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ScienceDirect
Environmental and Experimental Botany
Environmental and Experimental Botany 58 (2006) 188–196
www.elsevier.com/locate/envexpbot

Effects of foliar application of calcium nitrate on growth and physiological attributes of cowpea (*Vigna unguiculata* L. Walp.) grown under salt stress

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Received 12 March 2005; received in revised form 22 June 2005; accepted 30 August 2005

Abstract

Cowpea plants were grown in a glasshouse pot experiment to investigate the effects of NaCl salinity stress and foliar applications of $Ca(NO_3)_2$. The plants were subjected to the following four treatments: (1) control (nutrient solution alone), (2) 10 mmol L^{-1} $Ca(NO_3)_2$ as a foliar application + nutrient solution (FA + C), (3) 50 mmol L^{-1} NaCl + nutrient solution (NA + C), and (4) 50 mmol L^{-1} NaCl + 10 mmol L^{-1} $Ca(NO_3)_2$ as a foliar application + nutrient solution (NA + FA + C) twice weekly. The results showed that salt-stressed plants had less dry matter in the root and shoot, the concentrations of sodium and chloride in both plant organs increased, while those of Ca^{2+}, Mg^{2+}, and K^+ decreased in the high NaCl treatment. No significant differences in stomatal conductance, transpiration, net photosynthesis, and intercellular CO_2 were noted among treatments; hence, none of these variables was improved with the foliar $Ca(NO_3)_2$ sprays. However, chlorophyll fluorescence parameters of the NaCl-stressed plants had higher values following foliar $Ca(NO_3)_2$ sprays, suggesting that the spray was effective in partially alleviating adverse effects of salinity on these parameters. In conclusion, our overall results did not support the hypothesis that supplemental calcium would ameliorate the inhibitory effects of NaCl stress in cowpea plants.

Keywords: Salinity; Calcium nitrate; Foliar application; *Vigna unguiculata*

1. Introduction

Salinity is the basic environmental factor accounting for decreased crop productivity in many geographic areas, mainly in arid and semi-arid regions (Greenway and Munns, 1980). The total salt concentration of the nutrient solution has a strong impact on the commercial yield of horticultural crops in hydroponics (Sonneveld et al., 1999). The restriction of plant growth and productivity due to salinity is especially acute in arid and semi-arid regions (Kuznetsov and Shevyakova, 1997). Response to elevated salt may vary considerably among plant species as a function of their inherent salt tolerance (Savvas and Lenz, 2000). Salinity effects on plants are complex. The injurious effects of salinity are associated with water deficits, ionic imbalance, mineral nutrition, stomata behavior, photosynthetic efficiency, and carbon allocation and utilization (Greenway and Munns, 1980; Bohnert et al., 1995). The initial and primary effect of salinity, especially at moderate salinity concentrations, is due to its osmotic effects (Munns and Termaat, 1986; Jacoby, 1994). At the

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doi:10.1016/j.envexpbot.2005.08.003

whole plant level, ion concentrations in plant tissues increase as a result of salinity stress. Ion toxicity or nutrition deficiency could be caused by over dominance of a specific ion (Bernstein et al., 1974). Salinity poses several problems for plant growth and development, especially for glycophytes, by inducing physiological dysfunction (Shannon et al., 1994). Exposure to NaCl salinity affects water and ion transport processes in plants, which may change the nutritional status and ion balance (Läuchli and Epstein, 1990). These disorders may result from the effect of salinity on nutrient availability, competitive uptake, transport, or partitioning within the plant (Grattan and Grieve, 1999). Therefore, many experiments have been designed to study the effects of salinity on growth parameters and mineral nutrition of commercial crops, such as pepper (Chartzoulakis and Klapaki, 2000) and tomato (Pérez-Alfocea et al., 1996; Kaya et al., 2001). Salinity affects the crop during vegetative and reproductive stages; therefore, causes reductions in both dry biomass and crop yield (Aslam et al., 1993).

In the short term, salinity of water resources can be managed to optimize yield by maintaining salt stress at acceptable levels, depending on crop, soil type, and quality of irrigation water. In the long term, plant scientists may identify specific cellular mechanisms that account for yield reduction and then attempt to use natural selection or bioengineering to increase a plant's salt tolerance (Dalton et al., 2001). Investigations on tolerance of saline environments frequently point to restricted ion accumulation and organic solutes synthesis as major adaptations leading to salt resistance in glycophytes (Greenway and Munns, 1980). Moreover, there are multiple genes that seem to act in concert to increase salinity tolerance; and certain proteins involved in salinity stress protection have been recognized (Bohnert and Jensen, 1996).

Competition and interaction between Cl^- and NO_3, Na^+ and Ca^{2+} in the substrate as well as within the plant frequently lead to ion imbalances that may result in nutrient deficiencies (Grieve and Shannon, 1999). Other workers have linked NaCl stress with macronutrient deficiencies. For example, high NaCl concentration has been shown to induce calcium and nitrogen deficiencies in tomato and cucumber (Cerda and Martinez, 1998), wheat and barley (Ehret et al., 1990), maize (Evlagon et al., 1990), and tomato (Navarro et al., 2000). Salinity dominated by Na^+ salts not only reduces Ca^{2+} availability, but also reduces Ca^{2+} transport and mobility to growing regions of the plant, which affects the quality of both vegetative and reproductive organs. Salinity can directly affect nutrient uptake, such as Na^+ reducing Ca^{2+} uptake or Cl^- reducing NO_3 uptake (Grattan and Grieve, 1999).

Calcium has been shown to ameliorate adverse effects of salinity on plants (Ehret et al., 1990). Calcium is well known to have regulatory roles in metabolism (Cramer et al., 1986), and sodium ions may compete with calcium ions for membrane-binding sites. Therefore, it has been hypothesized that high calcium levels can protect the cell membrane from the adverse effects of salinity (Busch, 1995).

Although many studies show that salinity reduces nutrient uptake and accumulation or affects nutrient partitioning within the plant, there is little evidence to suggest that adding nutrients at levels above those considered optimal in non-saline environments improves crop yield (Grattan and Grieve, 1999).

Breeding for tolerance to salinity in crops has usually been limited by a lack of reliable traits for selection (Shannon, 1985; Noble and Rogers, 1992). Multiple genes seem to act in concert to increase salinity tolerance, and certain proteins involved in salinity stress protection have also been recognized (Bohnert and Jensen, 1996). An alternative strategy for coping with salinity could attempt to supplement Ca^{2+} and N where the growth medium is known to be, or may become, saline at some time during the crop growth cycle.

A pot experiment under greenhouse conditions was conducted. The objective was to study the effects of supplementing calcium nitrate via leaf sprays to investigate how to minimize the salt-induced deficiency in the cowpea plant through foliar $Ca(NO_3)_2$ application, evaluating the effects of root zone salinity on minerals and other growth and physiological parameters to study the effects of foliar sprays of calcium nitrate on salt-stressed and unstressed cowpea plants.

2. Material and methods

2.1. Plant material, treatments, and growth conditions

This experiment was conducted in the research area of the Scottish Crop Research Institute (SCRI), Dundee, Scotland, United Kingdom from February 27 to April 12, 2004. Cowpea (*Vigna unguiculata* L. Walp.) cv "Paceño" was grown in washed sand culture in pots under greenhouse conditions. Seeds were disinfected prior to sowing by immersing in calcium hypochlorite solution containing 5% active chloride for 5 min, and then washed three times with sterilized distilled water. Once seedlings emerged, they were watered with a commercial nutrient solution (20–20–20 N–P–K, Sangral, soluble fertilizer, William Sinclair Horticulture Ltd. Firth Road, Lincoln LN6 7AH, United Kingdom) at a dilution of 1:200. Treatments were initiated when the plants reached the first leaf stage, with a height of about 18 cm. The treatments were (1) control (nutrient solution alone), (2) 10 mmol L^{-1} $Ca(NO_3)_2$ as a foliar spray + nutrient solution (FA + C), (3) 50 mmol L^{-1} NaCl + nutrient solution (NA + C), and (4) 50 mmol L^{-1} NaCl + 10 mmol L^{-1} $Ca(NO_3)_2$ as a foliar spray + nutrient solution (NA + FA + C). Solution of $Ca(NO_3)_2$ was sprayed twice a week on the whole shoot of plants as a foliar spray. The volume of sprayed solution ranged from 6 to 20 mL per plant each time, depending on plant size or development. De-ionized water was sprayed as a control. The surface of the pot was covered with black plastic to minimize evaporation and to prevent algal growth and contamination by foliar sprays. The NaCl concentration was gradually elevated by 25 mmol L^{-1} daily until the desired

concentrations were reached. The composition of nutrient solution was (mg L^{-1}): 270 N, 31 P, 234 K, 200 Ca, 64 S, 48 Mg, 2.8 Fe, 1.2 Na, 0.5 Mn, 0.5 B, 0.02 Cu, 0.05 Zn, and 0.01 Mo. The pH of the solution was maintained close to 6.5 by adding H_2SO_4 or KOH. All plants were watered daily with an excess application of appropriate solutions to flush the pots, and drain the excess solution to maintain the level of salinity. The drained solution was collected to measure the electrical conductivity and verify that the salinity of the treatment solutions and the drained solutions were similar.

2.2. Physiological variables

Net photosynthesis (Pn, μmol m^2 s^{-1}), transpiration rate (E, μmol m^2 s^{-1}), stomatal conductance (gs, mmol m^2 s^{-1}), and intercellular CO_2 (μmol mol^{-1}) were measured on fully expanded leaves at 11, 15, 18, 22, and 25 days after starting the treatments. Pn, E, gs, and Ci were measured with a portable system (Ciras-1, PP system, Hitchin, Herts SG5 1 RT, United Kingdom). Succulence (S) was determined and calculated according the equation:

$$S = \frac{T_M - D_M}{D_M} \quad (1)$$

where T_M and D_M correspond to leaf turgid mass and dry mass, respectively.

2.3. Leaf relative water content

Leaf relative water content (LRWC) was calculated based on the methods of Larqué-Saavedra and Trejo-López (1990). One leave was collected from the mid-section of two plants per replicate to minimize age effects. The leaves were removed from the stem and then five disks of 1.5 cm^2 per leaf were obtained using a hole punch; then the disks were weighted to obtain fresh mass (F_M). In order to determine turgid mass (T_M), disks were floated in distilled water inside a closed Petri dish. During the imbibition period, leaf samples were weighted periodically, after gently wiping the water from the leaf surface with tissue paper until a steady state was achieved. To obtain dry mass (D_M), at the end of the imbibition period, leaf disks were placed in a pre-heated oven at 80 °C for 48 h. Values of F_M, T_M, and D_M were used to calculate LRWC using the equation:

$$\mathrm{LRWC}(\%) = \frac{F_M - D_M}{T_M - D_M} \times 100 \quad (2)$$

2.4. Chlorophyll fluorescence

Chlorophyll fluorescence (CF) parameters, minimal fluorescence (F_o), maximal fluorescence (F_m), variable fluorescence (F_v), and the ratio F_v/F_m of fully expanded leaves of two plants per treatment and replication were measured in vivo 0.5 h after darkness adaptation of the leaves, using a portable fluorometer plant efficiency analyzer (Hansatech Instruments, King's Lynn, Norfol, United Kingdom).

2.5. Ion uptake and transport

Specific absorption rate (SAR) of ions was calculated using the equation described by Hunt (1978):

$$\mathrm{SAR} = \left[\frac{M_2 - M_1}{t_2 - t_1}\right] \times \left[\frac{\ln \mathrm{Rw}_2 - \ln \mathrm{Rw}_1}{\mathrm{Rw}_2 - \mathrm{Rw}_1}\right] \quad (3)$$

where t_1 and t_2 correspond to the harvest times, M_1 and M_2 to the ion content of four whole plants at t_1 and t_2, respectively, and Rw_1 and Rw_2 to the dry weight of the root system of four plants. Similar equations were used to calculate the net transport rate (NTR) from the root to shoot of various ions. In these cases, M refers to the specified ion contained in the shoot.

Specific utilization rate (SUR) of ions expressed as the rate of dry weight increment per unit of absorbed nutrient was calculated using the equation described by Hunt (1978):

$$\mathrm{SUR} = \left[\frac{W_2 - W_1}{t_2 - t_1}\right] \times \left[\frac{\ln M_2 - \ln M_1}{M_2 - M_1}\right] \quad (4)$$

where t_1 and t_2 correspond to the harvest times, M_1 and M_2 to the ion content of four whole plants at t_1 and t_2, respectively, and W_1 and W_2 to the dry weight of the shoot system of four plants.

2.6. Ion concentration and determination of dry weight

Four randomly chosen plants per replicate were divided into shoots and roots, washed with distilled water to remove dust and other residues, such as foliar spray of $Ca(NO_3)_2$, and dried in an oven at 80 °C for 48 h to determine dry weights. The dried tissues (shoots and roots) were finely ground and stored in paper bags. The concentration of Na^+, Ca^{2+}, Mg^{2+}, and K^+ was determined by atomic absorption spectrophotometer (Shimadzu AA-660, Shimadzu, Kyoto, Japan) after digestion with H_2SO_4, HNO_3, and $HClO_4$. Chloride was extracted in boiling water and the concentration was determined by ion chromatography (Shimadzu HIC-6A, Shimadzu, Kyoto, Japan).

2.7. Experimental design and statistical analysis

Each treatment was replicated four times in a completely randomized design and each replicate had 10 plants (40 plants per treatment). Data were analyzed with ANOVA following the GLM procedures in SAS (1988). Differences among means of treatments were compared by Tukey's multiple range test at the 0.05 confidence level. When appropriate, the relative water content of leaves was subjected to arcsine transformation according to Sokal and Rohlf (1988).

Table 1
Effect of NaCl (50 mmol) with and without foliar application of calcium nitrate on dry matter, root/shoot ratio, leaf relative water content, and succulence in cowpea

Treatment	Shoot DM (g/plant)	Whole plant DM (g/plant)	Root/shoot ratio	Leaf relative water content (%)	Succulence
C	27.38 ab	30.16 ab	0.176 b	77.43 ab	11.16 ± 1.17
FA + C	29.41 a	33.50 a	0.127 c	81.33 a	10.66 ± 0.75
NA + C	18.56 c	21.59 b	0.211 a	77.45 ab	11.30 ± 1.08
NA + FA + C	20.60 bc	24.16 ab	0.169 b	69.45 b	11.41 ± 1.47

Means of four plants per replication. Means followed by the same letter in each column are not significantly different (Tukey $P = 0.05$). C: control; FA + C: foliar treatment; NA + C: sodium treatment; NA + FA + C: sodium + foliar treatment. Each value of succulence is the mean ± S.D. of two leaves per replication.

3. Results

3.1. Plant growth and leaf relative water content

Significant differences were observed among treatments for shoot and whole plant dry matter production. Both decreased compared with the FA + C and control (C) treatments, when a high NaCl (50 mmol L^{-1}) concentration was applied separately (Table 1). The ratio of root to shoot showed higher values in the salt treatment (NA + C) than in other treatments where FA + C had the lowest values. Relative water content of leaves showed significant differences among treatments, and varied in the order: FA + C followed by NA + C, C, and NA + FA + C (Table 1).

3.2. Physiological variables

Although succulence did not show significant differences among treatments, higher values were found in the NA + FA + C treatment followed by the NA + FA, C, and FA + C treatments (Table 1). The data on physiological variables are shown in Fig. 1. None of these variables differed significantly among treatments. However, transpiration and stomatal conductance in plants under NA + FA + C treatment were reduced at 15 and 18 days after starting treatments; plants under FA + C were reduced at 11 and 25 days after treatments; and plants under control were reduced at 22 days. On the other hand, both photosynthesis and intercellular CO_2 maintained reduced rates during the first 22 days, increasing at 25 days. Plants in the FA + C treatment showed lower values in both cases on most days after treatments, while those under NA + C showed higher values in most cases.

3.3. Chlorophyll fluorescence

Significant differences in the initial fluorescence (F_o), maximal fluorescence (F_m), and variable fluorescence (F_v) were noted among treatments, while the quantum yield of PSII, as indicated by F_v/F_m in the dark, was not affected by the treatments (Table 2). All chlorophyll fluorescence parameters showed higher values with foliar $Ca(NO_3)_2$ sprays on the NaCl-stressed plants (NA + FA + C treatment), followed in some cases by the control (C), (F_o), or NA + C treatment (F_m and F_v).

3.4. Ion concentration

Significant differences were also observed among treatments in the concentration of Ca^{2+}, Mg^{2+}, K^+, Na^+, and Cl^- in roots and shoots (except Ca^{2+} in shoots). Sodium and chloride concentrations increased significantly in both roots and shoots in the presence of NaCl and remained significantly higher than in the control or when foliar $Ca(NO_3)_2$ was applied. Values of Na^+ and Cl^- concentrations in roots and shoots in non-NaCl-stressed plants were lower in most cases with foliar $Ca(NO_3)_2$ applications; in NaCl-stressed plants, foliar $Ca(NO_3)_2$ application lowered shoot chloride only (Table 3). Concentrations of Ca^{2+} in roots decreased in the presence of NaCl. Similar results were observed for Mg^{2+} and K^+ concentrations in roots and shoots, which also decreased in most cases in the presence of NaCl, especially when foliar $Ca(NO_3)_2$ was applied in NaCl-stressed plants (Table 3).

3.5. Ion uptake, transport, and utilization

The specific absorption rate of Ca^{2+}, Mg^{2+}, Na^+, and Cl^- differed significantly among treatments. From the data on ion concentrations (Table 4), the SAR of Ca^{2+} and Mg^{2+} during the 19-day period was higher in C and FA + C treatments and decreased in the presence of NaCl, while the SAR of Na^+ and Cl^- were higher in NA + C and NA + FA + C treatments. The ion transport rate to shoots also varied significantly among treatments (Table 4). There were marked differences among treatments in the specific utilization rate for Ca^{2+}, Mg^{2+}, K^+, Na^+, and Cl^-, and the foliar spray with $Ca(NO_3)_2$ increased the SUR of Ca^{2+}, Mg^{2+}, K^+, Na^+, and Cl^- in non-stressed plants and for Ca^{2+}, Mg^{2+}, and K^+ in stressed plants (Table 4).

Table 2
Effect of NaCl (50 mmol) with and without foliar application of calcium nitrate on chlorophyll fluorescence parameters in cowpea

Treatment	F_o	F_m	F_v	F_v/F_m
C	311.66 ab	1846.75 b	1535.08 c	0.83 a
FA + C	282.00 b	1943.91 b	1661.91 bc	0.85 a
NA + C	292.58 ab	2028.83 b	1736.25 ab	0.85 a
NA + FA + C	364.08 a	2256.33 a	1892.25 a	0.83 a

Means of four plants per replication. Means followed by the same letter in each column are not significantly different (Tukey $P = 0.05$). C: control; FA + C: foliar treatment; NA + C: sodium treatment; NA + FA + C: sodium + foliar treatment.

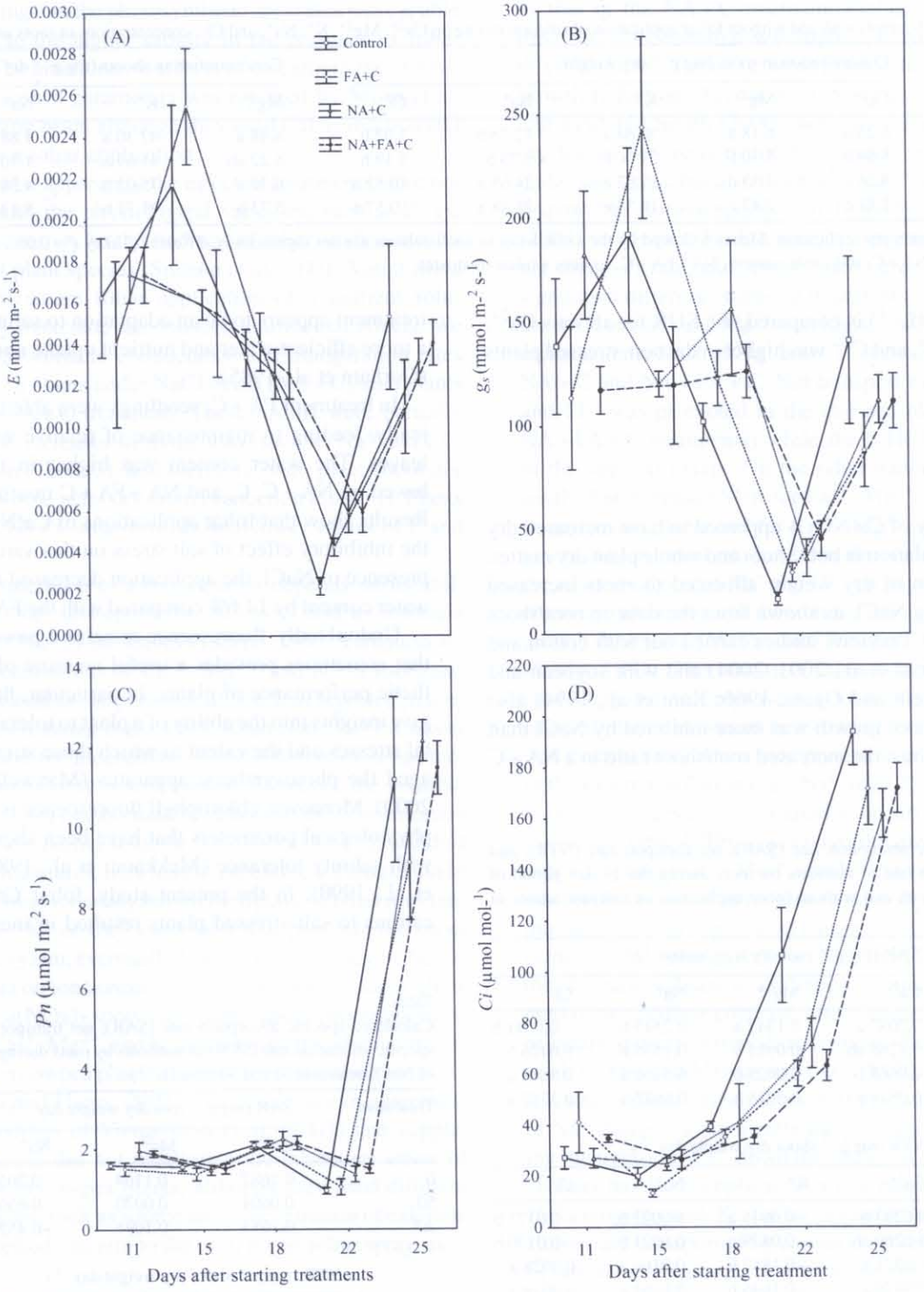

Fig. 1. Effect of NaCl (50 mM) with and without foliar application of calcium nitrate on: (A) transpiration, (B) stomatal conductance, (C) net photosynthesis, and (D) intercellular CO_2 in cowpea.

On the other hand, if SAR is compared between stressed and non-stressed plants (0 and 50 mmol NaCl L^{-1}), the SAR of Ca^{2+} and Mg^{2+} were lower in the stressed plants while the SAR of Na^+ and Cl^- was higher in the stressed plants (Table 5). The NTR for Ca^{2+}, Na^+, and Cl^- was promoted in the stressed plants (NA + C and NA + FA + C treatments) while that of K^+ was inhibited. Comparing the NTR between stressed and non-stressed plants (0 and 50 mmol NaCl L^{-1}), the NTR values for K^+ were both negative and lower in the stressed plants while those for Ca^{2+}, Na^+, and Cl^- were higher in the stressed plants. On the other hand, if SUR between stressed and non-stressed plants (0 and

Table 3
Effect of NaCl (50 mmol) with and without foliar application of calcium nitrate on Ca^{2+}, Mg^{2+}, K^+, Na^+, and Cl^- concentrations in roots and shoots of cowpea

Treatment	Concentration in roots (mg g^{-1} dry weight)					Concentration in shoots (mg g^{-1} dry weight)			
	Ca^{2+}	Mg^{2+}	K^+	Na^+	Cl^-	Mg^{2+}	K^+	Na^+	Cl^-
C	6.23 a	6.18 a	30.64 a	12.73 b	1.02 b	6.48 a	42.30 a	1.88 b	0.54 b
FA + C	5.08 b	5.30 b	27.00 b	9.73 b	1.19 b	6.22 ab	41.69 a	1.70 b	0.50 b
NA + C	3.26 c	2.53 c	17.52 c	24.65 a	10.52 a	6.30 a	35.02 b	4.58 a	5.79 a
NA + FA + C	2.81 c	2.42 c	16.58 c	25.48 a	10.57 a	5.73 b	31.72 b	5.13 a	5.04 a

Means of four plants per replication. Means followed by the same letter in each column are not significantly different (Tukey $P = 0.05$). C: control; FA + C: foliar treatment; NA + C: sodium treatment; NA + FA + C: sodium + foliar treatment.

50 mmol NaCl L^{-1}) is compared, the SUR for all ions Ca^{2+}, Mg^{2+}, K^+, Na^+, and Cl^- was higher in the non-stressed plants (Table 5).

4. Discussion

Foliar spray of $Ca(NO_3)_2$ appeared to have increased dry matter accumulation in both shoot and whole plant dry matter. The proportion of dry weight allocated to roots increased with increasing NaCl, as shown from the data on root/shoot ratio (Table 1). Previous studies carried out with cotton and prosopis (Meloni et al., 2001, 2004) and with soybean and alfalfa (Bernstein and Ogata, 1966; Kant et al., 1994) also showed that shoot growth was more inhibited by NaCl than root growth. Thus, the increased root/shoot ratio in a NA + C treatment appears to be an adaptation to salinity, resulting in a more efficient water and nutrient uptake under saline stress (Gorham et al., 1985).

In treatment FA + C, seedlings were able to adjust osmotically, leading to maintenance of relative water content of leaves. The water content was higher in the FA + C followed by NA + C, C, and NA + FA + C treatments (Table 1). Results show that foliar applications of $Ca(NO_3)_2$ overcome the inhibitory effect of salt stress on this variable, but in the presence of NaCl, the application decreased the leaf relative water content by 14.6% compared with the FA + C treatment.

Undoubtedly, fluorescence remains a powerful technique that sometimes provides a useful measure of the photosynthetic performance of plants. In particular, fluorescence can give insights into the ability of a plant to tolerate environmental stresses and the extent to which those stresses have damaged the photosynthetic apparatus (Maxwell and Johnson, 2000). Moreover, chlorophyll fluorescence is one of the few physiological parameters that have been shown to correlate with salinity tolerance (Mekkaoui et al., 1989; Monneveux et al., 1990). In the present study, foliar $Ca(NO_3)_2$ applications to salt-stressed plants resulted in increased fluores-

Table 4
Calculated specific absorption rate (SAR), net transport rate (NTR), and specific utilization rate of nutrients by roots during the 19-day period of NaCl treatment, with and without foliar application of calcium nitrate in cowpea

Treatment	SAR (mg g^{-1} root dry weight day^{-1})			
	Ca^{2+}	Mg^{2+}	Na^+	Cl^-
C	0.2087 a	0.1347 a	0.2545 b	−0.0041 b
FA + C	0.1286 ab	0.0981 a	0.1536 b	0.0426 b
NA + C	0.0668 b	0.0025 b	0.6460 a	0.3846 a
NA + FA + C	0.0541 b	0.0015 b	0.6677 a	0.3712 a
	NTR (mg g^{-1} shoot dry weight day^{-1})			
	Ca^{2+}	K^+	Na^+	Cl^-
C	0.0203 b	−0.0816 a	0.0023 b	−0.0175 b
FA + C	0.0260 ab	−0.0879 a	0.0021 b	−0.0130 b
NA + C	0.0371 a	−0.1852 b	0.0166 a	0.0028 a
NA + FA + C	0.0420 a	−0.1646 b	0.0164 a	0.0019 a
	SUR (mg g^{-1} shoot dry weight day^{-1})				
	Ca^{2+}	Mg^{2+}	K^+	Na^+	Cl^-
C	0.1100 ab	0.2327 ab	0.0258 ab	0.8049 a	0.8795 a
FA + C	0.1164 a	0.2639 a	0.0277 a	0.9656 a	0.8143 a
NA + C	0.0711 b	0.1516 b	0.0175 b	0.2769 b	0.1505 b
NA + FA + C	0.0860 ab	0.1903 ab	0.0209 ab	0.2677 b	0.1920 b

Means of four plants per replication. Means followed by the same letter in each column are not significantly different (Tukey $P = 0.05$). C: control; FA + C: foliar treatment; NA + C: sodium treatment; NA + FA + C: sodium + foliar treatment.

Table 5
Calculated specific absorption rate (SAR), net transport rate (NTR), and specific utilization rate (SUR) of nutrients by roots during the 19-day period of NaCl treatment (0 and 50 mmol L^{-1})

Treatment	SAR (mg g^{-1} root dry weight day^{-1})			
	Ca^{2+}	Mg^{2+}	Na^+	Cl^-
0	0.1687	0.1164	0.2041	0.0192
50	0.0604	0.0070	0.6568	0.3779
Δ[a]	0.1083	−0.1094	+0.4527	+0.3587
	NTR (mg g^{-1} shoot dry weight day^{-1})			
	Ca^{2+}	K^+	Na^+	Cl^-
0	0.0232	−0.0848	0.0022	−0.0153
50	0.0395	−0.1749	0.0165	0.0023
Δ	+0.0163	−0.0901	+0.0143	+0.0176
	SUR (mg g^{-1} shoot dry weight day^{-1})				
	Ca^{2+}	Mg^{2+}	K^+	Na^+	Cl^-
0	0.1132	0.2483	0.0268	0.8852	0.8469
50	0.0785	0.1710	0.0192	0.2723	0.1712
Δ	−0.0347	−0.0773	−0.0076	−0.6129	−0.6757

[a] Differences between ions at 50 and 0 mmol NaCl L^{-1}.

cence, showing that the photosynthetic apparatus was capable of adapting to the higher salinity in the presence of foliar $Ca(NO_3)_2$ The ameliorative effects of calcium in the chlorophyll fluorescence parameters was reported by Misra et al. (2001) in mung bean, although they used $CaCl_2$ in a nutrient solution, not a foliar application.

In our study, apparently the effect of treatments were not consistent with the physiological variables, such as *E*, gs, Pn, and Ci, contrary to the findings of other studies involving different plant species (Sultana et al., 2001; Vieira-Silva et al., 2003) where foliar application of a nutrient solution partially overcame salt-induced detrimental effects. We observed that stomatal conductance and transpiration rates were higher in plants under NaCl stress (NA + C) than plants under NA + FA + C treatment. These results were partially related to the increases in tissue ions, such as Na^+ and Cl^- accumulation (Table 3), and therefore, with plant shoot dry matter and whole plant dry matter reduction (Table 1). Plants under NA + FA + C treatment showed lower values of *E* and gs.

Salinity tolerance of a crop species depends on its ability to limit Na^+ absorption by the roots and to maintain low levels of Na^+ in the leaves (Tadano, 1983; Yamanouchi, 1995). Concentrations of Ca^{2+}, Mg^{2+}, and K^+ in roots and shoots decreased in the presence of NaCl, as reported in previous studies (Cramer et al., 1991; Khan et al., 1997; Lutts et al., 1999), and the mechanism of the differences in nutrient uptake under salinity is still unclear. However, the possible causes in cowpea plants could be related to the concentration of ions in the external solution (i.e., Na^+ and Cl^-), which if taken up at high rates, may lead to excessive accumulation in the tissue. These ions may inhibit the uptake of other ions into the root (i.e., K^+ or Ca^{2+}) and their transport into the shoot through the xylem, eventually leading to deficiency in the tissue. The data on ion concentrations in this study clearly show that foliar $Ca(NO_3)_2$ spray can not increase concentrations of nutrients (Ca^{2+}, Mg^{2+}, and K^+) to mitigate the adverse effects of salinity on cowpea plants as on other species (Sultana et al., 2001; Kaya and Higgs, 2002). Our results are in agreement with the findings of Vieira-Silva et al. (2003) that supplemental calcium did not ameliorate the inhibitory effect of NaCl stress in cowpea plants, although they used different methodologies, such as source and concentration of calcium, and they applied calcium to the root, not as foliar spray, as in our study.

On the other hand, the beneficial effects of calcium nutrition on NaCl-stressed plants are associated with the maintenance of cell membrane integrity, reducing Na^+, and favoring K^+ absorption in salt-stressed plants (Epstein, 1998). In this study, the addition of calcium did not favor K^+ absorption in salt-stressed plants because no significant differences were observed among treatments in the SAR. On the contrary, the addition of foliar $Ca(NO_3)_2$ increased Na^+ absorption (Table 4). Chloride concentration in roots was higher in plants subjected to foliar $Ca(NO_3)_2$ treatment, while in shoots, the concentration was relatively lower but statistically the same as that in the NA + C treatment (Table 3). In addition to this, Na^+ concentration was highest in roots and shoots in this treatment (Table 3), which could have contributed to growth reduction. According to Song and Fujiyama (1996) and Reid and Smith (2000), not only the content of Na^+ in the leaves was responsible for growth reduction in plants under salt stress, but also low Ca^{2+}, Mg^{2+}, and K^+ are jointly responsible. Similar results were found by Vieira-Silva et al. (2003) in cowpea. With respect to specific absorption rate, net transport rate, and specific utilization rate of nutrients, significant differences among treatments were found, where the SAR of Ca^{2+} and Mg^{2+} was higher in C and FA + C treatments and the SAR of Na^+ and Cl^- showed higher values in NA + C and NA + FA + C. Net transport rate for Ca^{2+}, Na^+, and Cl^- was promoted in the stressed plants (NA + C and NA + FA + C treatments) while the NTR for K^+ was lower in the stressed plants. On the other hand, foliar $Ca(NO_3)_2$ resulted in increased SUR for Ca^{2+}, Mg^{2+}, K^+, Na^+, and Cl^- in unstressed plants and for Ca^{2+}, Mg^{2+}, and K^+ in stressed plants. Much attention has been devoted to understanding the adverse effects of Na^+ and Cl^- on physiological and biochemical processes and how these ions contribute to plant growth inhibition (Munns and Termaat, 1986; Maas, 1993; Munns, 1993). The results of this study showed that foliar $Ca(NO_3)_2$ did not improve the SAR of Ca^{2+} and Mg^{2+} in stressed plants, as would be expected. On the other hand, it was evident that, when ions present at high concentrations in the external solution (i.e., Na^+ and Cl^-) were taken up at high rates, excessive accumulation occurred in the tissue, as showed in Table 4. This was a possible cause for selectivity of nutrient uptake (Ca^{2+}, Mg^{2+}, and K^+) because these ions may inhibit the uptake of other ions in the root and their transport into the shoot through the xylem, eventually leading to deficiency in the tissue. Likewise, Na^+ and Cl^- transport across the plasma membrane in a saline environment must be considered in two cellular contexts, after salt stress shock and after re-establishment of ionic homeostasis. According to Sultana et al. (2001) and Hasegawa et al. (2000), immediately after salt stress, the H^+ electrochemical gradient is altered. Influx of Na^+ dissipates the membrane potential, thereby facilitating the uptake of Cl^- down the chemical gradient. An anion channel has been implicated in this passive flux. Similarly, Na^+ competes with K^+ for intracellular influx because these cations are transported by common proteins. Therefore, one of our main objectives, how to minimize salt-induced nutrient deficiency in the cowpea plants through foliar $Ca(NO_3)_2$ application, was not achieved, suggesting that the mitigating effect of foliar treatment under saline conditions apply only to some species like strawberry (Kaya et al., 2002) and cucumber (Kaya and Higgs, 2002).

Acknowledgements

This research was supported by grants from the Royal Society and University of Dundee, England and the Programa

de Agricultura de Zonas Áridas of the Centro de Investigaciones Biológicas del Noroeste (CIBNOR).

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