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The study of the effect of salinity stress on the germination and the initial growth of cowpea (*Vigna unguiculata* L. Walp)

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Salinity is one of the most important problems in subtropical and tropical regions in the world, and it is a main factor limiting growth of plants. Regarding the existence of salinity in Khuzestan, a project was done to study the effects of various salts with different salinity levels on the germination and the growth of cowpea seedling in order to discover possible ways to increase its germination in salty soil. This test was done in a Completely Randomized Design with 3 replications in the Faculty of Agriculture, Laboratory of Shahid Chamran University of Ahvaz in 2010. The scholars considered the effects of $NaCo_3$, $NaHCo_3$, $CaCl_2$, NaCl (with 2,4,6 & 8 ds/m) and the distilled water as a control sample on percent (Gp) and the rate (Gr) of seed germination and also the length (Rl, Pl), cowpea seedlings, fresh and dry weight (Fwr, Fwp, Dwr, Dwp). The result showed that all of the growth indices usually decrease when the amount of salt increase ($p < 0.05$), but cowpea seed can bear on this salt with density to 8 ds/m (except $NaHCo_3$).With regard to results, we can conclude that cowpea cultivar is sensitive to salinity, so we suggested that making soil test to determine salinity before cultivating cowpeas in the farms of these areas.

Key words: Cowpea; growth; Salinity stress; Seed germination; seedling.
Abbreviation: Gp: Germination percentage, Gr: Germination rate, Rl: Radicle length, Pl: Plumule length, Fwr: Fresh weight of Radicle, Fwp: Fresh weight of Plumule, Dwr: Dry weight of Radicle, Dwp: Dry weight of Plumule.

Introduction

Salinity is one of the main and common stresses in the world, which is a major factor reducing total crop yields. It is believed that salinity reduces yield in about 50% of cultivated land (Flowers and Yeo, 1995; Maas, 1990). In Iran, approximately 25,000,000 ha of lands are affected by salinity. This amounts nearly 15 % of the total Iran area (Agricultural data collection, 1955). Salinization of agricultural soils is a worldwide concern, especially in irrigated lands, where water is the salt-transporting agent through soil profile. Saline

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soils present unfavourable conditions for seed germination and plant growth, limiting agricultural production. Irrigation induces an accumulation of salts at soil surface, affecting germination, stand, vegetative development, productivity and, at the worst cases, causes plant death (Silva and Pruski, 1997).

Salinity is defined as the presence of minerals at high levels (cations K, Mg, Ca, Na and anions No_3, Hco_3, So_4, Cl) in water and soil (Tanji, 1995). High salinity in the root zone affects the growth of many plant species. Total growth and leaf area are affected. Different plant species may have different tolerances to salinity. When soils in arid regions of the world are irrigated, solutes from the irrigation water can accumulate and eventually reach levels that have an adverse effect on plant growth. Current estimates indicate that 10 - 35% of the world's agricultural land is now affected with very significant areas becoming unusable each year. It is a world-wide problem, but most acute in North and Central Asia, South America and Australasia. Salinity can affect any process in the plant's life cycle, so salinity tolerance will involve a complex interplay of characters. Diverse methods have been used to reduce the salt affected soils and increase the quality of these soils, for example seraping (physical removal), flushing and leaching (Siyal *et al*., 2002) but these methods are characterized by their high costs. Therefore alternative measures are necessary. The legumes are very important both ecologically and agriculturally because of their ability to fix nitrogen in the root nodules in a symbiotic interaction with soil rhizobia. However, legumes are very sensitive to salinity and drought. Cowpea (*Vigna unguiculata* L. Walp.) is an important grain legume crop used as a fodder crop for livestock, as a green vegetable, and for dry beans (West and Francois, 1982). Many varieties are grown in tropical and sub-tropical agricultural areas of the world, where salinity is a yield-limiting factor. It is also cultivated as a dry land crop under different climatic conditions ranging from semi-arid to sub humid (Lush and Rawson, 1979). Cowpea is reported to have a good tolerance to heat and drought (Rachie and Roberts, 1974; Vasquez-Tello *et al*., 1990), and it has a high yield potential under irrigation (Turk *et al*., 1980). Cowpea is grown to obtain seeds and pods for human consumption, as a source of green manure and organic material on unproductive soils, primarily in semi-arid regions. Cowpea has a moderate tolerance to salinity, with a greater tolerance than corn, less than wheat, barley, sugar beet, and cotton (Hall and Frate, 1996). Effect of salinity on the germination, vegetative growth, and yield of cowpea has been studied (West and Francois, 1982; Imamul and Larher, 1983; Maas and Poss, 1989; Kannan and Ramani, 1988; Plaut *et al*., 1990; Larcher *et al*., 1990; Fernandes *et al*., 1994; Murillo-Amador *et al*., 2000).

Germination is the most sensitive stage of plant growth and poor germination in salinity caused by low establishment production and weak seedling and eventually would lead to reduce product. Sodium chloride and sodium carbonate are the dominant salts in saline areas (Ayer and West, 1985.) Anion Cl-ion is main limiting crop growth in saline areas (Kalbasi, 1980).

Recently, to expand worldwide crop production interest in desert agriculture has increased (Hutchinson and McGiffen, 2000). Statistically, Iran having approximately 697 hectares equivalent to 86.6 percent of the agriculture land under cultivation and production of cereals is one of the producers of these in the world (Department of Statistics and Information Department Agriculture, 2009). Different plants have different abilities saline conditions. Difference between salt tolerance not only form genus and species, also even within a species can be observed. Reduced germination rate and plant growth under salinity conditions depends on combine salt, salt concentration and stage of plant growth.

Since the salinity is one of the most important problems of tropical and subtropical regions (for example Khuzestan) and also according to the soil and irrigation water test, that showed the region has a variety of salt including sodium chloride, sodium sulphate, sodium carbonate, chloride calcium, etc. Therefore, the aim of this study was to investigate the effect of salinity stress on the germination and the initial growth of Cowpea (*Vigna unguiculata* L. Walp).

Materials and methods

Preparation of salts solution

Seed cultivar (Parasto cultivar) used in this experiment was a native mass. Cowpea seeds were collected from fields in southwest Iran (Ahvaz state: The latitude and longitude of Ahvaz is: 31° 19' 45" N / 48° 41' 28" E, also the city has an average elevation of 20 meters above sea level). Treatments included four types of salt and different levels of salinity, including four levels (2, 4, 6, 8 ds/m) and distilled water was used as control. Sodium chloride salts (NaCl), calcium chloride ($CaCl_2$), sodium carbonate ($NaCo_3$) and sodium hydrogen carbonate ($NaHCo_3$) were used as salt solution. For this purpose, using Formula 1 and 2, the amount of salt needed were determined to prepare salt solutions with the desired electrical conductivity (Rehman *et al*, 1994; Puppala *et al*., 1999) as follows:- 1) $\Omega = 0.36 \times Ec$ and 2) $\Omega = \sum (Ci) \times (RT)$.

These two formulas: Ω = osmotic potential, Ec = electrical conductivity based on ds/m, R = equivalent coefficient (0.083), C = Molarity, T = temperature based on Kelvin, and i is the number of ions.

Bioassays

This study was conducted as a completely randomized design at Shahid Chamran University of Ahvaz, Faculty of Agriculture in three repetitions in 2009–2010. To prevent the growth and activity of microorganisms, experimental dishes and twenty seeds surface were sterilized with the ratio of water: bleach solution (10:1) and were placed evenly on filter paper in sterilized 9 cm Petri dishes. Five milliliter solution from each salt was added to Petri dishes and distilled water used as control. Petri dishes were placed at growth chamber darkness at 25 ° C temperature. To prevent evaporation, dishes were closed. Treatments (salt solutions and the distilled water control) were arranged in a completely randomized design with three replications. After 7 days, germination percentage and germination rates were determined with number of germinated seeds and expressed as percentage. Radicle and plumule lengths were determined after 10 days by measuring seedlings. After measuring the radicle and plumule lengths, the seedlings were separated into plumule and radicle parts and Fresh weight of radicle and plumule were determined. The plants were then dried and dry weights recorded.

Statistical analysis: Data were computed statistically by applying probability using one way analysis of variance for each species with different concentrations where they were needed. Statistical analyses were performed using software of SAS and Excel.

Results

The results showed that salinity had a significant effect on germination percentage and rate, plumule and radicle length, plumule and radicle dry weight ($p < 0.05$).The values for germination percentage and rate, plumule and radicle length, plumule and radicle dry weight decreased with increasing (Table 1 and 2).

The effects of salinity on germination

Final germination percentage of seeds was significantly affected by salinity ($p < 0.05$) (Table 1 and 2). Seed Germination percentage showed that with increasing concentrations of each salt CaCL2, NaCl, NaCo3, NaHCo3 general trend over a significant decrease in the 5% level (Table 1 and Fig. 1 and 9).Germination did not occurred at 8 ds/m solution NaHCo3. Seed germination was highest in distilled water. Also, after the control (distilled water), salt NaCl showed highest percentage germination compared with other salts were observed (Table 1 and Fig. 1). Germination percentages were inversely related to salt concentration level. The percentage of germination significantly decreased in all varieties due to increasing salinity level. Salinity results in poor stand due to decrease in the rate of seed germination. Presumably, the osmotic effect due to salinity was the main inhibitory factor that reduced germination as indicated by Akbar and Ponnamperuma (1982).

The effects of salinity on germination rate

The rate of germination was the highest at the control (distilled water) and salt $NaCo_3$ treatment compared with other salts (Table 1 and 2, Fig. 2 and 10). The greatest reduction in germination was observed at concentrations of 8 ds/m $NaHCo_3$ salt. The decreasing tendency of germination rate due to increasing salinity was in the conformity with the reports of others (Mohammed *et al*., 1989; Khan *et al.,* 1997). The reduction of speed of germination at high salt levels might be mainly due to osmotic stress (Hakim *et al.,* 1988).

The effects of salinity on plumule and radicle length

Plumule length and radicle length of Cowpea seeds declined in all salt treatments relative to the control and with increase in salinity (p <% 5). The most plumule and radicle length was obtained in control treatment (distilled water) and the lowest plumule and radicle length was otained in Salt $NaHCo_3$ obtained (Fig. 3 and 4). Similarly, radical length was also decreased with increased salinity. Radicle length was more suppressed than plumule by salinity at each specific salt concentration level. The gradual decrease in root length with the increase in salinity as observed might be due to more inhibitory effect of NaCl salt to root growth compared that of shoot growth (Rahman *et al*., 2001).

The effects of salinity on fresh weight of plumule and radicle

Fresh weight of plumule and radicle was decrease with increasing salt concentrations ($p < 0.05$). The highest weight reduction in fresh weight of radicle was observed in salts $CaCl_2$, $NaHCo_3$ and lower weight reduction after the control treatment in salt NaCl. The maximum weight fresh plumule was observed in Salt $NaHCo_3$, lowest weight in salt, $CaCl_2$, $NaCo_3$, (Table 1 and 2, Fig. 5 and 6).

The effects of salinity on dry weight of plumule and radicle

Plumule dry weight was inversely related to salts concentration. The results indicated that in Cowpea seeds grow distilled water and seeds treated concentrations of salts was significant difference ($p < 0.05$).The greatest reduction weight was observed in salt $NaHCo_3$ (Table 1 and 2 and Fig. 7 and 8). Also, dry weight of radicle was inversely related to salt concentration, but was not significant difference in the 5% level between the different salts. It was relatively less sensitive to salt than plumule dry weight especially at higher salt concentrations.

Table 1. Influence of concentration of solution salts on the Germination and growth parameters in Cowpea

Treatments	Concentration (ds / m)	Germination percentage (%)	Germination rate	Radicle length (cm)	Plumule length (cm)	Fresh weight of Radicle (g)	Fresh weight of Plumule (g)	dry weight of Radicle (g)	dry weight of Plumule (g)
Control	0	89^{a}	0.825^{a}	4.80^{a}	6.50^{a}	0.059^{a}	0.891^{a}	0.032^{a}	0.192^{a}
	2	23.33^{bcdef}	0.592^{abcd}	1.84^{cdef}	3.81^{cdef}	0.032^{bcd}	0.621^{abcd}	0.004^{ab}	0.141^{abcd}
	4	20^{cdef}	0.568^{abcd}	1.08^{gf}	3.60^{cdef}	0.014^{efg}	0.503^{cd}	0.003^{ab}	0.114^{bcd}
$NaHCo_3$	6	15^{efg}	0.381^{cd}	0.718^{gh}	2.50^{fg}	0.013^{efg}	0.394^{d}	0.003^{b}	0.084^{d}
	8	0^{g}	0^{e}	0^{h}	0^{h}	0^{g}	0^{e}	0^{b}	0^{e}
	2	35^{bcd}	0.738^{ab}	2.426^{bcd}	4.74^{bc}	0.042^{b}	0.748^{abc}	0.005^{ab}	0.152^{abc}
	4	31.66^{bcdef}	0.730^{ab}	2.02^{bcdef}	4.67^{bc}	0.037^{bc}	0.720^{abc}	$0/004^{ab}$	0.141^{abcd}
$NaCo_3$	6	18.35^{defg}	0.690^{abc}	1.43^{egf}	3.56^{cdef}	0.018^{def}	0.564^{bcd}	$0/002^{b}$	0.119^{bcd}
	8	13.33^{gf}	0.533^{abcd}	0.583^{gh}	2.62^{efg}	0.014^{efg}	0.458^{cd}	$0/002^{b}$	0.092^{cd}
	2	43.33^{b}	0.615^{abcd}	4.19^{a}	5.20^{b}	0.045^{b}	0.593^{bcd}	$0/028^{a}$	0.131^{bcd}
	4	38.33^{bc}	0.609^{abcd}	2.86^{b}	4.06^{bcd}	0.032^{bcd}	0.501^{cd}	$0/018^{ab}$	0.127^{bcd}
NaCl	6	36.66^{bcd}	0.604^{abcd}	2.65^{bc}	3.89^{bcde}	0.031^{bcd}	0.489^{cd}	$0/010^{ab}$	0.119^{bcd}
	8	26.66^{bcdef}	0.492^{abcd}	2.28^{bcde}	3.09^{defg}	0.025^{cde}	0.468^{cd}	0.006^{ab}	0.117^{bcd}
	2	43.33^{b}	0.793^{a}	2.70^{bc}	4.08^{bcd}	0.030^{bcd}	0.788^{ab}	0.010^{ab}	0.170^{ab}
	4	36.66^{bcde}	0.696^{abc}	2.62^{bc}	3.88^{bcde}	0.026^{cde}	0.745^{abc}	0.008^{ab}	0.163^{ab}
$CaCl_2$	6	33.33^{bcde}	0.628^{abcd}	2.46^{bc}	3.73^{cdef}	0.019^{def}	0.588^{bcd}	0.007^{ab}	0.161^{ab}
	8	18.33^{defg}	0.339^{d}	1.50^{defg}	2^{g}	0.010^{fg}	0.136^{e}	0.002^{b}	0.081^{d}

Table 2. Comparison of salt used on the average germination percentage, germination rate, length, fresh and dry weight (radicle, plumule)

Treatments	Germination percentage (%)	Germinatin rate	Radicle length (cm)	Plumule length (cm)	Fresh weight of Radicle (g)	Fresh weight of Plumule (g)	dry weight of Radicle (g)	dry weight of Plumule (g)
Control	100^{a}	0.712^{a}	4.80^{a}	6.50^{a}	0.059^{a}	0.891^{a}	0.017^{a}	0.192^{a}
NaCl	40.41^{b}	0.580^{ab}	2.99^{b}	4.06^{b}	0.033^{b}	0.512^{bc}	0.015^{a}	0.123^{b}
$CaCl_2$	32.91^{bc}	0.614^{ab}	2.32^{c}	3.42^{b}	0.021^{d}	0.564^{b}	0.007^{a}	0.144^{b}
$NaCo_3$	27.91^{c}	0.673^{a}	1.61^{d}	3.89^{b}	0.028^{b}	0.622^{b}	0.004^{a}	$.126^{b}$
$NaHco_3$	14.58^{d}	0.385^{c}	0.910^{e}	2.48^{c}	0.014^{d}	0.379^{c}	0.002^{a}	0.084^{c}

Description of Tables: Comparison of averages has been done by use of ANOVA, analysis of variance; LSD test ($p < 0.05$) has been done. Letters which are relevant to the comparison of averages are comparable within their treatments.

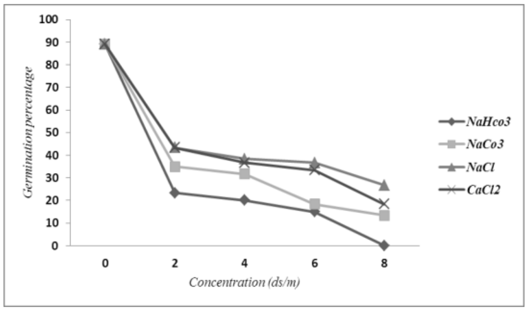

Fig. 1. Effects of the concentrations of salts on germination percentage

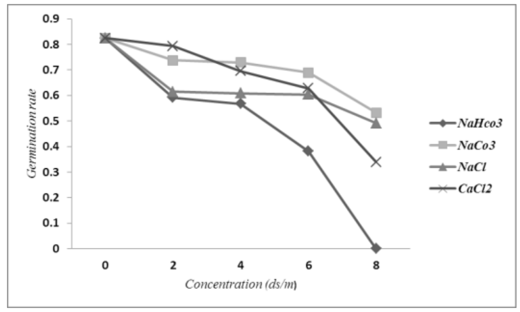

Fig. 2. Effects of the concentrations of salts on germination rate

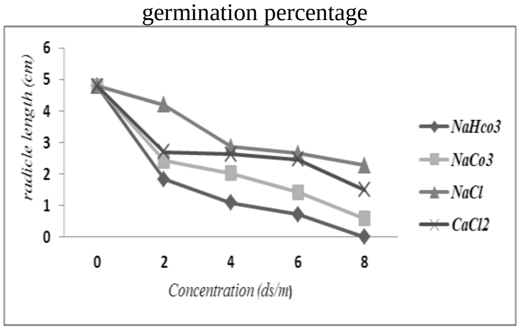

Fig. 3. Effects of the concentrations of salts on the radicle length

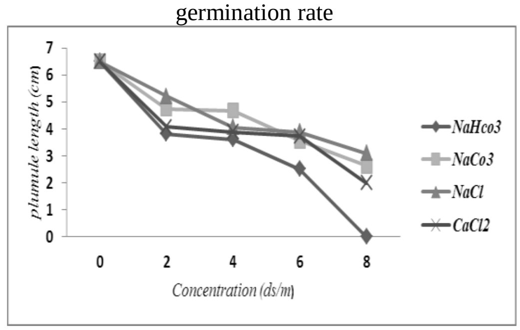

Fig. 4. Effects of the concentrations of salts on the Plumule length

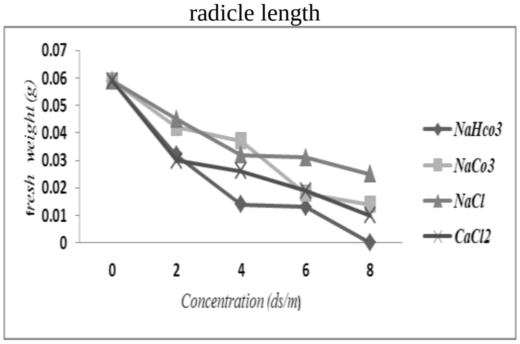

Fig. 5. Effects of the concentrations of salts on fresh weight of radicle

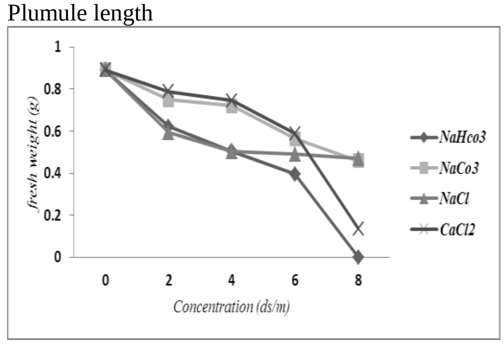

Fig. 6. Effects of the concentrations of salts on fresh weight of Plumule

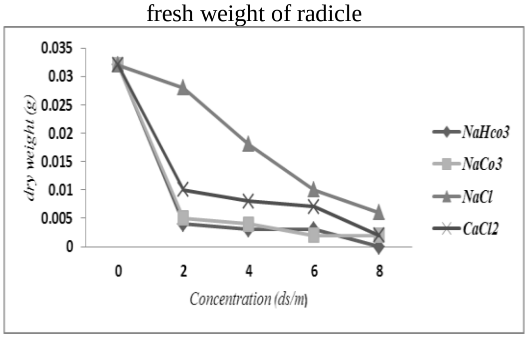

Fig. 7. Effects of the concentrations of salts on dry weight radicle

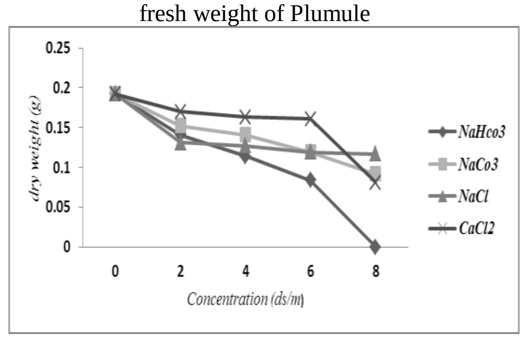

Fig. 8. Effects of the concentrations of salts on dry weight Plumule

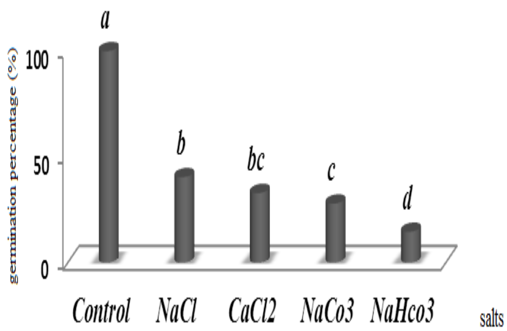

Fig. 9. Effects of different salts on germination percentage

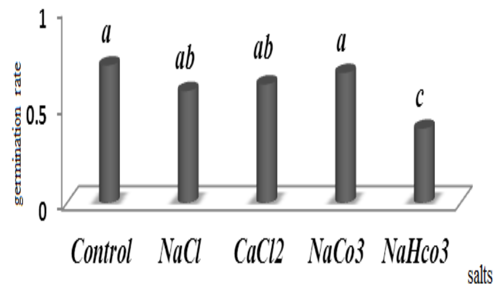

Fig. 10. Effects of different salts on germination rate

Discussions

In this study, effect of salts $CaCl_2$, NaCl, $NaCo_3$, $NaHCo_3$ on germination and growth parameters of Cowpea seeds were investigated. In this study, rate and germination as a criterion for seed germination of Cowpea, and length and fresh and dry weight of sprouts as a criterion the growth of Cowpea sprouts were studied. Results showed that the destructive effects NaHCo3 index measured was more than other salts (Table 1 and 2).The results of this study indicated that the seeds of Cowpea were susceptible to salinity at germination stage. Clyde *et al.* (2006) founded a highly significant reduction of seeds germination of Cowpea due to salinity. The Cowpea (*Vigna unguiculata* L. Walp) showed significant differences in its their tolerance to salinity (Murillo-Amador *et al*., 2006). Generally, the results indicated that the selection of salt tolerant genotypes may have an important role to establish salt adapted cropping systems. According to Greenway and Munns (1980), salt-tolerance in glycophytes associated with the ability to limit uptake and/or transport of saline ions (mainly Na+ and Cl) from the root to aerial parts. Similar results were found in rice, soybean, field bean, adzuki bean, pumpkin, and cucumber (Lo´pez *et al*., 1999), cucumber (Jones *et al*., 1989), and pepper (Chartzoulakis and Klapaki, 2000). Jamil and Rha (2007) observed that shoot length, root lengths and dry weight were decreased with increasing salt stress. It was concluded that germination and early seedling growth of seeds of Cowpea were inhibited by increasing salt concentration. No seed was germinated at 8 ds/m -1 (NaHCo3). Although exists a great amount of literature describing the effects of salinity in adult cowpea plants (Silva *et al*., 2003). However, its effects during seed germination in different cultivars still remain unclear. Due to this fact more studies with salt stressed seeds germination are necessary for the complete elucidation its effect on cowpea development.

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