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STUDY ON SALT TOLERANCE OF *HIPPOPHAE RHAMNOIDES* L. DURING GERMINATION

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The present investigation is an attempt to elucidate the salt tolerance of *H. rhamnoides* L. during germination. Salts of sodium, calcium and magnesium with their species anions chloride and sulphate were applied at various treatment levels. *H.rhamnoides* was found less tolerant of the salts of sodium, calcium and magnesium and sulphate salts of Na and Mg affected germination more than chloride salts.The interaction of sodium chloride and sodium sulphate with calcium chloride showed pronounced enhancement of seed germination in sodium sulphate treatment. This was possibly as a result of supression of sodium uptake and replacement of sulphate ions with that of chloride ions which showed less inhibitory effect when supplied individually.Germination of seeds also improved in magnesium chloride treatments on addition of calcium chloride. This was possibly due to an interference mechanism, where ions interfere directly with each other for the same uptake site.*H.rhamnoides* seeds were found equally tolerant to isotonic concentrations of sodium chloride or mannitol (non-electrolyte).It appeared that *H.rahmnoides* seeds can tolerate up to 20% salinity of sea water dilutions during germination.

Key words: Salt tolerance, Specific ion effect, Sea water, *Hippophae rhamnoides*.

Introduction

In saline environment the presence of excess salts severely affects seed germination either by impending water uptake or by toxic effects of salts entering into the seed. These effects have been summarized as (a) osmotic or (b) specific ion [1]. The osmotic effect is generally considrerd as the result of high osmotic potential (low water potential) of the soil, which in turn is caused by physico chemical factors of the soil. Manohar and Hyedecker [2] explained that the water stress can be produced either physically or chemically. Physically the soil matrix as a result of surface colloides create suction or water tension, which is now generally known as matrix potential. Chemically the presence of solutes in water results in lowering of the water availability (i.e. low water potential). The resultant of physical and chemical forces is commonly known as osmotic pressure or potential of the solution. Specific ion toxicities (influence of individual cations and anions) have been reported by many workers. Detailed work was carried out by Strognov [3] who classified halophytes into sulphate or chloride resistant types.

The germination of halophyte seeds in saline soils seem to be more important than their establishement. Many reports suggest that plants are less salinity tolerant as seedlings than at more mature stages of development [1,4,5]. The objectives of salt tolerance studies both in the field and laboratory is therefore to observe the limits of tolerance of seeds that successfully germinate and survive under saline conditions.

Hippophae rhamnoides a genus of the Family Eleagnaceae is of considerable economic importance becuase of its berries rich in ascorbic acid and also as stabilizer of unstable soil [9]. It has Euro-Asiatic distribution and occur on both inland and coastal sites. However, in Europe it grows mainly on coastal sand dunes and gravelly morainic detritis [6]. Inland it is found in Catalonia, the Pyrenees and occasionally north eastward to the Caucasus mountains and in the HinduKush-Himalaya range [6-8]. In Pakistan this species is inhabited in the mountainous Kuram valley region (non saline) which is in contrast to the coastal sand dunes in Europe, where salt spray frequently add salts in the soil causing surface salinity. Gorham [10] mentioned the effect of salt spray in rising the concentration of sodium, magnesium and chloride in dune soils at Blakeney Points, Norfolk.There is no comprehensive information of the salt tolerance of this species. A few studies indicated it to tbe salt tolerant [9]. However some earlier reports suggest it to be salt sensitive [11,12].

Owing to the economic importance, lack of information about its salt tolerance and adaptation in contrasting areas (saline and non-saline the present study was undertaken. The study would elucidate the response of *H. rhamnoides* to increasing salt concentrations or environmental stresses.

Materials and Methods

Experimental technique. Germination experiments were conducted in 9 cm diameter glass Petri dishes. Seeds were germinated on four layers of moist filter papers (Whatman no. 1). Seven ml of distilled water (control) or appropriate concen-

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trations of salt solutions were applied to Petri dishes. Seven ml of solution was considered to be suitable, since this does not submerge the seeds nor create abrupt changes of concerntration in test solution as a result of evaporation of water from the Petri dishes. Solutions were changed on alternate days after washing the Petri dishes with the appropriate solution to prevent the possiblity of contamination from seed exudate. Germination was recorded daily and the expriment was ended when there was no sign of germination for the three consecutive days. A seed was considered germinated when the radicle attained the length of 0.5 cm. For each concentration there were five replicates each replicate contained 20 seeds.

Germination experiments. Experiments were carried out at various concentrations of sodium calcium and magnesium salts of chloride and sulphate. Solutions were prepared in millimolar (mM) concentration by adding an appropriate amount of salt in de-ionized distilled water. The concentration of sodium salts were prepared in 25, 50, 100 and 150 mM, calcium and magnesium salts in 12.5, 25, 50, and 100 mM. The interaction of salts between calccium sodium and magnesium was also observed. All germination experiments were performed in dark incubators at 20 ± 1° without surface sterilization in order to simulate natural conditions.

An experiment to investigate the effect of sea water was carried out using sea water dilutions of 5, 10, 20, 40, 60 and 80% (v/v, natural sea water + distilled water). The experiment on the effect of osmotic pressure of the irrigating solution was conducted by using mannitol (non-electrolyte). The osmotic pressures in atmosphere (1 atm = 1.0135 bar) were obtained following the formula mentioned by Parmer and Moore (13)*viz:*

$$g = PVM / RT$$

where, g = gram of chemical required, P = osmotic pressure needed, M = molecular weight of the substance, R = 0.0802, T = absolute temperature.

H.rhamnoides inhabit coastal habitat and it is presumable that it tolerates salinity or osmotic stresses likely to occur. Therefore both experiments were conducted to understand the response of this species to sea water and somotic solutions. Analysis of variance for statstical significance of total germination was conducted following Bishop (14).

Result and Discussion

Effect of NaCl and Na_2SO_4 (a) Sodium chloride. Germination of seeds decreased with increase in concentration of NaCl solutions Fig.1a. Solutions of 25, 50 and 100 mM did not show significant reductions in germination within treatment levels. However comparative to the control significant reductions did occur (p=0.01 and 0.001) at 100 and 150 mM. The solutions of 100 and 150 mM delayed germination by 10 days, while germination occurred after 5 days in the control.

(b) Sodium sulphate. The germination of seeds in Na_2SO_4 solutions decreased remarkably above 25 mM concentrations Fig.1b. Highest germination occurred in the control and lowest in 100 mM, while it was completely suppresed in 150 mM Na_2SO_4 solutions. It appeared that sodium sulphate has a greater retarding influence on germination than sodium chloride (significance occurred at p=1% and 0.1% level).

Effect of $MgCl_2$ and $MgSO_4$ (a). Magnesium chloride. The germination percentage was high in low concentrations of $MgCl_2$ up to 25 mM but was significantly reduced at 50 mM (p=1%, Fig. 2a). The germination in the control was slightly lower than 12.5 mM $MgCl_2$. At 50 mM root development was severely affected, while seeds did not germinate at all in 100 mM $MgCl_2$.

(b) Magnesium sulphate. Germination of seeds in 12.5 mM $MgSO_4$ was insignificantly lower than the control, whereas it was significanly reduced at 25 and 50 mM relative

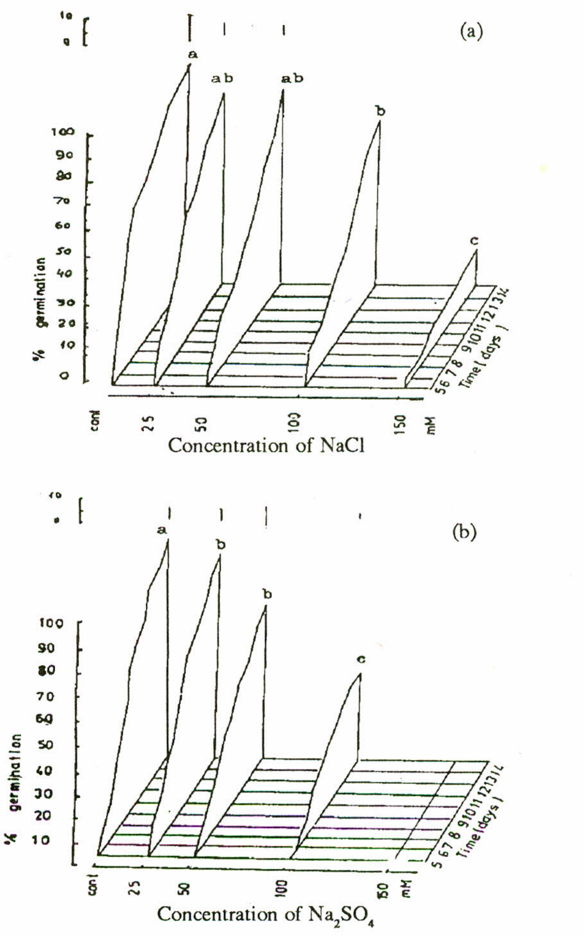

Fig. 1. The effect of chloride and sulphate salt of sodium on germination of *H. rhamnoides*. (a) sodium chloride (b) sodium sulphate. ±SD

to the control (p = 5% and 0.1% respectively). The solution of 100 mM completely prevented germination (Fig. 2b).

Effect of $CaCl_2$. The germination was higher in the lower concentrations of $CaCl_2$ (12.5 and 25 mM) compared to the control. A slight reduction in germination occurred in 50 mM followed by a pronounced reduction in the 100 mM solution (significance of the result occurred at p=5, 1 and 0.1%, Fig.3).

Owing to the insolubility of calcium sulphate salt it was difficult to maintain appropriate level of concentrations at 20°–1C. Hence, the experiment was not carried out with this salt.

Interaction of $CaCl_2$ with different concentrations of NaCl and Na_2SO_4 and its effect on germination. The germination of seeds in combined treatments of (NaCl 50 mM + $CaCl_2$ 25 mM) was slightly lower than the control. While, it was significantly less in the other combined treatments of (NaCl 100 mM + $CaCl_2$ 50 mM and NaCl 150 mM + $CaCl_2$ 100 mM) relative to the control Fig. 4a.

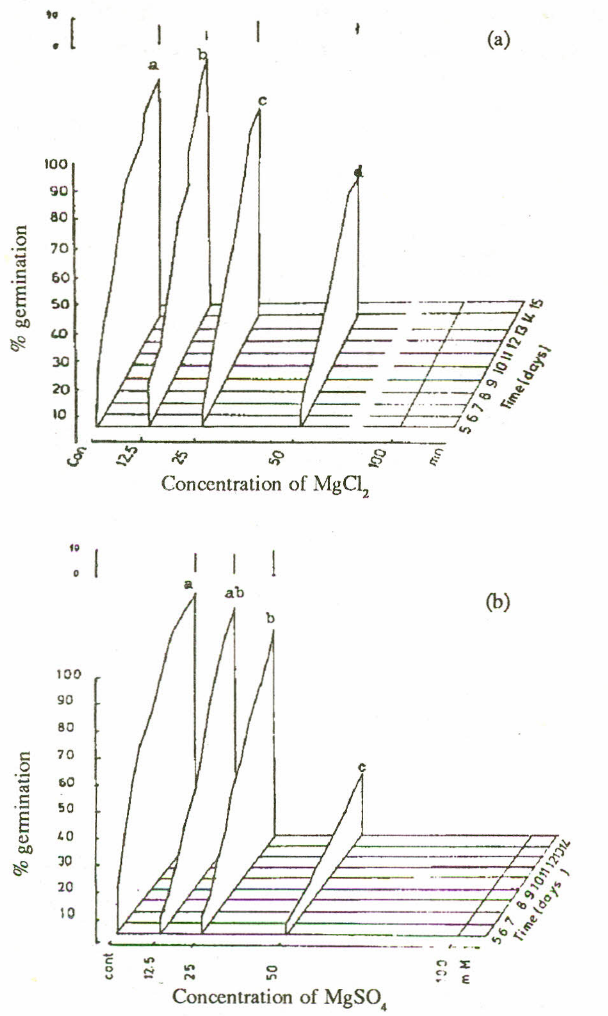

Fig. 2. The effect of chloride and sulphate salts of magnesium on germination *H. rhamnoides* (a) magnesium chloride (b) magnesium sulphate ±SD.

The germination of seeds in the combined treatments of Na_2SO_4 50 mM + $CaCl_2$ 25 mM, Na_2SO_4 100 mM + $CaCl_2$ 50 mM and Na_2SO_4 150 mM + $CaCl_2$ 100 mM) decreased at all levels significantly relative to the control. However it is notable that in individual salt treatments of sodium chloride and sodium sulphate the germination was severely affected at theses levels (Figs. 1a and 1b). The enhancement in germination on addition of calcium suggests that calcium interacts with sodium.

Interaction of $CaCl_2$ with $MgCl_2$ at different concentrations and its effect on germination. The germination of seeds in combined treatments of magnesium and calcium chlorides ($MgCl_2$ 25 mM +$CaCl_2$ 12.5, $MgCl_2$ 50 mM + $CaCl_2$ 25 mM, $MgCl_2$ 75 mM + $CaCl_2$ 50 mM and $MgCl_2$ 50 mM + $CaCl_2$ 50 mM) was significantly lower than the control (Fig. 4b). The addition of $CaCl_2$ at a concentration of 50 mM appeared to counteract the adverse effects of $MgCl_2$ on germination (compare Fig. 4b and 2ab).

Effect of sea water dilutions on germination. *H. rhamnoides* seeds showed high germination in dilutions of 5, 10, 20 and 40% sea water (v/v). Severe inhibitory effects of sea water occurred in 60% dilution (approximately equal to 20% salinity, when the salinity of sea water was found to be 35% on conductivity basis) and the dilution of 80% completely prevented germination (Fig.5).

Effect of mannitol on germination. The germination of seeds irrigated with mannitol solutions of -1 (-1.0135 bar), -2(-2.027 bar), -4(-4.05 bar) and -6 atm.(-6.08 bar) decreased gradually with increase in osmotic levels (Fig.6). Pronounced reduction in germination occurred in solutions of -8 atm.(-8.21 bar). The osmotic levels -2,-4,-6 and -8 affected root development by stunting roots.

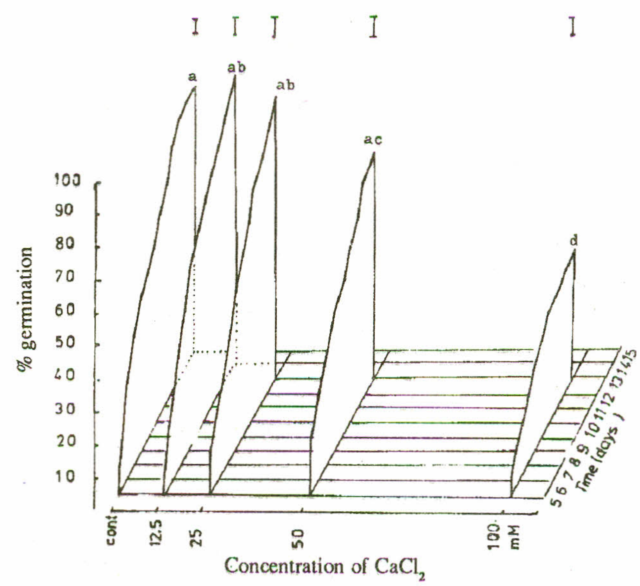

Fig. 3. The effect of calcium chloride on germination *H. rhamnoides* ±SD.

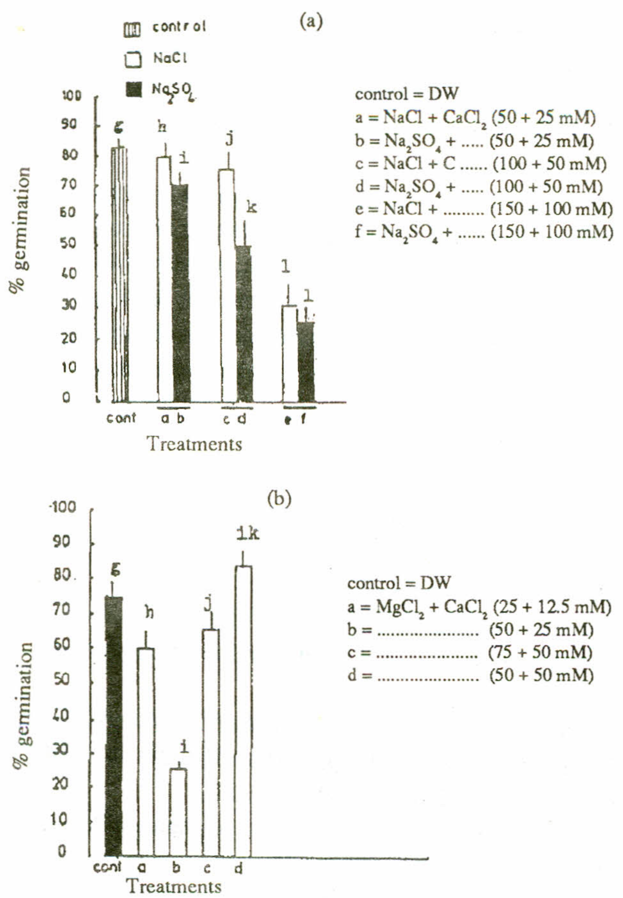

Fig. 4. The effect of the addition of calcium chloride to *H. rhamnoides* germinating in solutions of (a) NaCl and Na_2SO_4 (b) $MgCl_2$.

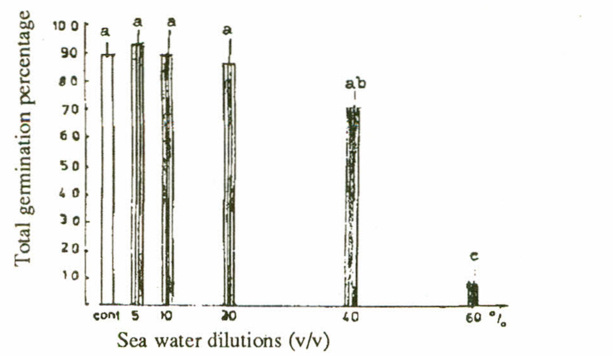

Fig. 5. The effect of sea water at a range of dilutions on the germination of *H. rhamnoides* .

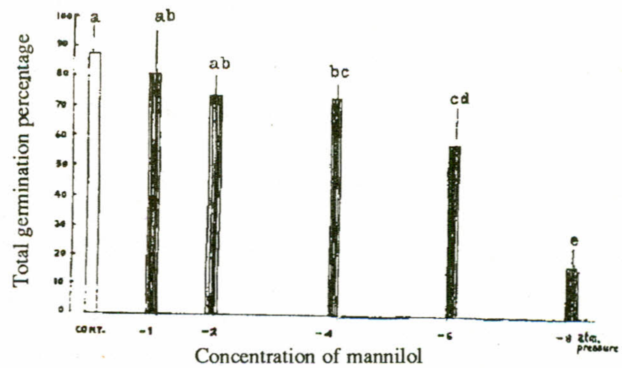

Fig. 6. The effect of mannitol solution of different concentrations on the germination of *H. rhamnoides* .

Experiments with seeds of *H. rhamnoides* demonstrated that it was less salt tolerant during germination and its degree of salt tolerance varied with different salt solutions. For example sulphate salts of Na and Mg affected germination more than chloride salts. The results on the effect of sodium chloride solutions is in line with the rsults of Workman and West [15] on *Euratia lanata,* Mirza and Mahmood [16] on *Phaseolus aureus* and Kayani and Rahman [17] on *Zea mays* who found a decrease and delay in germination with inccreasing concentrations of sodium chloride.

Germination of seed of *H.rhamnoides* was severely affected in sodium sulphate relative to the sodium chloride solutions. It seems that sulphate salts are more toxic than chloride salts for the germination of this species. The toxic effects of sulphate salts have been reported by many investigators. Younis and Hatata [18] use sodium potassium and magnesium salts of chloride and sulphate on *Triticum vulgare* and found that there was a specific ion effect. Radmann [19] found that sodium sulphate and magnesium chloride were more toxic than isotonic solutions of sodium chloride on the germination of *Medicago sativa.* Another report suggests that germination of grass species (*Panicum antidotale, Eragrostis lehmaniana, E.superba* and *E.curvula)* in soils contaminated by magnesium chloride or sodium sulphate would be less than in soils contaminated by calcium chloride at similar concentrations [20]. More recently Khan *et al.* [21] reported that germination retardation in wild desert species (*Achyranthus aspera, Peristrophe bicalyculata, Cassia holosericea* and *Prosopis juliflora*) was greater in sodium sulphate than sodium chloride. Similar results obtained for magnesium salts treatment which indicate less tolerance to sulphate salt than chloride.

The reslts on calcium chloride showed that there was slightly less germination of *H. rhamnoides* seeds in calcium chloride than in sodium chloride solutions of isotonic concentrations. Unlike the effect on root development in high concentrations of sodium chloride (blackening and stunting of radicle) calcium chloride caused no damage to the root.

The result on interaction of sodium chloride or sodium sulphate with calcium chloride showed significant improvement of germination. The germination improved more in sodium sulphate than in sodium chloride treatments. The enhancement of germination in sodium sulphate solutions on addition of calcium chloride is possibly because of reduced uptake of sodium and the replacement of sulphate ions with that of chloride ions (specific ion toxicity). The interionic influences or interaction between sodium and calcium have been reported by many workers [20-21]. For instance, Hyder and Greenway [22] have reported ameliorative role of calcium over the adverse effect of sodium on barley plants. Chaudhry and Wiebe [23] reported that isotonic concentrations of calcium chloride (dihydrate) showed improved germination of *Triticum vulgare* from 80 to 90% in 1% sodium chloride pretreated seeds. They also reported that 25% reduction in ^{22}Na uptake occurred on addition of calcium.

The result on interaction of calcium and magnesium suggests that calcium counteracts magnesium by ameliorating the deliterious effect of magnesium. The improvement of germination in magnesium chloride treatments on addiition of calcium chloride was possibly due to interference mechanism, where ions interfere directly with each other for same uptake site. This may also be considered as competitive inhibition i.e. when two chemically related ions compete for the same uptake site (eg. Na, K, Rb, Ca and Mg) [22]. Besides this selective absorption has also been reported in some species e.g. Rye plants can grown equally well at all Ca/Mg ratios owing to its preference for calcium [23]. We infer that improvement in germination on addition of calcium, to magnesium treatments is possibly due to selective intake of calcium by the seeds or competetive inhibition of magnesium. The present finding is partly in agreement with that obtained by Hyder and Yasmin [4] on *Sporobolus airoides*. They suggested that addition of calcium and sodium counteracted the deleterious effects of magnesium. They also infered that high magnesium concentration may cause disoraganisation of protoplasm resulting in depression of germination and the replacement of calcium could ameliorate this condition due to favourable effect of calcium on seed membranes.

H. rhamnoides showed considerable tolerance to sea water dilutions. Severe effect on germination occurred at 60% dilution (v/v approx. equal to 20% Salinity). Similar findings were obtained by Okusanya [26] who mentioned that cliff species *(Crithmium maritima,Daucus carola, Innula crithmoides and Spergularia rupicola)* tolerate up to 30% salinity of sea water concentration during germination. However *H.rhamnoides* was found to be less tolerant (up to 20% salinity) than those of the cliff species. The present finding also concurs with that obtained by Khan *et al*. [27] on *Prosopis juliflora* which showed insignificant differences in percentage germination up to 30% ammended sea water dilutions comparative to the control.

H.rhamnoides also showed tolerance to mannitol solutions. Reduction in germination was similar to that obtained in sodium chloride solutions (i.e. germination decreased with increasing levels of mannitol solutions). The results sugggests that *H.rhamnoides* is equally tolerant to sodium chloride salt solutions or osmotic potentials of the germinating medium. The present finding is in line with that obtained by Parmer and Moore [13] who found increased osmotic pressure levels decreased water absorption of and progressively delayed and reduced germination of two strains of maize (*Zea mays* strong and weak vigour).

As regards the inhabitance of *H.rhamnoides* in contrasting habitats (inland and coastal), we infer that although this species is low tolerant to individual salts concentrations, but, the interactions of salts, calcium in particular with magnesium and/or sodium plays an important role. It is obvious from its tolerance to sea water dilutions and the experiments on interaction of salts. A further study on seedling growth in culture solutions may reveal it vividly.

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