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Effect of salt stress on germination and seedling growth in rice genotypes

M. DJANAGUIRAMAN, R. RAMADASS AND D. DURGA DEVI
Department of Crop Physiology, Tamil Nadu Agricultural university, Coimbatore-641 003, Tamil Nadu.

Abstract : Five rice genotypes *viz.* White Ponni, ADT 38, ADT 39, IR 20 and TRY 1 were studied in laboratory for tolerance to salt stress. A salt level of 200 mM NaCl decreased the process of germination, root length, shoot length and vigour index. The tolerant genotype TRY 1 recorded a higher germination percentage, root length, shoot length and vigour index as compared to ADT 38, White Ponni, ADT 39 and IR 20. The amylase and dehydrogenase activity under salt level was more in tolerant genotype TRY 1, which accumulated less anthocyanin in its root.

Key Words: Root length, Shoot length, Vigour index, Salinity, Oryza sativa, Amylase, Dehydrogenase, Anthocyanin.

Introduction

Rice is least tolerant to salinity during seedling stage, but its tolerance increase with age and varieties differ in their tolerance to salinity (Frap, 1909). Available information on tolerance to salinity in rice at flowering stage is contradictory (Hayward and Bernstein, 1958). Early seedling stage in paddy has been reported to be more sensitive than later stage (Pearson, 1959) whereas Hayward and Bernstein (1958) reported that seedling and flowering stages of the crop are more sensitive to salinity while germination stage is most tolerant. NaCl decreased the germination especially the speed of germination. Inhibition of seeds in NaCl solution had a pronounced effect on germination potential. Increasing salt concentration reduced germination, though there were marked differences between the cultivars. Most cultivars would tolerate salinity levels upto 11 dSm^{-1} (Jagadev and Jena, 1993). Chloride dominated salinity proved more deleterious to seed germination and seedling growth as indicated by relatively more reduction in per cent germination, radicle and coleoptile length. The objective of the present study was to screen the rice genotypes for salt stress by assessing germination, root length, shoot length and vigour index.

Materials and Methods

A pot culture experiment was conducted in Tamil Nadu Agricultural University, Coimbatore during 2000. Five popular, medium duration (130 - 133 d) varieties namely White Ponni, IR 20, ADT 38, ADT 39 and TRY 1 are used for the study with five replication. ADT 38 and TRY 1 served as susceptible and tolerant check, respectively. About 25 seeds in each variety were placed in petridish containing 200 mM NaCl. In each petridish, 2 layers of filter paper were moistened with 10 ml of 200 mM NaCl solution. The germinability was recorded on the seventh day after placing. The number of seeds germinated was expressed as percentage under each treatment. The shoot and root length of the seedlings was measured from seven randomly selected seedlings in each treatment from each replication on the seventh day and expressed in cm. Vigour index was worked out according to the formula,

$$VI = \text{Germination \%} \times (\text{Root length in cm} + \text{Shoot length in cm})$$

Amylase activity of the seedlings was estimated by Bernfeld (1955) and the activity expressed as gram maltose h^{-1} g^{-1} fresh weight based on the amount of reducing sugars released. Amylase was extracted in precooled 20 per cent aqueous glycerol solution. The amount of reducing sugars released was estimated by dinitrosalicylic acid method. Dehydrogenase activity of the seeds was measured as per the method of Sato (1962). The colour developed in the tetrazolium treated seeds was extracted with 80% acetone by grinding the seeds in pestle and mortar. The extract was centrifuged at 3000 rpm for 15 minutes, and the colour was read at 480 nm using UV Spectrophotometer and expressed as OD at 480 mn (Beckman Spectrophotometer, Japan). Anthocyanin content was estimated as per the procedure adopted

Table 1. Effect of salt stress on germination, shoot length, root length, vigour index, in rice genotype

Varieties	Germination (%)		Shoot length (cm)		Root length (cm)		Vigour index	
	Control	200 mM	Control	200mM	Control	200 mM	Control	200 mM
TRY 1	94.50	92.50	10.60	8.74	10.46	10.10	1990.17	1742.70
ADT 39	94.50	80.50	9.94	8.66	9.30	7.70	1818.18	1316.98
White Ponni	91.00	73.50	10.42	7.72	9.36	7.10	1799.98	1089.27
IR 20	95.75	74.50	9.98	8.64	8.78	7.30	1796.27	1182.53
ADT 38	79.00	66.25	5.54	3.38	4.34	2.12	780.52	364.37
	SEd	CD(5%)	SEd	CD(5%)	SEd	CD(5%)	SEd	CD(5%)
Variety	0.6947	1.4091	0.0547	0.1107	0.0758	0.1533	18.02	36.43
Treatment	0.4011	0.8135	0.0346	0.0700	0.0480	00970	11.39	23.04
Variety x Treatment	0.9824	1.9928	0.0774	0.1565	0.1073	0.2160	25.49	51.52

Table 2. Effect of salt stress on dehydrogenase activity, amylase activity and anthocyanin accumulation in rice genotypes

Varieties	Dehydrogenase activity (Total OD at 480 nm)		Amylase activity (g maltose hr^{-1} g^{-1} fr.wt)		Anthocyanin content (unit g^{-1})	
	Control	200 mM	Control	200 mM	Control	200 mM
TRY 1	1.8033	0.9367	0.4267	0.2133	0.0182	0.0313
ADT 39	1.0967	0.9033	0.3000	0.1867	0.0078	0.0283
White Ponni	1.3567	0.5533	0.2600	0.1333	0.0102	0.0622
IR 20	1.0267	0.9000	0.2057	0.1697	0.0101	0.0261
ADT 38	0.9000	0.5333	0.1000	0.0767	0.0012	0.0465
	SEd	CD(5%)	SEd	CD(5%)	SEd	CD(5%)
Variety	0.0284	0.0581	0.0282	0.0588	0.00031	0.00063
Treatment	0.0367	0.0750	0.0178	0.0372	0.00024	0.00048
Variety x Treatment	0.0636	0.1300	0.0398	0.0832	0.00028	0.00056

by Kaliamoorthy and Rao (1994) from the root and expressed as units g^{-1} of fresh weight. Anthocyanin content of the root was extracted with 1% HCl - Methanol and the absorbance was recorded at 540 nm. One unit of anthocyanin content is arbitrarily defined as equivalent to an absorbance of 0.01. For the above bio-chemical analysis, the treatments were replicated four times and the mean was worked out. The data was analysed for its significance as per Panse and Sukhatme (1961).

Results and Discussion

Five varieties were evaluated for tolerance to salt stress under *in vitro* conditions. The germination per cent decreased, under salinity irrespective of the variety. The tolerant variety, TRY 1 recorded the highest germination percentage, whereas, the susceptible variety ADT 38 recorded the lowest germination percentage, while ADT 39, IR 20 and White Ponni recorded 80.50, 74.50 and 73.50 per cent germination, respectively (Table 1). The germination per cent in control was 79.00, 91.00, 94.50, 94.50 and 95.75 for varieties ADT 38, White Ponni, ADT 39, TRY 1 and IR 20, respectively. These varieties differed significantly. The difference due to salt concentration and varieties was also significant. The salt concentration reduced the shoot and root length significantly in all the varieties. The shoot length decreased from 10.6 cm to 8.74 cm in TRY 1, whereas in ADT 39 the decrease was from 9.3 cm to 8.6 cm. The highest reduction in shoot length was observed in ADT 38 which recorded 3.38 cm under salinity as compared to 5.54 cm in control. IR 20 recorded 8.64 cm as against 9.98 cm in control and White Ponni showed a decrease of 2.7 cm as compared to its absolute control. The root length ranged from 0.36 cm to 2.22 cm, irrespective of varieties. The maximum reduction of 2.22 cm was noticed in ADT 38, while the minimum reduction was in TRY 1 (0.36 cm). The difference due to varieties and salinity level was significant. The salt stress reduced the vigour index as compared to the control. The variation due to variety and salinity were found to be significant. TRY 1 showed a minimum decrease of 7 per cent compared to its control. The maximum decrease was observed in ADT 38 (53.6 per cent). It was followed by IR 20 (38.9 per cent), White Ponni (33.1 per cent) and ADT 39 (30.4 per cent) respectively.

Under 200 mM NaCl concentration, ADT 39 recorded the lowest anthocyanin accumulation of 0.0283 unit g^{-1} followed by IR 20 (0.0261 unit g^{-1}). Maximum accumulation was observed in White Ponni (0.0622 unit g^{-1}). Due to salinity, TRY 1, ADT 39, White Ponni, IR 20 and ADT 38 accumulated 171, 362, 609, 2.58 and 211.3 per cent increase over its control.

Amylase activity decreased under salt concentration and there existed significant difference between variety and levels of salinity. The highest activity of 0.2133 (g maltose hr^{-1} g^{-1}) was observed in TRY 1 and IR 20 recorded the least of 0.0697 (g maltose hr^{-1} g^{-1}), whereas their controls recorded 0.4267 and 0.2057 (g maltose hr^{-1} g^{-1}) respectively. ADT 39, White Ponni and ADT 38 recorded 0.3, 0.26 and 0.10 (g maltose hr^{-1} g^{-1}) respectively under salinity, whereas their controls recorded 0.186, 0.133 and 0.0767 (g maltose hr^{-1} g^{-1}) respectively.

Germination of seeds involve the activation of enzyme systems as well as mobilization of reserve foods and these process are adversely affected by NaCl (Levitt, 1980). Maximum germination percentage was observed in TRY 1, which shows the tolerant nature. The germinability of rice under high salt solution is an index of salt tolerance. The evaluation of salt tolerance under 200 mM has led to the identification of best genotypes showing high germinability. This is in accordance with the findings of Ahmed and Gupta (1991) who have reported that rice at germination was susceptible to salt stress. The same was supported by Gill and Singh (1985) that the tolerant rice genotypes showed faster germination than the sensitive varieties. Salinity delays germination but does not appreciably reduce the final germination percentage (Akbar and Yabuno, 1974). However, high salt solution reduced the germination percentage (Akbar, 1975). In the present study, the salinity was found to affect, the germination, root and shoot length. The shoot height and root length of the seedlings grown in salt solutions also showed decline indicating that the salt stress not only affected germination but also the growth of the seedlings. Salt toxicity effects in plants are clearly visible in both root and shoot growth (Amzallag and Lerner, 1994). The results indicated a decrease in trend in shoot height and root length as salinity increased. This is supported by the findings of Gill and Singh (1992). The salt stress was found to affect the dry matter production of the seedlings also indicating that the synthetic ability of the crop was affected. The same thing was observed in the present study also. The dehydrogenase activity declined under the salt stress indicating the hindrance of respiratory activity of the seedlings under salt environment. The genotypes TRY 1 followed by ADT 39 and IR 20 showed high germination under salt stress was found to possess relatively higher values for shoot and root growth and dehydrogenase and amylase activities, indicating their superiority over others for salt tolerance. This is in conformity with the findings of Mondal *et al.* (1988) and Ahmed and Gupta (1991) who reported that root length, shoot length was conspicuously affected by salt.

Anthocyanin are important flavonoids not only as colouring pigments but also play an important role in disease resistance as phytoalexins, and it accumulates in relation to salinity. Anthocyanin accumulates in response to higher salinity at different stages of seedling growth (Kaliamoorthy and Rao, 1994). Increase in anthocyanin accumulation may be a stress response and/or due to decreased mobilization of other nutrients into the primary root. Increase in anthocyanin accumulation leads to decreased water uptake, root growth and mobilization of reserve food materials from the endosperm to the growing root (Morgan, 1984). Tolerant variety TRY 1, ADT 39 and IR 20 accumulated less anthocyanin than susceptible one (White Ponni and ADT 38).

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