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Calcium and magnesium do not alleviate the toxic effect of sodium on the emergence and initial growth of castor, cotton, and safflower

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ABSTRACT

Castor (*Ricinus communis*), cotton (*Gossypium hirsutum*), and safflower (*Carthamus tinctorius*) are industrial crops frequently considered to be raised under high salinity of the soil or irrigation water. Sodium is the most common ion causing salinity, but other ions can also be found in toxic level. This experiment had the objective to evaluate if the presence of calcium and magnesium in the irrigation water alleviates the toxic effect of sodium in the emergence and initial growth of these three oilseed crops. Seeds were sown in trays for evaluation of emergence and in pots for evaluation of plant growth. The treatments consisted of simulations of the Na^+:Ca^{2+}:Mg^{2+} molar ratio found in the irrigation water of the Trans-Pecos region of the States of New Mexico and Texas, USA. The saline solutions were equivalent to 0, 50, 100, 150, 200, and 250% of the salt composition found in the reference water. Some solutions contained the three salts, while others contained only Na^+, and the electrical conductivity varied from 0.7 to 13.7 dS m^{-1} among treatments. For the analysis of plant growth, the treatments were imposed after seedling emergence, and the plants were harvested after 30 days.

In castor and safflower, the salinity effect was associated with the electrical conductivity rather than with the salt composition. The cotton genotype had been previously selected to be tolerant to Na^+, but it was sensitive to Ca^{2+} and Mg^{2+}. Safflower plants did not survive 30 days under exposure to salinity higher than 9.6 dS m^{-1} with any salt composition. In conclusion, Ca^{+2} and Mg^{+2} did not alleviate the toxic effect of Na^+, and the mechanisms of salt tolerance in cotton were ion-specific.

1. Introduction

Salt stress is an important constraint for agricultural production in many regions of the globe. High soil salinity can arise from natural causes or from human intervention, particularly under irrigated agriculture (Munns and Tester, 2008). Salinity affects plant production through osmotic stress, specific-ion toxicity, and nutritional imbalances (Kopittke, 2012; Munns and Tester, 2008; Wakeel, 2013). There are large differences in the tolerance to salinity among species because many mechanisms are used to protect vital organs and to exclude or compartmentalize salts.

Salts interfere with plant growth through two processes: initially, the growth slows due to osmotic stress, as the water uptake by root is impaired; later, the salts accumulate in toxic concentration in old leaves and cause its death (Munns and Tester, 2008). When initially exposed to high salt content, plant growth rapidly reduces due to osmotic (non-specific) effects. Over longer periods (days to weeks), individual salts may accumulate to toxic levels, thereby inducing specific-ion toxicities (Munns, 2002).

The most frequent salts affecting crops worldwide are Na^+ and Cl^-, but salinity can also be caused by K^+, Ca^{2+}, and Mg^{2+}, and to a lesser extent by sulfates and carbonates. The toxicity caused by Na^+ can be alleviated by other cations, such as K^+, Ca^{2+}, and Mg^{2+}. However, these cations have complex interactions in which K^+ seems to be the most important antagonist of Na^+, but it depends on the presence of Ca^{2+} or Mg^{2+} to be effective. In some situations, Ca^{2+}

Abbreviations: EC, electrical conductivity.

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Table 1
Description of the concentration of each ion and the electrical conductivity of the saline solutions.

Salt composition	NaCl (mMol)	$CaSO_4$ (mMol)	$MgSO_4$ (mMol)	Electrical conductivity ($dS\,m^{-1}$)
Control	–	–	–	0.7
	18	–	–	1.8
	36	–	–	3.9
Na^+	54	–	–	4.8
	71	–	–	6.1
	89	–	–	7.7
	18	11	6	3.6
	36	22	12	7.9
Na^{2+}, Ca^{2+}, and Mg^{2+}	54	33	17	9.6
	71	43	23	12.8
	89	54	29	13.7

has an apparent Na^+-alleviation effect, but this effect is still questionable, and the alleviation is observed in the cell uptake of Na^+ rather than in the growth reduction caused by this ion. Increased Na^+, K^+, and Mg^{2+} concentrations can reduce Ca^{2+} activity in the plasma membrane and induce deficiency of this nutrient (Kopittke et al., 2011; Kopittke, 2012; Munns and Tester, 2008; Tester and Davenport, 2003; Wakeel, 2013).

Castor (*Ricinus communis* L.), cotton (*Gossypium hirsutum* L.), and safflower (*Carthamus tinctorius* L.) are industrial oilseed crops that are often considered for cultivation in salt affected areas (Costa et al., 2013; Li et al., 2010b; Nobre et al., 2013; Silva et al., 2005; Tiwari et al., 2013; Yeilaghi et al., 2012). If their tolerance to salt stress is confirmed, these crops will be interesting options for regions where agricultural production suffers with this limiting factor.

The objective of this study was to evaluate if the presence of the cations Ca^{2+} and Mg^{2+} in the irrigation water alleviates the toxic effect of Na^+ on seedling emergence and initial growth of castor, cotton, and safflower plants.

2. Material and methods

The experiments were conducted in a greenhouse at Texas Tech University (Lubbock, TX, USA) in 2012. The treatments were designed as a simulation of the concentrations and proportions of major salts found in the irrigation water in the Trans-Pecos region in the State of Texas, USA (Ashworth, 1995). The treatments were defined as 50, 100, 150, 200, and 250% of the reference water, which had 36, 26, and 12 mM of Na^+, Ca^{+2}, and Mg^{+2}, respectively. The molar ratio was 1 Na^+:0.72 Ca^{2+}:0.33 Mg^{2+}. The same treatments were then repeated without inclusion of Ca^{2+} and Mg^{2+} (Table 1).

Saline solutions were prepared in 120-L plastic containers mixing tap water (0.004 $dS\,m^{-1}$) with NaCl, $CaSO_4$, and $MgSO_4$ in amounts to reach the assigned treatment (Table 1). A soluble fertilizer was mixed in equal dose to all the solutions in order to add 18 mM of N, 2 mM of P, and 4 mM of K. The control treatment was tap water with addition of the fertilizer. Because the K^+ concentration was fixed (because it was supplied as fertilizer), the molar ratio Na^+:K^+ among the solutions varied from 1:0.04 to 1:0.22. The electrical conductivity (EC) was measured after the solutions were prepared, and it varied from 0.7 to 13.7 $dS\,m^{-1}$ (Table 1). This solution was used for the experiments of seedling emergence and plant growth.

Studies on salinity should be preferentially based on the osmotic potential of the solutions rather than on the EC. However, EC has been used in most experiments with salinity because it is closely related to the osmolarity, and it is easier to measure (Ben-Gal et al., 2009). A correlation of 0.94 was found between the osmotic potential (varying from −0.029 to −0.485 MPa) and the electrical conductivity (varying from 0.40 to 14.35 $dS\,m^{-1}$) in solutions with varying contents of Na and Ca (Ben-Gal et al., 2009).

The study was conducted with castor seeds of the cv. Brigham, which is the first commercial variety selected for reduced ricin content (Auld et al., 2003), cotton line DN-1, which was previously selected for tolerance to high NaCl among wild cotton accesses in a hydroponic system (Castillo, 2011), and safflower line 672, which was selected for winter planting in the breeding program of Texas Tech University (Oswalt and Auld, 2011).

2.1. Seedling emergence

Plastic trays were filled with an 8-cm layer of the substrate Metromix® (vermiculite, bark, peat moss, and coarse perlite). The test in castor was made with four replications of 40 seeds per tray, and in safflower, it was made with nine replications of 20 seeds. The seedling emergence was not tested in cotton. Trays were arranged in a completely randomized design. Seeds were buried 3-cm deep (castor) or 1-cm deep (safflower), covered with substrate, and irrigated daily with the respective saline solution. Emerged seedlings were counted daily and discarded (clipped). They were assumed as emerged when the cotyledons were out of the soil.

After sowing, data was taken over 20 days in castor and 9 days in safflower. The percentage of emergence and the time for emergence of 50% of the seeds were calculated. The time for 50% of emergence of castor seedlings was calculated by interpolation in order to include the fraction of day. The equation was $t_{50\%} = t_{d-1} + (50 - e_{d-1})/(e_d - e_{d-1})$, in which $t_{50\%}$ is the time for emergence of 50% of the seeds, t_{d-1} is the day before 50% was reached, e_{d-1} is the emergence (%) observed in t_{d-1}, and e_d is the emergence (%) in the day it was ≥50%. In safflower, the same calculation was made considering the threshold of 40%, because some plots did not reach 50% of emergence.

2.2. Plant growth

The experiment was conducted in 12-L pots in a greenhouse with controlled temperature (28 ± 3 °C). The substrate was made of soil from the top soil layer (0–15 cm) collected from the Experimental Farm of Texas Tech University (Lubbock, TX). The soil had 2500 $mg\,kg^{-1}$ of Ca^{2+} and 520 $mg\,kg^{-1}$ of K^+. The pots of the same species were arranged in a completely randomized design with four replications. Five seeds were sowed in each pot, and irrigated with tap water. The salt treatments begun immediately after the first seedling emerged. The pot was daily irrigated with the respective saline solution in a volume enough for allowing at least 20% of drainage. Ben-Gal et al. (2009) employed a similar method (daily irrigation with 20% drainage) and confirmed that the technique worked properly because the salinity in the drainage water was stable, and the Na^+ and Ca^{2+} content were always proportional (twice) to the amounts added through irrigation.

Destructive analyses were conducted at 30 days after emergence. The reproductive structures (flowers, racemes) and dead plants were counted. Leaf area was measured twice using a Li-Cor LI-3100 m. Roots were carefully washed from the soil. Dry weight of leaves, stems, and roots were taken after oven-drying for three days at 80 °C. Shoot/root ratio was calculated.

2.3. Statistical analysis

The data on castor and cotton (all cations) was analyzed by polynomial regression using the linear model ($y = ax + b$) in function of the EC of the irrigation water. The data on safflower and cotton (Na^+) plant growth was analyzed using the model of inverse first order ($y = a/x + b$). The slope significance in both models was tested with t test ($p < 0.05$). Equations were calculated separately for the