Available online at www.sciencedirect.com
SCIENCE DIRECT®
Scientia Horticulturae 108 (2006) 423–431
SCIENTIA HORTICULTURAE
www.elsevier.com/locate/scihorti

Effect of NaCl salinity in the genotypic variation of cowpea (*Vigna unguiculata*) during early vegetative growth

Bernardo Murillo-Amador [a,*], Enrique Troyo-Diéguez [a], José Luis García-Hernández [a], Raúl López-Aguilar [a], Narciso Ysac Ávila-Serrano [b], Sergio Zamora-Salgado [b], Edgar Omar Rueda-Puente [c], Cengiz Kaya [d]

[a] *Centro de Investigaciones Biológicas del Noroeste, S.C. Mar Bermejo No. 195, Col. Playa Palo de Santa Rita., La Paz, Baja California Sur 23090, Mexico*
[b] *Universidad Autónoma de Baja California Sur, La Paz, Baja California Sur, Mexico*
[c] *Universidad de Sonora, Campus Santa Ana. Sonora, Mexico*
[d] *Harran University, Faculty of Agriculture, Horticulture Department, Sanliurfa, Turkey*

Received 25 August 2004; received in revised form 25 January 2006; accepted 17 February 2006

Abstract

Twenty-five genotypes of cowpea (*Vigna unguiculata* L. Walp.) were tested for salt-tolerance at the vegetative growth stage in pot in the greenhouse experiments at salinity levels of 0, 85, and 170 mM NaCl. Plant survival was the main criterion for classifying genotypes. Other criteria included the ion concentration (Na^+ and Cl^-) in root and shoot and biomass accumulation. Four local accessions (‘Paceño’, ‘Tardón’, ‘Sonorense’, and ‘Cuarenteño’), three accessions from California (‘CB46’, ‘CB27’, and ‘CB3’), and one accession from the International Institute of Tropical Agriculture (IITA) (‘IT82D-889’) survived at concentrations of both 85 and 170 mM NaCl and were classified as salt-tolerant, while ‘IT96D-666’, ‘IT89KD-288’, and ‘IT93K-734’ from IITA were classified as salt-sensitive. One local accession (‘Sesenteño’), three accessions from IITA (‘PEPH-V Wes-85’, ‘IT86D-719’, and ‘IT95K-1090-12’), and one accession from California (‘CB5’) were classified as moderately salt-tolerant. Eight accessions from IITA (‘IT96D-733’, ‘IT90K-277-2’, ‘IT91K-93-10’, ‘IT91K-118-20’, ‘IT90K-284-2’, ‘IT95K-1088-4’, ‘IT89KD-391’, and ‘IT94K-437-1’) and one from California (‘CB88’) were classified as moderately salt-sensitive. Biomass was affected by both 85 and 170 mM NaCl in all groups of genotypes, however, salt-tolerant and moderately salt-tolerant genotypes showed higher biomass than genotypes classified as moderately salt-sensitive and salt-sensitive. In all genotypes Cl^- concentration was higher in shoots than roots and increased as salinity increased. Similarly Na^+ concentration increased with increasing salinity. However, in salt-tolerant and moderately salt-tolerant genotypes, Na^+ concentration was more in roots than shoots, while in moderately salt-sensitive and salt-sensitive genotypes, Na^+ was higher in shoots than roots.

Keywords: *Vigna unguiculata*; Plant survival; Sodium; Dry matter production

1. Introduction

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 soil salinity is a yield-limiting factor. It is also cultivated as a dryland crop under different climatic conditions ranging from semi-arid to subhumid (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 but less than wheat, barley, sugar beet, or cotton (Hall and Frate, 1996). The effect of salinity on the germination, vegetative growth, or yield of cowpea has been studied (West and Francois, 1982; Imamul Huq and Larher, 1983; Maas and Poss, 1989; Kannan and Ramani, 1988; Plaut et al., 1990; Larcher et al., 1990; Fernandes de Melo et al., 1994; Murillo-Amador et al., 2000, 2001); however, there are

* Corresponding author. Tel.: +52 612 123 84 84x3440; fax: +52 612 123 85 25.
E-mail address: bmurillo04@cibnor.mx (B. Murillo-Amador).

0304-4238/$ – see front matter
doi:10.1016/j.scienta.2006.02.010

no data about screening or selection criteria on the effect of salinity on the early seedling growth. Screens are designed to differentiate genetic effects from random environmental effects, and few attempts have been developed which had resulted in the selection of salt-tolerance varieties. Unfortunately few screening and selection procedures have proven successful for salt-tolerance as changes occur in specific tissues throughout the plant's life cycle (Shannon, 1997). The few screens methods for salt-tolerance have been accomplished on a number of crops, using various parameters including germination and emergence, survival, growth rate, chlorosis or leaf damage, and the genotypic differences within species (Shannon, 1997). According to Flowers and Yeo (1997) developing the procedure may itself be a difficult option, due to the complexity of environment by genotype interactions and ontogenetic drift in the response to salt, but the procedure is conceptually simple: to expose a group of genotypes to salinity, in order to choose the ones that perform the best. On the other hand, screening and selection for any character are desired at developmental stages as early as possible because plant growth is positively correlated with quantity or commercial quality of the marketed product. In this sense, a screening technique was recently developed to screen cowpea genotypes for salt-tolerance during germination and emergence stages (Murillo-Amador et al., 2000, 2001). However, the effectiveness of such screens varies but the main emphasis seems to be in balance with a controlled environment, so that the screening techniques are reliable, against the uncertainty of variation in natural conditions in the field. The objective of this research was to evaluate the effect of salinity on the early vegetative growth of cowpea genotypes and develop a greenhouse screening method, based on the survival percentage, ion composition, and dry matter production which allows a quick and easy-to-measure screening tool for genotypic differences in salinity tolerance.

Table 1
Cowpea genotypes evaluated for the effect of NaCl salinity during vegetative growth

Genotypes	Origin
Paceño	Traditional variety, La Paz, B.C.S., México
Tardón	Traditional variety, San Isidro, B.C.S., México
Sonorense	Traditional variety, Hermosillo, Sonora, México
Cuarenteño	Traditional variety, Todos Santos, B.C.S., México
CB46	California cultivar, University of California, Riverside, USA
CB88	California cultivar, University of California, Riverside, USA
CB27	California cultivar, University of California, Riverside, USA
CB3	California cultivar, University of California, Riverside, USA
CB5	California cultivar, University of California, Riverside, USA
PEPH-V Wes-85	Texas cultivar, University of Texas A&M, USA
IT86D-719	Nigerian breeding line, IITA, Ibadan, Nigeria
IT96D-666	Nigerian breeding line, IITA, Ibadan, Nigeria
IT89KD-288	Nigerian breeding line, IITA, Ibadan, Nigeria
IT82-889	Nigerian breeding line, IITA, Ibadan, Nigeria
IT93K-734	Nigerian breeding line, IITA, Ibadan, Nigeria
IT96D-733	Nigerian breeding line, IITA, Ibadan, Nigeria
IT89KD-391	Nigerian breeding line, IITA, Ibadan, Nigeria
IT90K-277-2	Nigerian breeding line, IITA, Ibadan, Nigeria
IT91K-93-10	Nigerian breeding line, IITA, Ibadan, Nigeria
IT94K-437-1	Nigerian breeding line, IITA, Ibadan, Nigeria
IT95K-1090-12	Nigerian breeding line, IITA, Ibadan, Nigeria
IT95K-1088-4	Nigerian breeding line, IITA, Ibadan, Nigeria
IT91K-118-20	Nigerian breeding line, IITA, Ibadan, Nigeria
IT90K-284-2	Nigerian breeding line, IITA, Ibadan, Nigeria
Sesenteño	Traditional variety, La Paz, B.C.S., México

2. Materials and methods

2.1. Plant material, treatments, and growth conditions

The experiment was conducted under greenhouse with natural conditions, from May to July 2001 at the Centro de Investigaciones Biológicas del Noroeste, S.C. (CIBNOR), 17 km NW of La Paz, Mexico (24°08′N, 110°24′W), located in an arid zone of Baja California Sur. A field screening was done previously, where the 38 genotypes studied were planted at the same time and under the same environmental conditions to get enough seeds with the same vigor and the same lot, selecting 25 genotypes with the same or similar maturity length, color, and other seed parameters (Table 1).

For seedlings emergence seeds were previously disinfected by immersion in calcium hypochlorite solution containing 5% active chloride during 5 min, after they were then washed three times with sterilized distilled water. Afterwards seeds were placed in sterilized peat pellets—Jiffy-7 (Jiffypots, Jiffy Products (N.B.) Ltd., Shippegan, Canada), inside sterilized Petri dishes (150 mm × 15 mm) and were watered with distilled water during seven days. Seedling emergence stage was conducted in a growth chamber (Percival, Model MB-60B, Controlled Environment, Percival Manufacturing Company, USA) at 25 ± 1 °C, 12 h of daylength, and 80% of relative humidity to prevent high evaporation of the saline medium. Seeds were considered to be emerged when the seedling appeared through the jiffypot surface. Three days after emergence, seedlings were moved to a greenhouse and transferred to a plastic pot (13 cm top diameter, 8 cm bottom diameter × 10 cm deep, with one hole in the bottom) filled with inert material (Canadian sphagnum peat moss which is a natural, organic soil conditioner that regulates moisture and air around plant roots for ideal growing conditions; Sunshine, Sun Gro Horticulture Canada Ltd.). We maintained water potential values of −0.1, −0.5, and −0.8 MPa for 0, 85 and 170 mM NaCl, respectively, which was measured weekly psychometrically using a Wescor Model HR-33T microvoltmeter (Wescor Inc., Logan, UT). Eight pots were used for each genotype and each saline treatment with one plant per pot. The salt treatments were: 0, 85, and 170 mM NaCl, which were applied after transferring the pots to a naturally illuminated greenhouse when the seedlings had the primary leaves developed. All plants were watered daily with an excess of appropriate saline solution with nutrients containing (mg/l) 220 N, 40 P, 200 K^+, 140 Ca^{2+}, 42 Mg^{2+}, 4 Fe^{3+}, 1.25 Mn^{4+}, 0.18 B, 0.23 Zn^{2+}, and 0.25 Cu^{2+}. Watered with an excess of solution permitted to flush the pots allowing draining the excess of solution to maintain the level of salinity. All drained solutions were collected to measure the electrical conductivity and verify that the salinity of the treatment solutions and the drained solutions was similar. The pH of all treatments solutions was maintained close to 6.5 by adding H_2SO_4 or KOH.

Average temperature inside greenhouse was 16 °C/33 °C (night/day); humidity was 29% in average during the day and 59% at night. Plants were grown in an average of 12-h photoperiod of 279 ± 23 μmol m^{-2} s^{-1} photosynthetically active radiation. The length of the experiment was 62 days.

2.2. Experimental design and statistical analysis

Experimental design was a factorial treatment set of 25 genotypes and three salinity treatments were arranged in a completely randomized design with four replicates of two plants per replication. Data were subjected to analysis of variance, using the ANOVA and GLM procedure in SAS (SAS, 1990). Also correlation and regression analyses were performed.

2.3. Selection criteria for salt-tolerance classification

The plant survival percentage at 85 and 170 mM NaCl was used to classify the genotypes as salt-tolerant, moderately salt-tolerant, moderately salt-sensitive, and salt-sensitive, following the next criteria: at 170 mM it is expected that at least 30% of plants must survive to considerer the genotypes as salt-tolerant while that the salt-sensitive genotypes are based on the mortality percentage at 85 mM NaCl following the next criteria: when 10% of a total of plants were dead at this saline treatment, the number of dead plants of each genotype were counted; those genotypes with the biggest number of dead plants were chosen and selected as salt-sensitive genotypes. All genotypes were grouped in four categories according to their response to the effects of salinity on plant survival at 85 and 170 mM NaCl following the next criteria: (1) salt-tolerant genotypes, showing >50% plant survival in both 85 and 170 mM NaCl; (2) moderately salt-tolerant, showing >25 and <50% plant survival at 170 mM NaCl and >50% plant survival at 85 mmol NaCl; (3) moderately salt-sensitive, showing >75% plant survival at 85 mM NaCl and 0% plant survival at 170 mM NaCl; (4) salt-sensitive genotypes, showing <70% plant survival at 85 mM NaCl and 0% plant survival at 170 mM NaCl. Finally, to corroborate this classification, Na^+ and Cl^- concentrations in both roots and shoots and dry matter production (biomass) at each group of genotypes were evaluated.

2.4. Tissue analysis

Plants were separated into roots and shoots and dry weight was determined. Length of roots and shoots, number of trifoliate leaves (data not showed), and biomass (dry weight of roots and shoots) were determined when a study plant died in each saline treatment. Before dry weights measurement, plant tissues were rinsed in tap water during 1 min and washed with distilled water. Dry weight was estimated after drying at 80 °C for 48 h. For mineral analysis, dry plant material was ground in a blender (Braun 4-041 Model KSM-2) and digested in a mixture of H_2SO_4 and $HClO_4$ acids (1:10:4). Concentration of Na^+ of the digest was determined by atomic absorption spectrophotometry (Shimadzu AA-660, Shimadzu, Kyoto, Japan). Chloride was extracted in boiling water and determined by ion chromatography (Shimadzu HIC-6A, Shimadzu).

3. Results

3.1. Plant survival

After week of treatment application, the leaves of plants of some genotypes subjected to 170 mM NaCl started showing necrosis and wilting and the plants eventually began to die after 13 days. The primary leaves showed more damage than the first and second trifoliate leaves. After two weeks, the leaves of plants of some genotypes in 85 mM NaCl started showing necrotic spots which slightly spread, wilting and leaves of plants of some genotypes dried up and plants began to die. A gradual death of leaves in both 85 and 170 mM NaCl continued to reach 70% of mortality at 170 mM NaCl, after 62 days of applying this saline treatment, while 10% of mortality at 85 mM NaCl was reached after 45 days. Thirteen of 25 genotypes showed some seedlings at the end of the experiment (62 days) at 170 mM NaCl (Table 2). Seedlings of only 10 genotypes showed mortality percentage of 12.5–62.5% at 85 mM NaCl (Table 2). 'Sonorense', 'CB3',

Table 2
Classification of cowpea genotypes based on the percentage of surviving seedlings at 85 and 170 mM NaCl during vegetative growth

Group 1 salt-tolerant	Surviving (%) at mM NaCl		Group 2 moderately salt-tolerant	Surviving (%) at mM NaCl		Group 3 moderately salt-sensitive	Surviving (%) at mM NaCl		Group 4 salt-sensitive	Surviving (%) at mM NaCl	
	85	170		85	170		85	170		85	170
Sonorense	100.0	100.0	PEPH-V Wes-85	100.0	37.5	IT96D-733	100.0	0.0	IT93K-734	62.5	0.0
CB3	100.0	100.0	IT86D-719	100.0	37.5	IT90K-277-2	100.0	0.0	IT96D-666	50.0	0.0
CB27	100.0	87.5	CB5	100.0	25.0	IT91K-93-10	100.0	0.0	IT89KD-288	37.5	0.0
Cuarenteño	100.0	75.0	IT95K-1090-12	100.0	25.0	IT91K-118-20	100.0	0.0			
CB46	100.0	62.5	Sesenteño	87.5	25.0	IT90K-284-2	100.0	0.0			
Paceño	100.0	62.5				IT95K-1088-4	87.5	0.0			
IT82D-889	87.5	62.5				CB88	87.5	0.0			
Tardón	87.5	50.0				IT89KD-391	87.5	0.0			
						IT94K-437-1	75.0	0.0			

'CB27', 'Cuarenteño', 'CB46', 'Paceño', 'IT82D-889', and 'Tardón' showed more than 50% of surviving seedlings in both 85 and 170 mM NaCl and were classified as salt-tolerant (Table 2). 'PEPH-V Wes-85', 'IT86D-719', 'CB5', 'IT95K-1090-12', and 'Sesenteño' showed 25–49% of seedlings surviving at 170 mM NaCl/L and more than 50% at 85 mM NaCl and these genotypes were classified as moderately salt-tolerant (Table 2). One group of nine genotypes showed more than 75% of seedlings surviving at 85 mM NaCl and 0% at 170 mM NaCl and was classified as moderately salt-sensitive (Table 2). The results suggest that 'CB88' and 'CB5' were salt-tolerant at germination and emergence but 'CB88' was salt-sensitive at the early vegetative growth stage and 'CB5' was moderately salt-tolerant at the early vegetative growth stage. The genotypes classified as salt-sensitive were 'IT93K-734', 'IT96D-666' and 'IT89KD-288', and had less than 70% of seedlings

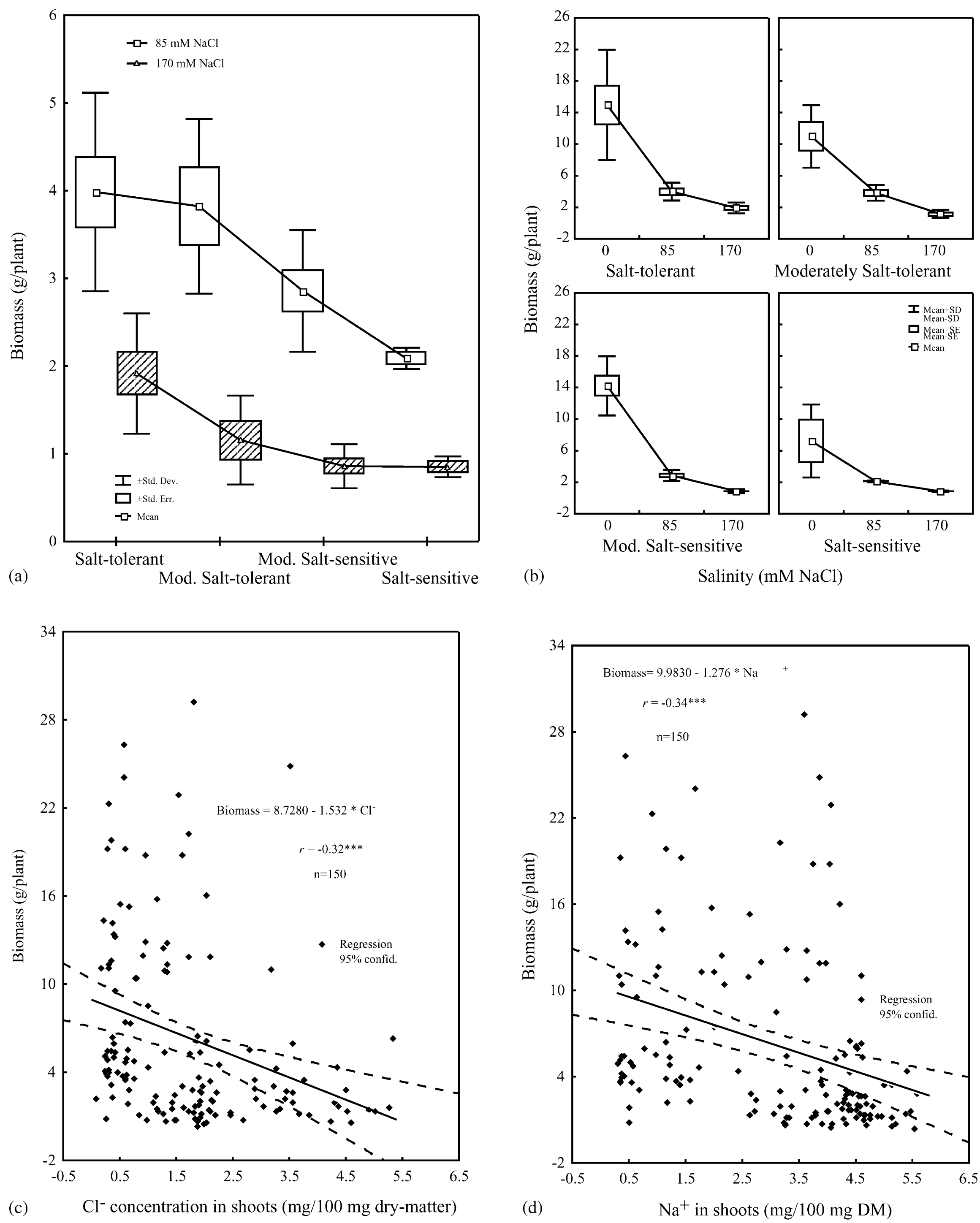

Fig. 1. Genotypic differences of cowpea genotypes under saline treatments on: (a) dry matter production (biomass), (b) effects of external NaCl salinity on biomass of salt-tolerant, moderately salt-tolerant, moderately salt-sensitive, and salt-sensitive, (c) relationship between Cl^- in shoots and biomass, and (d) relationship between Na^+ in shoots and biomass.

surviving at 85 mM NaCl (Table 2). In the present study some plants of several genotypes could grow at moderately high salinity of 170 mM NaCl for few weeks but probably did not survive up to maturity; therefore, further studies in advanced stages of the plant growth are required.

3.2. Biomass

Biomass decreased more severely at 170 mM NaCl than in 85 mM NaCl in all groups of genotypes (Fig. 1(a)) and decreased as salinity increased (Fig. 1(b)). There was a strong and significant linear correlation between either Na^+ or Cl^- concentrations in shoots and biomass with a strong reduction in biomass in response to a significative increase in both Na^+ and Cl^- concentrations in shoots (Fig. 1(c and d)). In all four groups of genotypes, both 85 and 170 mM NaCl affected dry matter production, with an average reduction for all groups of 28 and 10% as compared to the control at 85 and 170 mM NaCl, respectively. Genotypes classified as salt-tolerant and moderately salt-tolerant showed higher biomass than genotypes

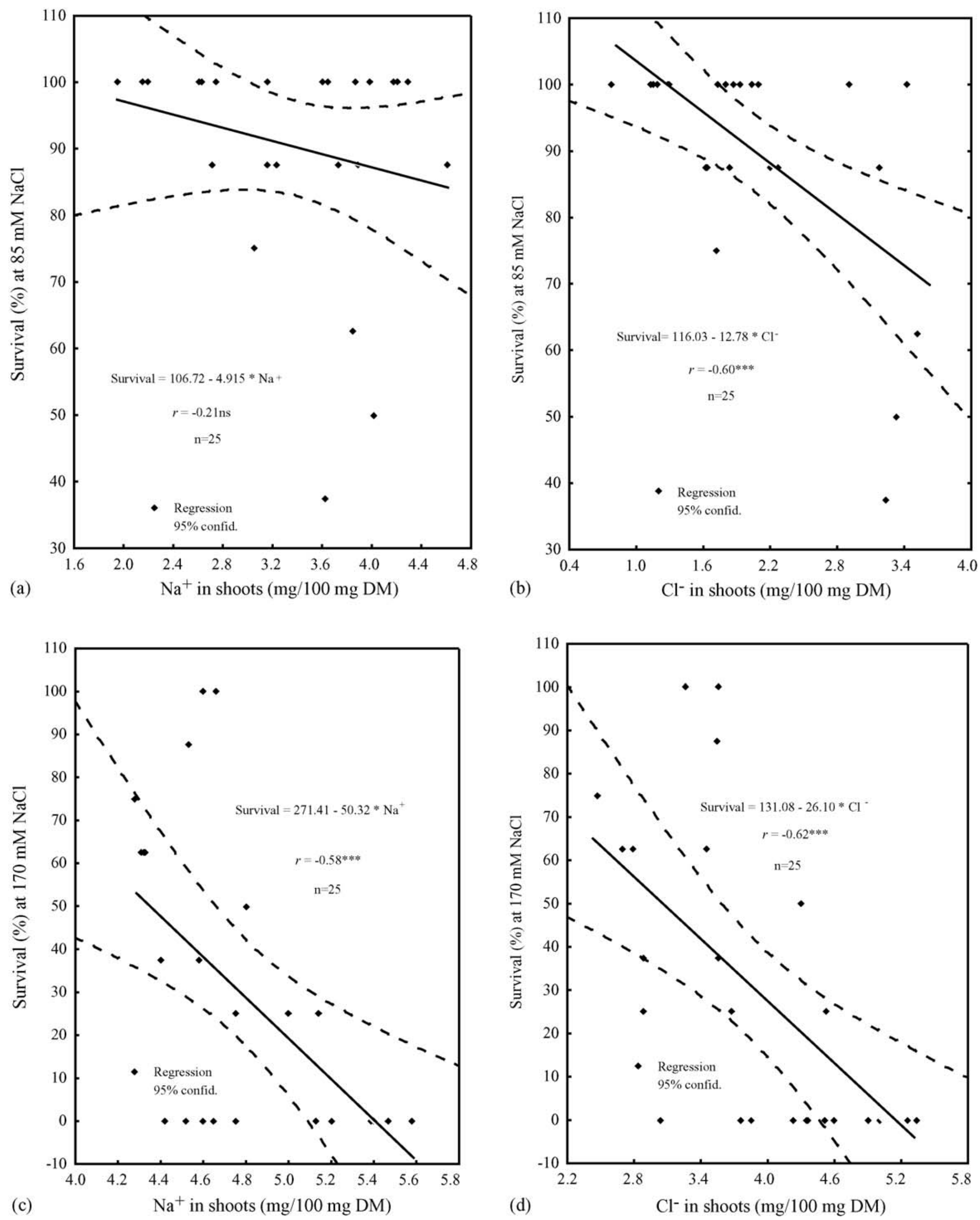

Fig. 2. (a–d) Relationship between sodium and chloride concentration and varietal tolerance in cowpea genotypes under two saline treatments.

classified as moderately salt-sensitive and salt-sensitive in both 85 and 170 mM NaCl.

3.3. Sodium and chloride concentrations associated to survival

The relationship between sodium concentration and seedling survival in cowpea at 85 mM NaCl (45 days after treatment application) was low and not statistically significant ($r = -0.21$, $p = 0.30$, $n = 25$) (Fig. 2(a)). However, Cl^- uptake showed a significant relationship with seedling survival ($r = -0.60$, $p = 0.002$, $n = 25$) (Fig. 2(b)). Both Na^+ and Cl^- uptake at 170 mM NaCl after 62 days of treatment application showed a high relationship with survival (Na^+, $r = -0.58$, $p = 0.003$; Cl^-, $r = -0.62$, $p = 0.0.001$, $n = 25$) (Fig. 2(c and d)). According to the classification of genotypes, Na^+ concentration was higher in roots than shoots in salt-tolerant and moderately salt-tolerant genotypes (Fig. 3(a)), while in moderately salt-sensitive and salt-sensitive genotypes, Na^+ was higher in shoots than roots (Fig. 3(a)). In all grouped genotypes, Na^+ increased in both roots and shoots as salinity increased (Fig. 4(a and b)). On the other hand, Cl^- concentration was higher in shoots than in roots in all grouped genotypes, being higher in most cases in moderately and salt-sensitive genotypes (Fig. 3(b)) and this concentration increased in all grouped genotypes in both roots and shoots as salinity increased (Fig. 4(c and d)). Average Na^+ content in roots + shoots of all groups of genotypes was higher than Cl^- content in roots + shoots (Fig. 3). Because all groups of genotypes showed a higher Cl^- content in shoots than roots (Fig. 3(b)) this shows that cowpea is unable to exclude Cl^- ions from the shoots. The increased concentration of both Na^+ and Cl^- in roots and shoots in all genotypes as salinity increased (Fig. 4) may be related to the reduced rates of photosynthesis because in this study, there was partial closure of stomata as salinity increased (data not shown). The maintenance of a higher dry matter production in both 85 and 170 mM NaCl of salt-tolerant and moderately salt-tolerant genotypes seemed to be a good indicator of the salt-tolerance of this genotype.

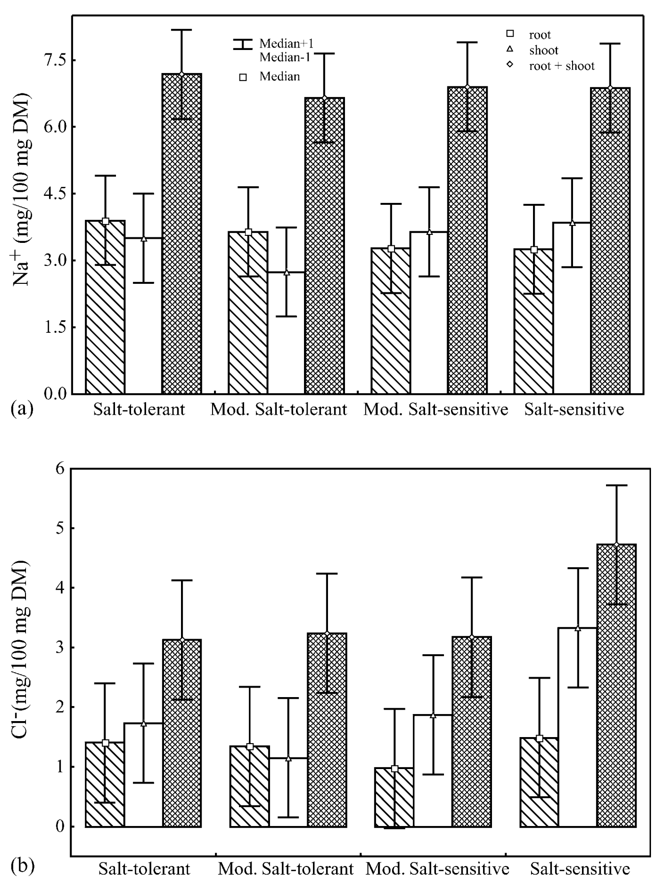

Fig. 3. Effect of NaCl salinity on: (a) Na^+ and (b) Cl^- concentrations in different plant tissues of salt-tolerant, moderately salt-tolerant, moderately salt-sensitive, and salt-sensitive cowpea genotypes.

4. Discussion

4.1. Plant survival

The fact that some genotypes as ‘Paceño’, ‘CB46’, ‘CB88’, ‘CB5’, ‘Cuarenteño’, ‘CB27’, and ‘CB3’ classified as salt-tolerant at germination, emergence (Murillo-Amador et al., 2000, 2001), or in the early vegetative growth stage evidenced the sensitivity of cowpea to salinity changes during its growth and development, and that these differences may include the effect of environmental factors as temperature, soil moisture content, and some other variables as oxygen concentration, light, and soil temperature. Our study evidenced that plant survival was more sensitive to salinity than other stages such as germination or emergence (Murillo-Amador et al., 2000, 2001); this is obvious from plant counts over a prolonged period (45 and 62 days), which showed a high mortality due to build up of salinity. Our results are in agreement with Maas and Grieve (1990) because we found that salt-tolerance during germination, emergence, and seedling growth varies considerably among crops, but also among genotypes of a certain species as cowpea, e.g. ‘Sonorense’ was salt-sensitive at germination and emergence stage but was tolerant at the early vegetative growth stage. Nevertheless, there are some known exceptions including cotton, sugar beets, sorghum, and safflower, which are more sensitive during germination; others crops as barley, corn, rice, and wheat are most sensitive during early seedling growth and then become increasingly tolerant during later stages (Maas, 1986). In the case of cowpea, it is most sensitive to salinity during germination, early vegetative growth or prior the floral bud formation but exhibits most tolerance during pod filling or later stages (Maas and Poss, 1989; Hall and Frate, 1996). In this sense, although plant survival to the farmer has little value because this parameter does not consider economic yield, plant survival on saline soil has been widely used by ecologists to evaluate the tolerance of native species growing on uncultivated lands; indirectly this criterion is potentially useful, because plant breeders can use it as the basis for selecting salt-tolerant lines and cultivars (Maas, 1981).

4.2. Biomass

The results of reduction of dry matter production in the present study are in agreement with Greenway and Munns

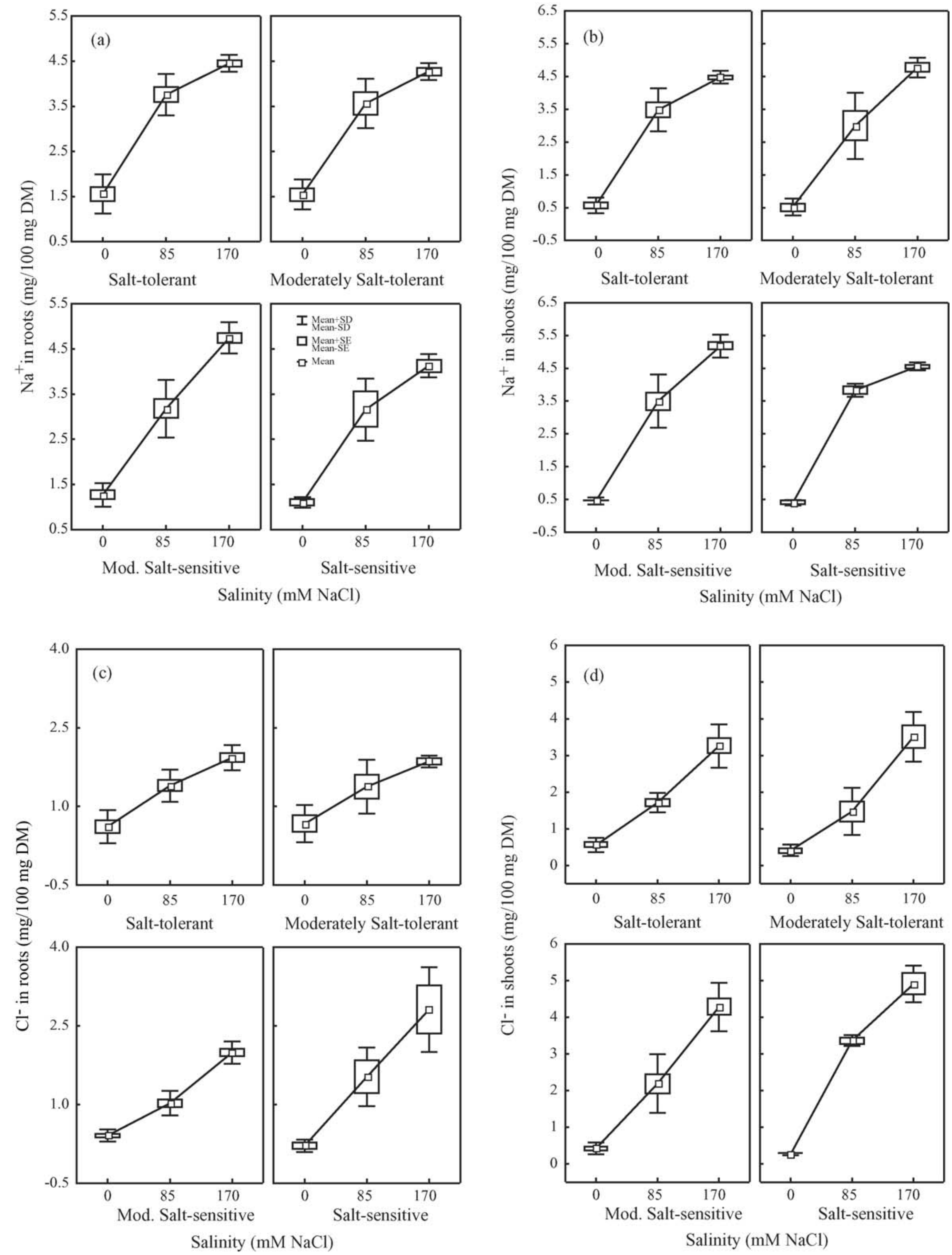

Fig. 4. The effect of external NaCl salinity on: (a) Na^+ in roots, (b) Na^+ in shoots, (c) Cl^- in roots, and (d) Cl^- in shoots of salt-tolerant, moderately salt-tolerant, moderately salt-sensitive, and salt-sensitive cowpea genotypes.

(1980) who showed that these results could be a combined effect of osmotic stress, which is more harmful to plants during the succulence seedling stage and the higher ion uptake (Dumbroff and Cooper, 1974). The differences of biomass production between salt-tolerant and salt-sensitive genotypes confirm that the first criteria based on plants survival enable cowpea genotypes to be ranked for salinity tolerance according to plant survival and dry matter production. Similar results were found by Murillo-Amador et al. (2001) because genotypes tolerant had higher biomass at 170 mM NaCl than genotypes salt-sensitive during emergence stage. The reduction of dry matter as salinity increased (Fig. 1(b)) could be due to a higher accumulation of Na^+ and Cl^- in shoots but the fact that salt-sensitive genotypes showed higher Cl^- shows that seemingly this reduction is due to Cl^- than Na^+ as shown in Fig. 4(c and d), where reduction in biomass is more affected at low Cl^- concentration than higher Na^+ concentration. Similar results were found by West and Francois (1982) who showed that Cl^- was the ion that significantly affected salinity. In this sense, high Cl^- concentrations in shoots are associated with chlorosis and death, and these injuries occur even when Na^+ in the shoots is low, e.g. in avocado (Bingham et al., 1968), grapevines (Bernstein et al., 1969), in fruit tress and many other woody plants (Bernstein, 1975).

4.3. Sodium and chloride concentrations

The results of the present study showed a high variation in both individual and varietal in Na^+ and Cl^- transport in both saline treatments (Fig. 2). According to Greenway and Munns (1980), salt-tolerance in glycophytes is 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 by Flowers and Yeo (1981, 1986), in rice, soybean, field bean, adzuki bean, pumpkin, and cucumber (López et al., 1999), lettuce (Shannon et al., 1983), mungbean (Salim and Pitman, 1988), dicotyledonous halophytes (Glenn and O'Leary, 1984), turfgrasses (Marcum and Murdoch, 1990), sugar beet (Marschner et al., 1981), cucumber (Jones et al., 1989), pepper (Chartzoulakis and Klapaki, 2000), and maize (Schubert and Läuchli, 1990). On the other hand, ion effects have been considered to be related to salt-tolerance (Abel and Mackenzie, 1964; Ashraf et al., 1989). For the relationship between salt-tolerance and ion effects, it was suggested that plant species differ in the degree of Na^+ and Cl^- toxicity affecting their growth. The present study showed significant differences among the groups of genotypes in the mineral distribution in both organs (Fig. 3(a and b)) and the fact that salt-tolerant and moderately salt-tolerant cowpea genotypes sequestered Na^+ mainly in the roots to protect the aerial part agree with the results of Jacoby (1964), Lessani and Marschner (1978), Fernandes de Melo et al. (1994), and consequently, *V. unguiculata*, similar to other beans (Seeman and Critchley, 1985) could be an effective excluder of Na^+ (West and Francois, 1982; Fernandes de Melo et al., 1994). The results showed that moderately salt-sensitive and salt-sensitive genotypes increased Na^+ content in shoots, so these genotypes could be considered as includer of Na^+. Although in the present study Na^+ content was higher than Cl^- in both organs (Fig. 3), the salt-tolerant and moderately salt-tolerant genotypes seemed to prevent the transport of Na^+ to the aerial part in order to avoid the deleterious effects of salt on plant metabolism such as demonstrated in other species by Marschner (1986) and Fernandes de Melo et al. (1994).

In conclusion, the present study confirms the utility of plant survival as an early screening tool for salt-tolerance and that with the genetic variability among cowpea genotypes, it is possible to find salt-tolerant and salt-sensitive genotypes, based on the plant survival, ions content, and dry matter production. There are wide differences in responses to salinity among the genotypes under study and previous growth stages, particularly between the genotypes from different origin (Nigeria, USA, and México). However, our results provide guidelines for the selection of salt-tolerant cowpea genotypes and this information is relevant and very important to breeders and plant physiologists interested in improving salt-tolerance of cowpea and to the farmers that grow cowpea in the areas where the soil and groundwater have high content of NaCl, such as in the northwest region of Mexico. A refinement of current screening tool could be desirable to facilitate germplasm evaluation.

Acknowledgments

This research was supported by grant ZA1.1. from CIBNOR, S.C. The authors greatly appreciate the technical assistance of Alvaro González, Lidia Hirales, Carmen Mercado, and Mario Benson.

References

Abel, G.H., Mackenzie, A.J., 1964. Salt tolerance of soybean varieties (*Glycine max* (L.) Merril) during germination and later growth. Crop Sci. 4, 157–161.

Ashraf, M., McNeilly, T., Bradshaw, A.D., 1989. The potential for evolution of tolerance to NaCl, $CaCl_2$, $MgCl_2$ and seawater in four grass species. New Phytol. 112, 245–254.

Bernstein, L., Ehlig, C.F., Clark, R.A., 1969. Effect of grape rootstocks on chloride accumulation in leaves. J. Am. Soc. Hortic. Sci. 94, 584–590.

Bernstein, L., 1975. Effects of salinity and sodicity on plant growth. Annu. Rev. Phytopathol. 13, 295–312.

Bingham, F.T., Fenn, L.B., Oertli, J.J., 1968. A sand culture study of chloride toxicity to mature avocado trees. Proc. Soil Sci. Soc. Am. 32, 249–252.

Chartzoulakis, K., Klapaki, G., 2000. Response of two greenhouse pepper hybrids to NaCl salinity during different growth stages. Sci. Hortic. 86, 247–260.

Dumbroff, E.B., Cooper, A., 1974. Effects of salt stress applied in balanced nutrient solutions at several stages during growth of tomato. Bot. Gaz. 13, 219–224.

Fernandes de Melo, D., Jolivet, Y., Rocha, A.F., Gomes Filho, E., Silva, L.M., Dizengremel, P., 1994. Effect of salt stress on mitocondrial energy metabolism of *Vigna unguiculata* cultivars differing in NaCl tolerance. Plant Physiol. Biochem. 32, 405–412.

Flowers, T.J., Yeo, A.R., 1981. Variability in the resistance of sodium chloride salinity within rice varieties. New Phytol. 88, 363–373.

Flowers, T.J., Yeo, A.R., 1986. Ion relations of plants under drought and salinity. Aust. J. Plant Physiol. 13, 75–91.

Flowers, T.J., Yeo, A.R., 1997. Breeding for salt resistance in plants. In: Jaiwal, P.K., Singh, R.P., Gulati, A. (Eds.), Strategies for Improving Salt Tolerance in Higher Plants. Science Publishers, Inc., pp. 247–264.

Glenn, E.P., O'Leary, J.W., 1984. Relationship between salt accumulation and water content of dicotyledonous halophytes. Plant, Cell Environ. 7, 253–261.

Greenway, H., Munns, H., 1980. Mechanisms of salt tolerance in nonhalophytes. Annu. Rev. Plant Physiol. 31, 149–190.

Hall, E.A., Frate, A.C., 1996. Blackeye Bean Production in California. University of California, Division of Agriculture and Natural Resources, 23 pp. (Publication 21518).

Imamul Huq, S.M., Larher, F., 1983. Osmoregulation in higher plants: effects of maintaining a constant Na:Ca ratio on the growth, ion balance and organic solute status of NaCl stressed cowpea (*Vigna sinensis* L.) Z. Pflanzenernaehr Bd. 113, 163–176.

Jacoby, B., 1964. Function of bean roots and stems in sodium retention. Plant Physiol. 39, 445–449.

Jones, R.W., Pike, L.M., Yourman, L.F., 1989. Salinity influences cucumber growth and yield. J. Am. Soc. Hortic. Sci. 114, 547–551.

Kannan, S., Ramani, S., 1988. Evaluation of salt tolerance in cowpea and tobacco: effects of NaCl on growth, relative turgidity and photosynthesis. J. Plant Nutr. 11, 435–448.

Larcher, W., Wagner, J., Thammathaworn, A., 1990. Effects of superimposed temperature stress on in vivo chlorophyll fluorescence of *Vigna unguiculata* under saline stress. J. Plant Physiol. 136, 92–102.

Lessani, H., Marschner, H., 1978. Relation between salt tolerance and long distance transport of sodium and chloride in various crop species. Aust. J. Plant Physiol. 5, 27–37.

López, A.R., Yamada, S., Yamanouchi, M., 1999. Comparison of sodium uptake by and transport in detached plant parts among several crops. Soil Sci. Plant Nutr. 45, 659–668.

Lush, W.M., Rawson, H.M., 1979. Effects of domestication and region of origin on leaf gas exchange in cowpea (*Vigna unguiculata* (L.) Walp.) Photosynthetica 13, 419.

Maas, E.V., 1981. Salinity and crop productivity. California Plant and Soil Conference. New Horizons in Agriculture. Sponsored by California Chapter American Society of Agronomy, January 28–30. Sacramento Inn, Sacramento, CA, 10 pp.

Maas, E.V., 1986. Salt tolerance of plants. Appl. Agric. Res. 1, 12–26.

Maas, E.V., Poss, J.A., 1989. Salt sensitive of cowpea at various growth stages. Irrig. Sci. 10, 313–320.

Maas, E.V., Grieve, C.M., 1990. Salt tolerance of plants at different growth stages. In: Proceedings of the International Conference, Tando Jam, Pakistan, January 7–11, p. 27.

Marcum, K.B., Murdoch, C.L., 1990. Growth responses, ion relations, and osmotic adaptations of eleven C_4 turfgrasses to salinity. Agron. J. 82, 892–896.

Marschner, H., Kylin, A., Kuiper, P.J.C., 1981. Differences in salt tolerance of three sugar beet genotypes. Physiol. Plant. 51, 234–238.

Marschner, H., 1986. Beneficial mineral elements. In: Marschner, H. (Ed.), Mineral Nutrition of Higher Plants, 10. Academic Press, London, pp. 341–368.

Murillo-Amador, B., Troyo-Diéguez, E., Jones, H.G., Ayala-Chairez, F., Tinoco-Ojanguren, C.L., López-Cortés, A., 2000. Screening and classification of cowpea genotypes for salt tolerance during germination. PYTON Int. J. Exp. Bot. 67, 71–84.

Murillo-Amador, B., Troyo-Diéguez, E., López-Cortés, A., Jones, H.G., Ayala-Chairez, F., Tinoco-Ojanguren, C.L., 2001. Salt tolerance of cowpea genotypes in the emergence stage. Aust. J. Exp. Agric. 41, 1–8.

Plaut, Z., Grieve, C.M., Maas, E.V., 1990. Salinity effects on CO_2 assimilation and diffusive conductance of cowpea leaves. Physiol. Plant. 79, 31–38.

Rachie, K.O., Roberts, L.M., 1974. Grain legumes of the lowland tropics. Adv. Agron. 26, 62–67.

Salim, M., Pitman, M.G., 1988. Salinity tolerance of mungbean (*Vigna radiata* L.) seed production. Biol. Plant. 30, 53–57.

SAS, 1990. SAS/STAT. In: User's Guide. Statistics, SAS, Institute, Cary, NC, EEUU, 1028 pp.

Seeman, J.D., Critchley, C., 1985. Effects of salt stress on the growth, ion content, stomatal behavior and photosynthetic capacity of a salt-sensitive species, *Phaseolus vulgaris* L. Planta 164, 151–162.

Schubert, S., Läuchli, A., 1990. Sodium exclusion mechanisms at the root surface of two maize cultivars. Plant Soil 123, 205–209.

Shannon, M.C., McVreight, J.D., Draper, J.H., 1983. Screening tests for salt tolerance in lettuce. J. Am. Soc. Hortic. Sci. 108, 225–230.

Shannon, M.C., 1997. Genetics of salt tolerance in higher plants. In: Jaiwal, P.K., Singh, R.P., Gulati, A. (Eds.), Strategies for Improving Salt Tolerance in Higher Plants. Science Publishers, Inc., pp. 265–288.

Turk, K.J., Hall, A.E., Asbell, C.W., 1980. Drought adaptation of cowpea. I. Influence of drought on seed yield. Agron. J. 72, 413–420.

Vasquez-Tello, A., Zuily-Fodil, Y., Pham-Thi, A.T., Vieira-Da Silva, J.B., 1990. Electrolyte and Pi leakages and soluble sugar content as physiological tests for screening resistance to water stress in *Phaseolus* and *Vigna* species. J. Exp. Bot. 41, 827–832.

West, D.W., Francois, L.E., 1982. Effects of salinity on germination, growth and yield of cowpea. Irrig. Sci. 3, 169–175.