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Effect of NaCl and PEG Induced Osmotic Potentials on Germination and Early Seedling Growth of Rice Cultivars Differing in Salt Tolerance

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Abstract: The effect of reduced osmotic potentials on germination and early seedling growth of four rice cultivars differing in salt tolerance were studied using iso-osmotic solutions (0, -0.232, -0.457, -0.677, -0.906 and -1.129 MPa) of NaCl and polyethylene glycol (PEG 4000). Seed germination and early seedling growth were assessed using four replicates of 25 seeds at 21± 1°C in the dark using paper towel method. Onset of germination, germination rate and seedling growth, all declined with increasing concentrations of both NaCl and PEG, the former being more inhibitory. Germination and growth processes were mainly affected at and above -0.457 MPa osmotic potential in both NaCl and PEG. Rice cultivars differed greatly in their tolerance to salt and water stress. However, the differences were well pronounced in NaCl but less so in PEG. The imposition of water stress by PEG for 9 days did not permanently inhibit germination or induce dormancy. However, salt stress appeared to be lethal than the equivalent osmotic potentials of PEG. Salt tolerant cultivars (V2 and BR23) performed consistently better under salt stress and consistently poor under osmotic stress compared with salt sensitive cultivars (V1 and IR8). These results suggested that the salt tolerance of rice cultivars is probably determined by their ability to withstand excessive Na^+ and Cl^- ions rather than their ability of water stress tolerance.

Key words: Rice, osmotic potential, germination, NaCl and PEG

Introduction

Soil water potential is an important environmental factor in saline areas controlling germination and seedling establishment. The regulation of seed germination by such environmental factors is important in both ecological and agricultural context as the interactions between environmental stress and endogenous adaptation mechanisms determine whether a particular seed will germinate under given conditions (Falleri, 1994). Fully imbibed, non-dormant seeds can be expected to initiate radicle growth after a lag period related to temperature. If the water potential of the imbibition medium is reduced, germination will be delayed or prevented depending upon the extent of reduction in water potential (Falleri, 1994).

The deleterious effects of salinity on germination and early seedling growth is well established (Greenway and Munns, 1980; Bewley and Black, 1982). However, opinion regarding the mode of deleterious effects of salinity on germination and early seedling growth differ among the researchers. Some workers describe the effect in terms of osmotic potential (Greenway and Munns, 1980; Bewley and Black, 1982); while others explain it as a toxic effect (Hampson and Simpson, 1990a, b). Some also explain the effect of NaCl on germination and early seedling growth as having both osmotic and toxic components (Katembe *et al.*, 1998).

Although a few studies have been conducted on wheat, barley, pulses, halophytes etc. information about rice is scanty. The degree to which salinity affects the germination and early seedling growth of rice by an osmotic effect or specific ion toxicity or both and whether it differs between cultivars differing in salt tolerance is still a subject of study. One technique for studying the effect of water stress on germination and early seedling growth is to simulate stress conditions using artificial solutions to provide variable osmotic potentials (Taylor *et al.*, 1982). If NaCl has only an osmotic effect, iso-osmotic NaCl and PEG solutions should affect the seeds in the same manner and to the same extent.

The present work was initiated to determine the effect of iso-osmotic solutions of NaCl and PEG on germination and early seedling growth of two types of rice cultivars differing in salt tolerance and to differentiate the osmotic effect from the toxic effect through comparison of NaCl and metabolically inactive osmoticum, polyethylene glycol (PEG 4000).

Materials and Methods

The study was conducted at the University of Aberdeen, UK during February to March, 2001. Four rice cultivars (BR1192-2B-35 = V1, BR5828-11-1-4 = V2, IR8 and BR23) differing in salt tolerance were assessed in this experiment. V1 and IR8 represented salt-sensitive while, V2 and BR23 represented salt-tolerant cultivars (Alam, 2001).

Different levels of reduced water potentials were imposed using different concentrations of NaCl and polyethylene glycol 4000 (PEG 4000), as shown below;

Table 1: Water potentials of NaCl solution and PEG 4000 at 22° C in terms of mM of NaCl and %PEG 4000

NaCl (mM)	Osmotic potential (Mpa)	% PEG 4000 (Based on average molecular weight of 3350)
0	0	0
50	-0.232	8.267
100	-0.457	14.789
150	-0.677	19.624
200	-0.906	23.036
250	-1.129	24.770

(Adapted from Mexal *et al.*, 1975).

Seed germination was assessed using four replicates of 25 seeds in a randomized complete block design (RBD). Prior to the germination test, seeds were surface sterilized in 1% sodium hypochloride solution for one minute. Then they were rinsed in distilled water for two one minute periods. The 25 seeds were placed on 80 cm length paper towel (18.50 cm wide), folded to give two layers of towel and pre-moistened with either 30 ml of distilled water or the experimental solution. The seeds were covered by another fold of 40 cm length paper towel, pre-moistened with 10 ml of the respective experimental solution. Each towel was then rolled and partially immersed in a beaker containing 10 ml of the appropriate osmotic solution. Each beaker was then kept in a tightly closed polythene bag and placed in a germination room at 21± 1°C in the dark. Germination was recorded every day for 9d. Seeds were considered germinated when both the plumule and radicle had extended to more than 2

mm. Final count of germination and plumue and radicle lengths were recorded at 9th day.

After completion of the germination test (9 d), any ungerminated seeds were washed well to remove any NaCl or PEG on the seed surface and placed for germination in distilled water. Germination counts were recorded daily for a further 8 d.

Germination rate (GR) was calculated as $GR = 1/T_{50}$ and T_{50} (= mean germination time, MGT) was calculated with the following equation of Younsheng and Sziklai (1985).

$$MGT = \sum nd / N.$$

Where, n is the number of germinated seeds on each day, d is the number of days from the beginning of the test and N is the total number of germinated seeds.

The data were analysed using standard statistical procedures. All percentage germination data were transformed to arc sine values before statistical analysis.

Results

Final germination: Final germinations were significantly reduced in NaCl and PEG 4000 (Fig. 1). When the first two points were excluded, approximately linear fits ($r = 0.917$, $P < 0.001$ for NaCl and $r = 0.902$, $P < 0.001$ for PEG 4000) were obtained to the remaining points. NaCl was less inhibitory permitting greater germination than PEG. The response of the salt-sensitive and tolerant cultivars differed in NaCl but they responded similarly in iso-osmotic solutions of PEG. There was a little reduction in germination in iso-osmotic solutions of both NaCl and PEG above -0.457 MPa. At osmotic potentials of -0.677 and -0.906 MPa germination of salt-sensitive cultivars was reduced to about 74 and 20% respectively and it was completely inhibited at -1.129 MPa in NaCl. Salt-tolerant cultivars however, maintained about 90 and 70% germination respectively at osmotic potentials -0.906 and -1.129 MPa. In contrast, a large reduction in germination of these cultivars was observed in iso-osmotic solutions of PEG after -0.457 MPa. Final percent germination in PEG did not exceed 14% in osmotic potential -0.677 MPa in all cultivars and it was completely inhibited at osmotic potential -0.906 MPa. Cultivars differed significantly in their response to salt and osmotic stress and the differences were pronounced at and above -0.677 MPa osmotic potentials. BR23 was highly tolerant to salt stress but highly sensitive to osmotic stress. V2 was highly tolerant to salt stress and moderately sensitive to osmotic stress. In contrast, IR8 was highly sensitive to salt stress but was the most tolerant cultivar to osmotic stress. V1 was moderately sensitive to osmotic stress and highly sensitive to salt stress.

After 9 days the un-germinated seeds were rinsed and transferred to a paper towel with distilled water to check for possible inhibition or permanent damage caused by NaCl and PEG. The response of the seeds was rapid except for the salt-tolerant cultivars, which had been in NaCl. Maximum germination was achieved within 2 or 3 days. However, when total germination was compared with germination percentage at 0 MPa highly significant differences were found between cultivars and osmoticum and their interaction was also significant (Tables 2a, 2b). PEG did not permanently inhibit germination whereas NaCl did, especially in salt-tolerant cultivars. For any osmotic potential, recovery was higher in PEG than NaCl. None of the seeds of salt-tolerant cultivars (V2 and BR23) recovered from high NaCl concentration. On average, germination during recovery period were about 67 and 87% for V1, 87 and 91% for IR8, 0 and 85% for V2 and 0 and 96% for BR23 respectively in NaCl and PEG.

Germination rate (GR): The effect of osmotic potential was more noticeable on GR than on germination. A moderate osmotic potential of -0.457 MPa caused a significant reduction in GR. GR was inversely linearly related to osmotic potential both in NaCl ($r = 0.948$, $P < 0.001$) and PEG ($r = 0.981$, $P < 0.001$). However, the rate of reduction in GR was significantly higher in PEG than its iso-osmotic solutions of NaCl under all osmotic potentials (Fig. 2). GR also differed between cultivars in their response to salt stress. Salt-tolerant cultivars V2 and BR23, had consistently higher GR in salt solutions. In PEG, differences in GR between cultivars were not pronounced except BR23 which was the most sensitive cultivar to osmotic stress (data not presented).

Plumule length (PL): The plumule length of rice was more sensitive to salt and PEG than either germination or GR. Weak solutions of NaCl and PEG (-0.232 MPa) reduced PL to about 84 and 73% respectively, whereas osmotic potentials of -0.232 MPa had no effect on germination and GR (Table 3). In general, PLs were progressively reduced as osmotic potentials increased and the rate of reduction was greater in PEG ($r = 0.983$, $P < 0.001$) than in iso-osmotic solutions of NaCl ($r = 0.972$, $P < 0.001$). On an average, at -0.232, -0.457 and -0.677 MPa osmotic potentials, PLs were reduced to about 85, 50 and 30% in NaCl and 70, 30 and 10% in PEG respectively to control. At -0.906 and -1.129 MPa osmotic potentials, PL in NaCl was reduced to about 20 and 5% respectively to control. Salt-tolerant cultivars (V2 and BR23) performed consistently better in salt solutions but consistently worse in PEG. In contrast, salt-sensitive cultivars (V1 and IR8) performed poorly in NaCl, but in PEG their PLs were significantly longer than V2 and BR23 at comparable osmotic potentials of NaCl. BR23 was identified as the most sensitive cultivar to osmotic stress. Its PLs were reduced to about 90 and 55% in NaCl at -0.232 and -0.457 MPa whereas, it was reduced to respectively 50 and 15% in comparable solutions of PEG and failed to germinate at -0.677 MPa. V1 and IR8 were equally tolerant to water stress and equally sensitive to salt stress.

Radicle length (RL): Like PL, RL was also progressively reduced with increasing osmotic potentials (Table 4). However, unlike PL, the reductions in RLs were similar in NaCl and PEG under lower osmotic potentials (up to -0.677 MPa). They varied between cultivars as well as between osmotica. On an average at -0.232, -0.457 and -0.677 MPa, RL was reduced to about 85, 65 and 45% in NaCl and 75, 60 and 30% in PEG respectively compared with control values. Cultivar responses of RL also were similar to those of PL. Salt-tolerant cultivars V2 and BR23 consistently had shorter radicles than salt-sensitive cultivars V1 and IR8 in PEG although V2 and BR23 had consistently longer radicles at any comparable osmotic potentials in NaCl. BR23 in particular, was the most sensitive cultivar to osmotic stress, while V1 was the most tolerant cultivar to osmotic stress having RL of about 55% to the control value at -0.677 MPa. However, V1 was equally sensitive to salt stress, as was IR8.

Discussion

With reducing osmotic potentials, both NaCl and PEG delayed the onset of germination, reduced final percent germination, decreased germination rate and plumule and radicle extension. Such responses have been reported by many workers over a wide range of halophytic and glycophytic plant species (Hampson and Simpson, 1990a, b; Falleri, 1994; Huang and Redmann, 1995; Katembe *et al.*, 1998). The general view is that a decrease in water potential gradient between seeds and their surrounding media adversely affects seed germination and subsequent growth processes. The physical process of water uptake leads to the activation of metabolic processes as the dormancy of the seed is broken following hydration (Katembe *et al.*, 1998). Elevated NaCl and PEG concentrations slowed down water uptake by seeds, thereby inhibiting the germination process. Reduced fresh weight in elevated iso-osmotic solutions of both NaCl and PEG (data not presented) indicated reduced water uptake by the germinating seeds (Alam, 2001).

For all the treatments, PEG was found to be more inhibitory to germination, GR, plumule and radicle growth compared with iso-osmotic NaCl solutions. These results agree with those of

Table 2a: Effect of NaCl on percent germination of different rice cultivars during germination and recovery period

Osmotic potential	V1		IR8		V2		BR23	
(MPa)	GP	RP	GP	RP	GP	RP	GP	RP
0	95	0	100	0	97	0	100	0
-0.232	91	0	98	0	95	0	99	0
-0.457	84	0	98	0	96	0	99	0
-0.677	74	0	74	87	92	0	99	0
-0.906	18	69	23	87	88	0	95	0
-1.129	0	65	0	86	77	0	68	0
Mean		67		87		0		0

Table 2b: Effect of PEG on percent germination of different rice cultivars during germination and recovery period

Osmotic potential	V1		IR8		V2		BR23	
(MPa)	GP	RP	GP	RP	GP	RP	GP	RP
0	95	0	97	0	97	0	100	0
-0.232	97	0	95	0	93	0	98	0
-0.457	89	0	94	0	89	0	87	0
-0.677	6	80	14	90	6	84	0	95
-0.906	0	79	0	91	0	86	0	96
-1.129	0	75	0	93	0	85	0	98
Mean		78		91		85		96

LSD values, osmotic potential = 3.29, osmoticum = 1.90, cultivar = 2.69, osmoticum* cultivar = 3.80, osmotic potential* cultivar = 6.58, osmotic potential* osmoticum* cultivar = 9.30. GP = germination (%) during germination period, RP = germination (%) during recovery period.

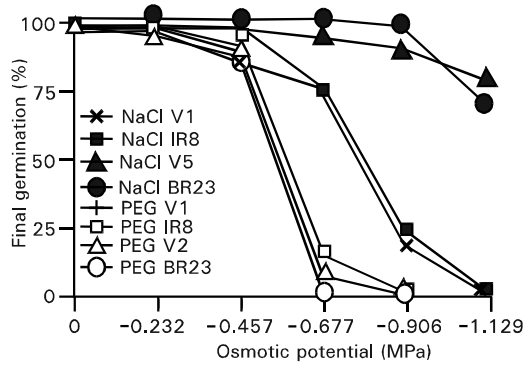

Fig. 1: Effect of NaCL and PEG on germination of different cultivars

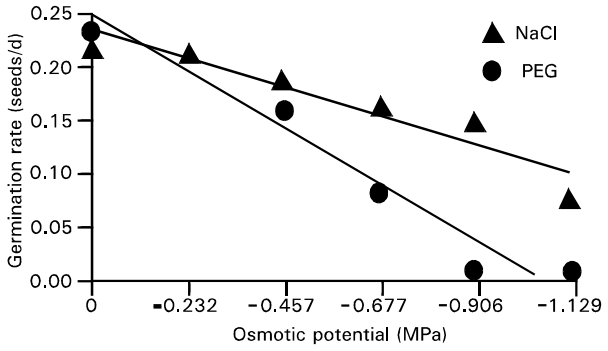

Fig. 2: Effect of NaCL and PEG on germination of different cultivars (average of four cultivars)

Hampson and Simpson (1990a, b) for wheat, as well as Huang and Redmann (1995) for barley and *Brassica*, Gulzar and Khan (2001) for *Aeluropus lagopoides*. However, such findings are not universal. Roundy *et al.* (1985) and Katembe *et al.* (1998) observed the opposite in wildrye, wheat grass and *Atriplex* species probably due to the tolerance of these plant species (halophytic) to high osmotic stress. The explanation of the higher inhibitory effect of PEG than NaCl lies in ion or solute entry into the seed. Unlike PEG, NaCl may readily cross the cell membrane into the cytoplasm of the cell unless an active metabolic pump prevents accumulation of the ions (Katembe *et al.*, 1998). Entering ions lower the seed osmotic potential which facilitates hydration of the seed by allowing a higher seed matric potential than the osmotic potential of the solution surrounding the seed (Roundy *et al.*, 1985). In PEG, hydrolysis of storage compounds could lower the internal osmotic potentials of the seed sufficiently for water entry (Hampson and Simpson, 1990a).

Imposition of osmotic stress to -0.677 MPa and above with PEG for 9 days did not substantially reduce viability or induce dormancy in rice seeds (as measured by the recovery test). However, salt stress at equivalent osmotic potentials appeared to be lethal especially to the salt-tolerant cultivars and also to a large proportion of the salt-sensitive cultivars. This is in agreement with Hampson and Simpson (1990a) in wheat and suggests that glycophyte seeds can remain viable for a considerable period under water stress but not under salt stress. The reason for the differing lethality between osmoticum as well as between cultivars again could be the ion entry and indicating that NaCl has a toxic effect in addition to an osmotic effect. Apparently the entry of salts speeds hydration, but seeds make this osmotic adjustment at the risk of specific ion toxicities which cause damage to seed metabolism in some way (Shannon and Francois, 1977). Specific ion toxicity of the Na^+ and Cl^- ions on the cell membrane, cytoplasm and/or nuclei of the cell may partly be responsible for the fact that NaCl is more lethal than iso-osmotic concentrations of PEG (Katembe *et al.*, 1998). NaCl treated seeds therefore, show poor recovery. Moreover, the presence of Na^+ and Cl^- ions in the cell may induce changes in protein activity because ions affect the structure of the hydration water which surrounds the protein molecule (Waisel, 1972). Cramer *et al.* (1987) commented that dividing cells may be sensitive to unfavourable ion ratios because they are non vacuolated and therefore, can not compartmentalise the ions easily. The same may be true for the germinating seeds. At higher salt concentrations, (-0.906 MPa and above) all or most (> 75%) of the seeds of the salt-sensitive cultivars did not initiate germination and so avoided the adverse effects of salt. In contrast, seeds of salt-tolerant cultivars, due to their inherent ability to withstand such salt stress, did initiate germination and were affected subsequently by the toxic effect of salt and failed to germinate during the recovery period. Onset of mitosis following germination for seedling establishment usually occurs when the radicle is about 6-8 mm long. This elongation is achieved by cell expansion supported by imbibition (Katembe *et al.*, 1998). Ion entry that facilitated water uptake and initial phase of cell elongation more in salt-tolerant than in salt-sensitive cultivars might become fatal to the cell division process which is vitally important for successful germination. Although rice seeds were able to recover well from soaking in high osmotic potentials in PEG, their recovery from salt solution was poor. None of the seeds of salt-sensitive cultivars V1 and IR8, germinated at -1.129 MPa in either NaCl and PEG. However, the recovery of V1 and IR8 respectively by about 65 and 86% from NaCl and by 75 and 93% from PEG (Tables 2a, 2b) showed that NaCl had an osmotic effect in addition to a toxic effect especially at higher salt concentrations. The germination rate (GR) was more affected by moderate osmotic potential (-0.457 MPa) than final germination percent. Moreover, seedling growth of rice was more sensitive to osmotic potentials than germination and GR, with significant effects even at -0.232 MPa. In contrast, osmotic potential of -0.232 MPa had no effect on germination and GR. These results are supported by other studies on wheat (Hampson and Simpson, 1990a) and barley (Huang and Redman, 1995). Growth probably requires more turgor pressure and lower osmotic potentials than germination (Hadas and Stibbe, 1973).

Plumule and radicle growth were progressively reduced as osmotic potentials decreased both in NaCl and PEG. However, although the

Table 3: Effect of NaCl and PEG on plumule length of different rice cultivars 9 days after seed placement for germination

Osmotic potential	NaCl					PEG				
(Mpa)	V1	IR8	V2	BR23	Mean	V1	IR8	V2	BR23	Mean
0 (PL)	2.78	3.67	4.26	5.13	3.96	2.61	3.58	4.26	5.13	3.89
-0.232 (% C)	80.00	84.00	82.00	89.00	84.00	105.00	70.00	65.00	52.00	73.00
-0.457 (% C)	53.00	40.00	58.00	54.00	51.00	48.00	23.00	23.00	16.00	28.00
-0.677 (% C)	21.00	20.00	43.00	35.00	30.00	15.00	13.00	9.00	0.00	9.00
-0.906 (% C)	15.00	15.00	30.00	20.00	20.00	0.00	0.00	0.00	0.00	0.00
-1.129 (% C)	0.00	0.00	18.00	8.00	7.00	0.00	0.00	0.00	0.00	0.00

LSD values, osmotic potential = 2.71, osmoticum = 1.56, cultivar = 2.21, osmoticum*cultivar = 3.13, osmotic potential*cultivar = 5.41, osmotic potential* osmoticum*cultivar = 7.65. PL = plumule length in control (cm), %C = percent values to the control, 0 = no germination.

Table 4: Effect of NaCl and PEG on radicle length of different rice cultivars 9 days after seed placement for germination

Osmotic potential	NaCl					PEG				
(Mpa)	V1	IR8	V2	BR23	Mean	V1	IR8	V2	BR23	Mean
0 (RL)	5.54	7.23	7.05	7.69	6.88	5.61	6.67	7.05	7.69	6.75
-0.232 (% C)	87.00	97.00	80.00	90.00	89.00	79.00	74.00	85.00	67.00	76.00
-0.457 (% C)	57.00	55.00	74.00	68.00	63.00	63.00	51.00	65.00	57.00	59.00
-0.677 (% C)	38.00	38.00	54.00	48.00	44.00	50.00	34.00	29.00	0.00	28.00
-0.906 (% C)	23.00	22.00	38.00	30.00	28.00	0.00	0.00	0.00	0.00	0.00
-1.129 (% C)	0.00	0.00	23.00	14.00	9.00	0.00	0.00	0.00	0.00	0.00

LSD values, osmotic potential = 4.11, osmoticum = 2.38, cultivar = 3.36, osmoticum*cultivar = 4.75, osmotic potential*cultivar = 8.23, osmotic potential* osmoticum*cultivar = 11.64. RL = radicle length in control (cm), %C = percent values to the control, 0 = no germination.

plumule growth was much lower in PEG than NaCl, radicle growth was similar. These findings are not consistent with those of Roundy *et al.* (1985) and Hampson and Simpson (1990b). Since Ca is important in regulating the membrane permeability and can be displaced from plasmalemma of root cells by Na^+ ions (Cramer *et al.*, 1985) and as membrane leakage is much higher in NaCl than iso-osmotic solutions of PEG (Hampson and Simpson, 1990b), greater growth of plumules and radicles was expected in PEG than NaCl. The explanation lies in the methodology used in this study. They used Petri-dishes to germinate the seeds but paper towels were used in the present study which was later known to contain Ca (Alam, 2001). The Ca in the paper towel might had protected the plumule and radicle growth to some extent from the adverse effects of NaCl.

Cultivar differences in final percent germination were clear at and above -0.677 MPa in NaCl but less so in PEG (Fig. 1). This suggests that rice cultivars might be very sensitive to osmotic stress and the differences between cultivars are minimal. Nevertheless, there were significant differences between cultivars. Germination of BR23 was completely inhibited and that of the other three cultivars varied between 6-14% at -0.677 MPa in PEG. These differences in response to osmotic stress tolerance could be genetic i.e. seeds of different cultivars might have different critical water potentials or hydration levels below which the physical processes of germination are slowed or prevented as similarly observed in seeds of different plant species (Hadas and Stibbe, 1973; Naylor, 1991).

These results indicate that salt tolerance of rice cultivars is probably determined by their ability to withstand the excessive Na^+ and Cl^- ions not by their ability to tolerate osmotic stress. Therefore, study on the compartmentation of Na^+ and Cl^- ions in different parts of the seeds during germination and early seedling growth may be needed to explain the mode of action of NaCl on seed germination and early seedling growth. Considerable cultivar differences are likely to exit in rice in response to salt stress but differences under osmotic stress are minimal. The implications of this for plant breeders are that rice cultivars tolerant of low osmotic pressure in PEG would be likely to be salt-tolerant, but that a large number of salt-tolerant lines might be missed by screening in PEG. The appropriate strategy is to screen in salt solution.

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