

Germination parameters: The petri-dishes were examined daily and the number of germinated seeds was recorded. A seed was considered to be germinated after rupturing of seed coat, emergence of plumule and radicle and were >2mm long.

Germination percentage: The percentage of germination was calculated using the formula

Germination (%)

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

Seedling vigour index (SVI): Seedling vigour index can be expressed by the following equation

Seedling vigour index = (Average shoot length + average root length) × germination percentage

Root and shoot length measurement: Five seedlings were randomly selected from each petri-dish and the length of root and shoot was measured after 7 days.

Statistical analysis: All the data collected was analyzed by using analysis of variance (ANOVA) technique. Data analysis was carried out according to the statistical procedure described by the computer package MSTAT-C. Means were separated by using the Duncan's Multiple Range Test (DMRT) and Least Significant Differences (LSD) at 5% level of significance.

Results and Discussion

Based on the statistical analysis of data, on comparison with control, there was a significant decrease in imbibition rate of all the genotypes except C20R, BK-49-76, IR58025B, CSR-30 and EPLT-104 under 120mM salt stress at both 24 hrs and 48 hrs duration (Table-1). It was observed that the imbibition rate was recorded highest mean in FL-478 and lowest mean in BCW-56. In FL-478, though imbibition rate is high, the root length is less in germination stage and shoot length is less whereas in BCW-56, the length of root is more in germination stage, shoot length is less during germination, even when the imbibition rate is low. The genotypes EPLT 109, EPLT 104 and IBL 57 showed lesser root and shoot growth at germination stage but showed moderate level of tolerance initially under imbibed conditions. The genotype RPHR 111-3 showed tolerance in germination stage even though found to be sensitive at imbibition state. It was found that the tolerance differed initially surviving in the imposed salinity state and further emergence. The susceptible genotype IR-28 has better root growth in germination stage; however it is susceptible at early stage screening. The genotype BCW 56, IR 58025B, APMS6B showed susceptibility on par with IR28 in both the stages whereas BK49-76, BK36-167, INDAM 300-007, AjayaR, FL-478 and DRRH-2 showed tolerance at both the initial and final stages of screening. The genotype RPHR-1004 showed high root length and less shoot length in both the germination while RPHR-1005, C20R and DRRH-3 showed moderate root length and less shoot length in germination stage and more root length and moderate shoot length in initial screening stage. However RPHR-611-1 has more root length and moderate shoot length .The imbibition rate of salt tolerant genotypes FL-478, CSR-30, Pokkali, and heat tolerant genotypes Nagina22 and Bala remained same within 24-48 hours of soaking. The root length mean of FL-478 and IR-28 is found to be superlative at 120mM concentration of imposed salinity on 10^{th} day where as the shoot length mean was found to be higher only for FL-478 and Bala. The imbibition rate didn’t differ much for the most of the genotypes screened and it was moderate between 0.5 -0.8. Based on the screening parameters the hybrid lines DRRH-2, RPHR-1005 are likely found to be moderately tolerant for salt in the primary stage of salinity stress.

In developing salt tolerant cultivars, rice breeding programs are making efforts to evaluate diverse germplasm to enhance their utility (Ismail *et al.*, 2007). Gregorio *et al.* (1997) emphasized that salinity symptoms were prominent on reduction in root growth, stunted growth, stem thickness leading to complete cessation of growth and dying of seedlings. Rice is relatively tolerant during germination, becomes very sensitive during early seedling stage (Singh *et al.*, 2004). The importance of the seedling or sprouting stage cannot be undermined as it affects crop establishment. Delayed differentiation of root and shoot reduction in seedling vigor is seen with increase in salt concentration. The shoot growth was found to be more inhibited than root growth (Dubey and Sharma, 1989). Pareek *et al.* (1998) observed delayed germination, reduction in germination percentage, reduced growth of primary root and shoot axis. Severe effects of salinity on germination and seedling growth were reported by Chakrabarty and Chattopadhyay *et al.*, 2000. The gradual decrease in root length with the increase in salinity as observed due to more inhibitory effect of NaCl salt to root growth compared to that of shoot growth (Rahman *et al.*, 2001). We have screened 34 rice cultivars at 120mM of salt concentration, a significant reduction was observed in shoot length and root length in germination stage for 5days and also observed moderate reduction in root and shoot length. With increase of salinity stress, reduction of root length, shoot length, dry weight of root and dry weight of shoot was observed by Roy *et al.* (2002). Abdelhamid *et al.* (2010) who reported significant differences due to salinity levels and genotypes among faba bean genotypes to seedling shoot to

Table-1 : The hybrid parental lines were screened for salinity tolerance at seedling stage with modified yoshida salt solution (control & 120mM NaCl).

Genotypes									5th day	10th day	5th day	10th day	5th day	10th day	5th day	10th day
	control	control	120mM	120mM	control	control	120mM	120mM	control	control	120mM	120mM	control	control	120mM	120mM
	IR at 24h	IR at 24h	IR at 24h	IR at 24h	IR at 48h	IR at 48h	IR at 48h	IR at 48h	RL Mean	RL Mean	RL Mean	RL Mean	SL Mean	SL Mean	SL Mean	SL Mean
BCW-56	0.33	0.33	0.32	0.32	0.35	0.35	0.32	0.32	5.16	5.6	5.27	5.7	4.25	4.8	2.55	4.0
EPLT-109	0.55	0.55	0.54	0.54	0.59	0.59	0.57	0.57	5.65	6.2	4.97	5.8	4.76	7.1	3.38	5.3
EPLT-104	0.57	0.57	0.59	0.59	0.61	0.61	0.61	0.61	5.14	7.0	4.16	5.2	4.36	6.0	1.75	4.2
RPHR-111-3	0.60	0.60	0.56	0.56	0.66	0.66	0.58	0.58	6.1	6.3	3.72	6.0	5.05	5.9	2	3.2
RPHR-1096	0.58	0.58	0.56	0.56	0.62	0.62	0.58	0.58	6.36	6.6	4.16	5.7	4.89	6.2	2.13	4.5
RPHR-619-2	0.59	0.59	0.54	0.54	0.69	0.69	0.56	0.56	6.92	7.1	4.5	5.3	4.52	6.3	2.54	4.9
RPHR-1004	0.58	0.58	0.61	0.61	0.64	0.64	0.63	0.63	4.8	4.9	5.49	7.5	4.3	5.9	2.58	4.4
RPHR-1005	0.56	0.56	0.50	0.50	0.62	0.62	0.52	0.52	7.01	7.6	4.83	8.4	5.21	7.8	2.12	5.2
SC5-2-2-1	0.62	0.62	0.54	0.54	0.68	0.68	0.54	0.54	5.3	6.0	5.33	6.5	4.69	6.3	2.55	4.0
BK-52-104	0.46	0.46	0.45	0.45	0.49	0.49	0.47	0.47	7.46	6.9	4.37	7.1	4.82	6.7	2.51	5.7
RPHR-611-1	0.79	0.79	0.69	0.69	0.87	0.87	0.72	0.72	5.3	6.7	5.62	7.3	5.33	7.2	3.53	5.3
C20R	0.72	0.72	0.79	0.79	0.81	0.81	0.83	0.83	7.11	7.1	5.64	7.3	5.14	6.5	3.31	4.9
BK-35-155	0.81	0.81	0.80	0.80	0.89	0.89	0.83	0.83	4.54	4.5	5.94	6.0	4.26	6.2	3	4.1
BK-49-43	0.47	0.47	0.48	0.48	0.52	0.52	0.50	0.50	7.33	9.5	5.31	6.4	4.99	5.6	2.82	4.5
BK-36-167	0.58	0.58	0.55	0.55	0.59	0.59	0.57	0.57	7.98	8.7	4.47	6.0	4.44	6.4	2.21	4.1
BK-49-80	0.51	0.51	0.49	0.49	0.58	0.58	0.51	0.51	7.26	8.0	5.27	6.2	4.34	6.8	2.64	4.6

BK-49-76	0.45	0.45	0.49	0.49	0.49	0.49	0.51	0.51	4.96	8.6	5.07	5.9	4.26	6.8	2.25	4.4
BK-49-77	0.50	0.50	0.48	0.48	0.57	0.57	0.50	0.50	6.7	7.1	5.94	6.7	4.44	6.5	2.83	4.3
BK-49-78	0.43	0.43	0.46	0.46	0.50	0.50	0.48	0.48	6.02	7.0	5.08	5.8	6.32	6.6	2.13	4.7
AJAYA-R	0.71	0.71	0.69	0.69	0.75	0.75	0.71	0.71	6.18	5.6	5.68	7.4	6.18	7.0	2.68	5.1
IBL-57	0.51	0.51	0.45	0.45	0.53	0.53	0.45	0.45	5.76	8.4	3.64	4.3	3.81	5.9	1.275	3.1
APMS-6B	0.54	0.54	0.53	0.53	0.61	0.61	0.54	0.54	4.32	4.4	4.06	6.4	2.52	5.7	1.59	4.2
IR58025B	0.66	0.66	0.69	0.69	0.69	0.69	0.72	0.72	6.24	6.8	2.99	5.1	4.02	5.7	2.07	4.3
KRH-2	0.68	0.68	0.67	0.67	0.70	0.70	0.71	0.71	4.03	5.7	4.39	6.7	3.76	5.7	1.83	4.1
DRRH-2	0.82	0.82	0.71	0.71	0.91	0.91	0.79	0.79	5.27	5.5	6.05	6.6	5.06	7.2	3.65	6.7
DRRH-3	0.54	0.54	0.56	0.56	0.60	0.60	0.59	0.59	6.55	6.6	6.04	7.5	5.22	6.4	2.99	5.7
PA-6444	0.75	0.75	0.74	0.74	0.78	0.78	0.76	0.76	5.83	7.5	4.3	5.7	3.52	5.1	2.3	4.5
INDAM300-007	0.80	0.80	0.80	0.80	0.91	0.91	0.83	0.83	6.92	9.1	4.75	5.5	5.53	7.5	3.13	5.5
FL-478	0.92	0.92	0.86	0.86	1.03	1.03	0.90	0.90	5.47	5.2	4.56	7.4	5.6	6.9	2.67	5.0
IR-28	0.78	0.78	0.79	0.79	0.83	0.83	0.77	0.77	6.73	9.2	5.71	8.3	4.03	6.5	2.1	4.3
CSR-30	0.74	0.74	0.81	0.81	0.76	0.76	0.82	0.82	3.775	6.8	4.12	6.2	3.41	6.0	1.93	4.6
POKKALI	0.82	0.82	0.75	0.75	0.85	0.85	0.76	0.76	4.92	7.2	2.57	4.5	3.68	8.0	0.95	3.7
Nagina22	0.52	0.52	0.52	0.52	0.53	0.53	0.52	0.52	4.78	6.8	3.71	5.0	4.5	6.6	2.1	4.7
Bala	0.75	0.75	0.72	0.72	0.78	0.78	0.75	0.75	5.63	6.6	5.15	5.8	6.41	7.9	3.31	5.6
Mean	0.634	0.634	0.618	0.618	0.687	0.687	0.640	0.640	5.889	6.874	4.775	6.286	4.648	6.514	2.450	4.641
Standard Deviation	0.131	0.131	0.126	0.126	0.142	0.142	0.131	0.131	1.066	1.292	0.881	1.009	0.850	0.695	0.637	0.732
Minimum	0.430	0.430	0.450	0.450	0.490	0.490	0.450	0.450	3.775	4.400	2.570	4.263	2.520	5.080	0.950	3.138
Maximum	0.920	0.920	0.860	0.860	1.030	1.030	0.900	0.900	7.980	9.480	6.050	8.410	6.410	8.011	3.650	6.680

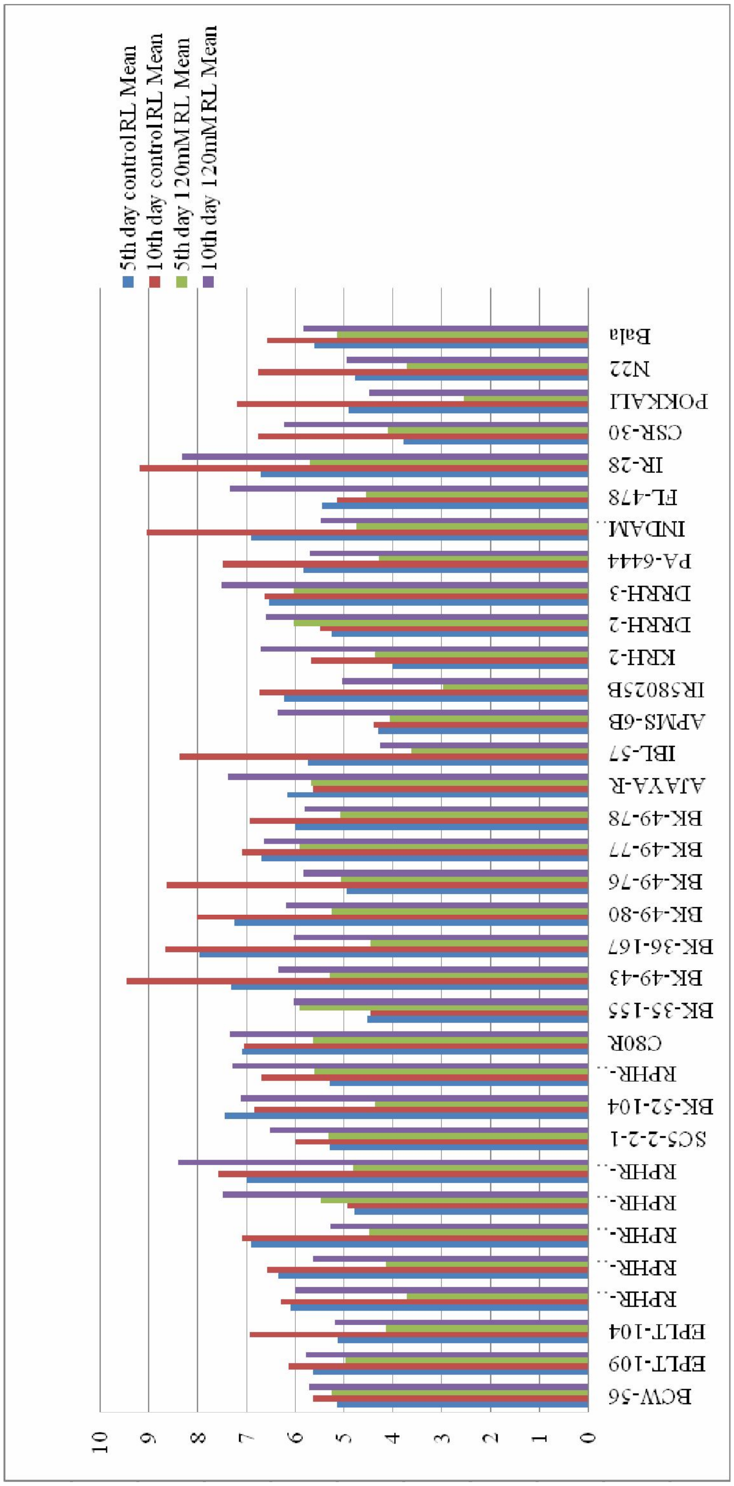

Fig.-1 : Performance of genotypes for root length under salt stress at germination stage.

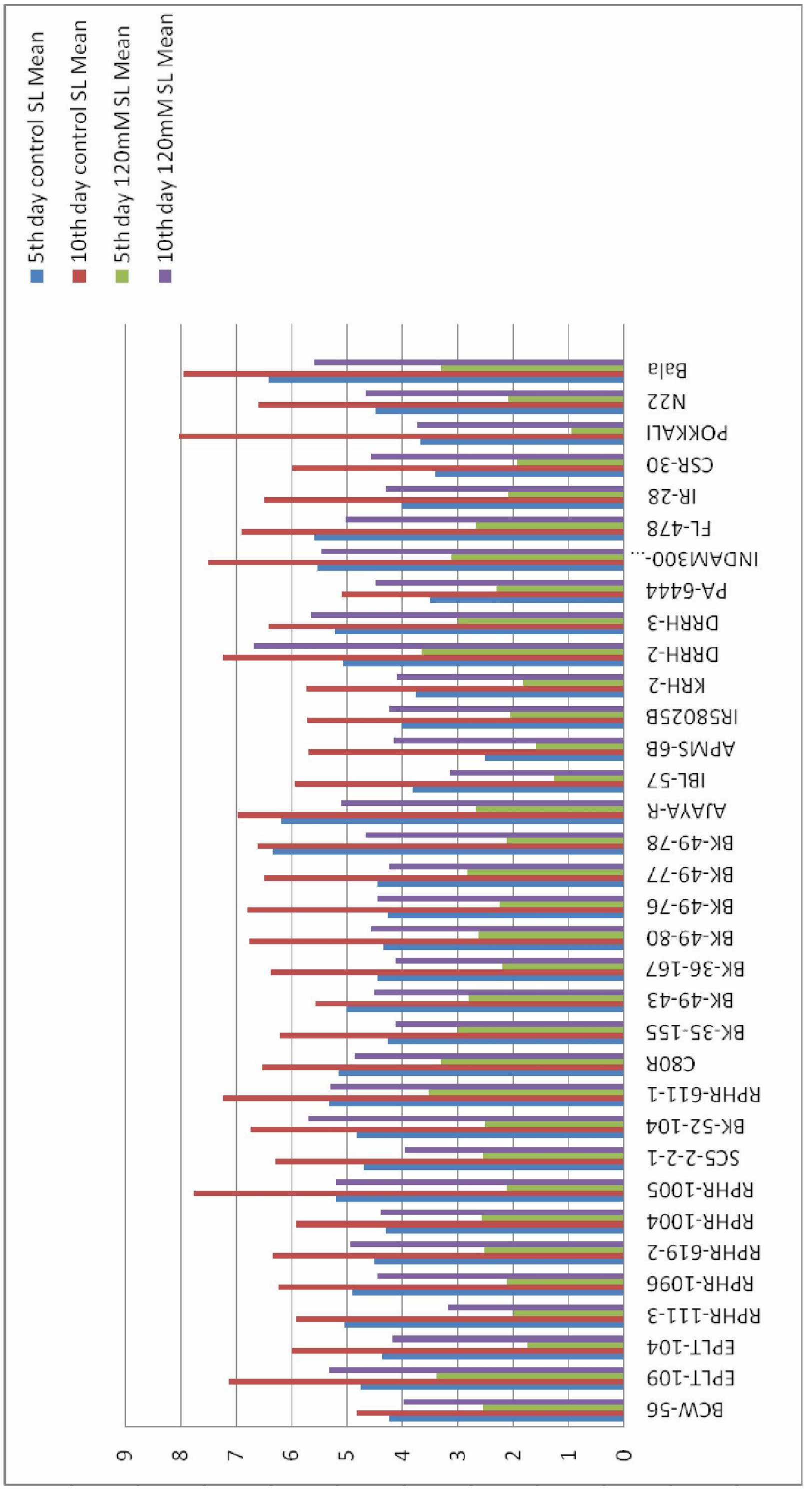

Fig.-2 : Performance of genotypes for shoot length under salt stress at germination stage.

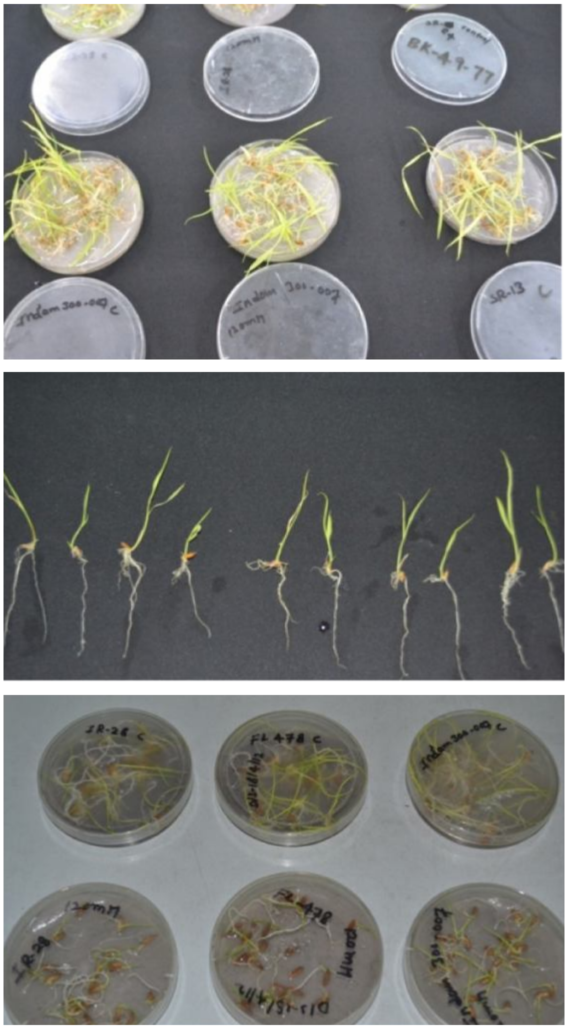

Fig.-3 : Variation in germination among the genotypes screened under salt solution.

root ratio. According to their findings seedling shoot to root ratio was highly reduced at higher salinity levels and genotypes showed variation in their response to salinity. Geressu and Gezahagn (2008) they reported significant difference for salinity treatments and treatment interactions for seedling shoot to root ratio of sorghum genotypes. Jamil and Rha (2007) also screened transgenic lines of rice at early seedling stage and observed reduction of shoot and root length in all the lines. Rice seedling growth and survival rate for low salinity threshold values have been reported for cultivar M-202 (Zeng Ling *et al.*, 2000). Based on the above results it clearly conferred that salinity tolerance differed within the earlier and later stages of germination during the imposed salinity stress. Present study clearly indicated most of genotypes were tolerance to salinity at germination stage and an indicator for raising nursery in salt affected soils.

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