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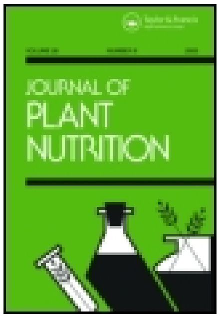

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Soybean Response and Ion Accumulation under Sprinkler Irrigation with Sodium-rich Saline Water

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ABSTRACT

The magnitude of crop growth and yield depends on the salinity level, the toxic ions present, and the irrigation system used. In order to study the effect of saline sprinkler irrigation on soybean growth and ionic accumulation in plant tissues a pot experiment was set up. There were three irrigation water quality treatments [electrical conductivity (EC) 0, 2, and 4 dS m^{-1}]**.** Soybean aerial biomass was 25% lower than the Control when irrigation salinity was 4 dS m^{-1}. Clearly salinity entering via leaves affected the grain filling stage and severely reduced soybean grain production (80% reduction) when salinity in irrigation water surpassed 2 dS m^{-1}. Sprinkler irrigation aggravates soybean's low salinity tolerance and restricts its cropping in such conditions. For early stages two linear relationships between leaf chloride (Cl^-) concentration

(Y= 14.2-2x) or potassium (K^+)/ sodium (Na^+) ratio (Y=5.3x-3.4) and soybean grain yield were found. Both relationships may be used as diagnostic tools for soybean growing under saline sprinkler irrigation.

Keywords: environmental stresses, water quality, soybean

INTRODUCTION

In order to achieve high crop yields, irrigation is used in many agricultural areas throughout the world, even in humid production areas. Supplying water during periods of drought, the so called "supplemental irrigation" is suited for those areas. Nevertheless reduced availability of water resources results in increased use of marginal quality water, mainly saline waters (Ayers and Wescot, 1985; Shalhevet, 1992). The use of these water sources triggers water stress, ion toxicity and nutritional imbalances on crops as well as land salinization. The overall effect of salts on crops has been thoroughly studied for a long time. Chlorides (Cl^-) mainly associated with sodium (Na^+) predominates among soluble salts in many locations and its toxic effect on plants has also been carefully studied, from molecular to agronomical scales (Ayers and Wescot, 1985; Balestrasse et al., 2008). The magnitude of the detrimental effect of irrigation water on crop growth and yield depends on the salinity level and the excess of toxic ions such as Na^+ and Cl^- (Maas and Hoffman 1977). Both elements decrease plant growth because of specific toxicities and ionic effects on plant metabolism. Salinity also disturbs nutrient relations, transport and partition within plant (Hu and Schmidhalter, 2005). The negative effects of soil salinity are also related to the different irrigation systems, i.e., drip, furrow and sprinkler irrigation (Bernstein and

Francois 1973). Moreover, it has been long known that the use of low quality water on sprinkler irrigation poses the potential problem of salt absorption by leaves. This fact enhances toxicity effects when compared to those found under technologies that apply water over soil surface, as furrow or drip irrigation (Bernstein and Francois, 1973; Grattan et al., 1994; Maas, 1985).

Most research about crops salt tolerance was carried out with saline or salinized soils (Ayers and Wescot, 1985). Among crops, soybean (*Glycine max L. Merrill*) is known to be moderately tolerant to salinity, with threshold values ranging from 4 dS m^{-1} to 5 dS m^{-1} (Katerji et al., 2003; Katerji et al., 2000; Maas and Hoffman, 1977; Shalhevet et al., 1995), beyond which growth and yield are drastically reduced. Additionally, soybean is especially sensitive to ion toxicity and Cl^- injury (Abel and MacKenzie, 1964; Parker et al., 1983). Soybean is at present one of the main crops of the world. It has exceptional nutritional characteristics and ability to grow under a wide range of environmental conditions and management systems. Currently, USA, Brazil, Argentina and China account for almost 90% of the world's soybean production, with Argentina being the first country to export soybean and its products (oil, expellers, etc) (USDA, 2008). Soybean's production has doubled in the last two decades in parallel with increasing food demand worldwide (Eickhout et al., 2006). The expansion of this crop has been documented also on saline soils in various parts of the world (Beecher, 1994; Essa, 2002; Scanlon et al., 2005). In Argentina, initially soybean was grown in Mollisols of the agricultural region of the Pampas but at present it is also being grown in marginal and semiarid lands, prone to periodical droughts. To cope with this disadvantage, soybean is sprinkler irrigated on large fields, covering periodic summer droughts and the irrigation water applied shows a wide range of salinity degrees (Lavado, 2009). The soybean area subjected to supplemental irrigation in the country varies, according to the water regime of the year, but currently covers over 20000 ha, in semiarid regions (Díaz Zorita, personal communication).

Soybean accumulated four times more Na^+ and Cl^- from irrigation water using sprinkler irrigation than using other irrigation methods (Wang et al., 2002). Ion absorption from the above-canopy sprinkler irrigation may be more pronounced in soybean than in other crops, because of its foliar morphology; this is the reason why plant salt accumulation is higher when compared to drip or furrow irrigation, even when good quality water is used (Wang et al., 2002; Grieve et al., 2003). However, the potential deleterious effects of saline sprinkler irrigation on soybean yields are not well documented and the relationship between ion uptake and accumulation, and grain yield are still unknown. On the other hand, Na^+ "per se" was not considered a better indicator of salt sensitivity and ionic imbalance caused by salinity than the potassium (K^+)/sodium ratio (K^+/Na^+) ratio in alfalfa (Isla and Aragüés, 2009). Our objective was to study the effect of sprinkler irrigation with salty water on soybean growth, development parameters and yield, and ionic accumulation in plant tissues. One practical purpose was to obtain indicators for the early detection of salt effects on crop yield. In order to do that, we mimicked the field environment and technology under controlled conditions.

MATERIALS AND METHODS

A pot experiment was established following a completely randomized design with three treatments and 12 replicates for each treatment. Water with three different salinity levels (0, 2, and 4 dS m^{-1}) was applied to the soybean plants by mimicking above-canopy sprinkler irrigation.

Water salinity level was obtained by addition of enough sodium chloride (NaCl) to distilled water in order to reach the desired electrical conductivity (EC) level for each treatment at every irrigation event. All treatments received the same volume of water (800 mL per pot every 72 hours), irrigation was applied during the early day. In order to avoid salt accumulation within the pots, surplus water was added to provide a leaching fraction of about 0.2 soil field capacity.

Plants were grown under a rain shelter that excluded rainfall but allowed plants to be exposed to natural light and temperature conditions. Three soybean seeds (‘NA 4613’ cultivar, maturity group IV) that were previously inoculated were sown in 8 L plastic pots filled with a soil mixture composed by 30% sand and 70% A horizon of a sandy loam Typic Argiudoll soil. The final size particle distribution was 13% clay, 12%, silt, 74% sand. Following standard techniques (Sparks et al., 1996) the growing media composition was: 12.6 g kg^{-1} of organic carbon (Walkley and Black method); a 7.6 pH; 32.8 mg kg^{-1} available phosphorus (P) (Kurtz and Bray method) and 0.38 dS m^{-1} (soil saturation extract) EC_S. Pots were thinned to one plant per pot after 15 days of seeding. To prevent any nutritional deficit, each pot received 2 g of triple superphosphate and 0.125 g of an all micronutrients mix before sowing. Every 30 days all pots had 1 g of a soluble fertilizer (N:P:K 25-10-10) added.

At bloom (R1-R2); pod (R3-R4) and maturity (R8) stages (50, 70 and 130 days after sowing, respectively), plant and soil mixture samples were collected from four pots from each treatment. Soil EC was measured en 1:2.5 soil:water extracts (Sparks et al., 1996). Plant height (main shoot only), number of pods, and number of grains were recorded. Aerial biomass, previously rinsed with distilled water, was divided into leaves, shoots, pods, and grains. Roots were washed, sieved and harvested. All vegetative samples were dried at 60ºC for 72 hours and then weighed. Chloride, sodium and potassium content on sieved and homogenized samples of roots, leaves, shoots, pods and grains were determined. Cl^- was extracted from all vegetative samples (150 mg) with 10 mL distilled water in a hot bath (60°C for 30 min) and filtered (Drew and Saker, 1984). Filtrates were used to determine Cl^- by volumetric titration (AOAC, 1965). For Na^+ and K^+ analysis 500 mg of vegetative material was digested in 10 mL of perchloric acid ($HClO_4$) (70%) and 5 mL of concentrated nitric acid (HNO_3) (AOAC, 1965) and determined by atomic absorption spectrophotometer (USEPA, 2006).

Data was analyzed using individual analysis of variance (ANOVA) for each sampling date (R1-R2; R3-R4; R8). When significant differences were found, a comparison of means test (Tukey) was applied. In order to identify the relationship between measured ion content and soybean grain yield regression analysis was used, and the best-fit linear regression was chosen (Table Curve 2D; AISN Software Inc, 2000).

RESULTS

Soil salinity increased rapidly for both salinity treatments (Table 1). Soil EC did not increase further over time. Although no Ca was applied in the irrigation water, Na^+ absorption ratio within the soil solution ranged from 0.8 and 1.1 for control and treatment 4.

No differences in plant height were noticeable in any treatment at early stages of the crop cycle but after stage R3-R4 lower heights were recorded for both saline treatments (treatments 2 and 4) (Figure 1). Soybean biomass responds unevenly to salinity. The total biomass was reduced at early stages only in treatment 4 (Figure 2). Additionally, no significant differences in leaves and shoots biomass production were found between Control and treatment 2, whereas in treatment 4 they were 25% lower in average. Roots were more affected than aerial biomass; for both salinity treatments the reduction in root weight was twice the one exhibited by aerial parts of the plant, 20 % and 50 % for treatment 2 and 4, respectively, as compared with the Control. Soybean pod and grain number and weight show a pattern similar to other vegetative parts (Table 2): a 20% reduction in pod number and weight was found for treatment 4 but no differences between Control and treatment 2 were detected. On the contrary, grain production was significantly affected in both salinity treatments (2 and 4). The yield loss was more than 27 and 83 %, respectively than that obtained under Control irrigation.

Both leaf Na^+ and Cl^- concentration increased as salinity in irrigation water augmented (Table 3). Leaf Na^+ concentration was 2.8-fold greater for treatment 2 and 3.9 times higher for treatment 4, than Control at maturity (R8). Leaf Cl^- concentration for both salinity treatments exceeded 3.4 and 5.8 times the Control treatment. Along the crop cycle, foliar content of Na^+ increased according to the salinity applied to plants; however it remained unchanged for the control plants, foliar K^+ content, conversely, remained constant in all three treatments along the crop cycle. Distribution pattern of ions in the plant parts showed some differences: plant roots showed the highest Na^+ root content ranging from 3.68 to 4.14 mg g^{-1} of dry matter (DM) on Treatment 2 and from 6.54 to 9.78 mg g^{-1} DM on Treatment 4. Leaves and shoots had similar contents averaging 3.08 and 4.79 mg g^{-1} DM for Treatment 2 and Treatment 4, respectively. On the contrary, Cl^- was mainly accumulated in aerial parts, following the decreasing concentration order leaves > shoots > roots. In this case roots accumulated 0.6 – 1.28 mg g^{-1} DM.

A significant negative linear relationship between leaf Cl^- concentration at an early reproductive stage (R1-R2) and soybean yield was found (Figure 3A). Conversely the relationship found between K^+/Na^+ ratio and yield was positive (Figure 3B). The different linear regression models between leaf Cl^- concentration and soybean yield and K^+/Na^+ ratio and yield for the remaining stages (R3-R4 and R8) are presented in Table 4.

DISCUSSION

Soil salinization is a common consequence of saline irrigation. Our results agree with Wu et al. (2001) who found for a similar range in water salinity to ours, soil plots reached comparable EC values to those applied in irrigation water, when proper drainage was set up. The effect of salinity on vegetative components followed the general known pattern. Plant height reduction agrees with data from Essa (2002) and Shalhevet et al. (1995). The obtained results are consistent with results from other authors showing soybean's low tolerance for salts, in this case 2 dS m^{-1} This EC value is half the one determined as a threshold for soybean under flood, furrow or drip irrigation systems found either in early research (Maas, 1986; Maas and Hoffman, 1977) as well as in recent studies (Bustingorri and Lavado, 2011; Chang et al., 1994; Katerji et al., 2003; Kao et al., 2006; Shalhevet et al., 1995). Saline sprinkler irrigation had a more severe effect on reproductive organs than on vegetative ones, which has not been reported in previous studies. When salinity in irrigation water surpassed 2 dS m^{-1}, pod production was slightly affected whereas grain production was, on average, half the one obtained under non-saline irrigation. In this case late reproductive stages were more critical than initial ones. Rao et al.

(2008) found higher reductions in number of spikelet and rice grain yield than in number of tillers and straw yield. For soybean, the low yield obtained under saline sprinkler irrigation was due to an important reduction of pod and seed production. Salinity may have had an adverse effect on pollen viability resulting in flower sterility and high yield loss for soybean as already found for rice (Rao et al., 2008).

Although sprinkler irrigation water caused a rapid soil salinization witch also affected soybean plants. Salt damage in soybean results from the accumulation of chloride and sodium in stems and leaves (Parker et al., 1983; Wang and Shannon, 1999). Since Na^+ and Cl^- are known to be absorbed by leaves (Maas et al., 1982), high levels of these toxic ions were expected to be found in aerial tissue, especially leaves. Leaf chloride values obtained in this research (1 to 6 mg g^{-1} DM) were higher than those found in previous studies, ranging from 0.13 to 4.45 mg g^{-1} DM (Bustingorri and Lavado, 2011; Essa, 2002; Jeong-Dong et al., 2008) where salinity was applied to plant roots, either by soil salinity or hydroponic. The proportional increase in leaf Cl^- and Na^+ concentration found in this experiment are within the range reported by Grieve et al. (2003). They also found that Cl^- and Na^+ in leaves could reach concentrations 10 and 8-folds higher than those where the ions enter only by root uptake, due to direct foliar absorption of these ions. Sodium exclusion from leaves by its retention in roots or the retranslocation of Na^+ and Cl^- absorbed by leaves to shoots and roots is a characteristic response to salinity of many leguminous species (Läuchli, 1984). These processes contribute to the control of Cl^- and Na^+ toxicity in certain soybean cultivars, such as “Lee” (Abel and MacKenzie, 1964). Supporting these finding, Grieve et al. (2003) found lower Na^+ contents in leaves than other plant parts in a “Lee” related cultivars. In our studied cultivar roots had the higher Na^+ contents, but Na^+

concentration in leaves and shoots remained also high along the crop cycle. Conversely Cl^- was mainly accumulated in aerial plant parts (90 % of overall Cl^- content). This inability to exclude Cl^- specially and also partially Na^+ from leaves explains this cultivar's low salt tolerance, thus the high yield loss. This finding, in agreement with previous knowledge about different genetic background to cope with salinity among soybean cultivars (Abel and MacKenzie, 1964; Jeong-Dong et al., 2008), opens a door for genetic improvement of this crop in relation to salinity.

In our case the K^+/Na^+ ratio showed a good relation with soybean yield, as found by Isla and Aragüés (2009) on alfalfa. However, compared with this more salt tolerant species (Maas and Hoffman, 1977) soybean shows lower grain yields with K^+/Na^+ foliar ratios lower than the threshold of 2, found by them in alfalfa. The use of leaf tissue concentration at early stages (R1-R2) may be used as a diagnostic tool because of the close relationship found between Cl^- and K^+/Na^+ in leaves and soybean grain yield. The other stages are too late to be useful for that purpose.

CONCLUSIONS

Sprinkler irrigation with saline waters affects soybean growth and salts entering the plant through the leaves magnify soybean's low tolerance to salinity and limit its cropping in such conditions. The effect is more severe for the reproductive component of grain yield than for vegetative components. When comparing the result from our study with those reported in literature it can be seen that soybean cultivars show a differential ability to exclude toxic ions from leaves to roots. In this work soybean accumulated Na^+ and Cl^- in leaves, which explains the high impact that sprinkler irrigation has on grain yield. The relationship found between either leaf Cl^- concentration or K^+/Na^+ ratio (both in early crop growth stages) and grain yield may be used as a diagnostic tool for estimating soybean yield loss when salts entering via leaves are the main stress factor.

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Table 1. Mean and standard deviation soil EC values measured along the soybean cycle (1:2.5 extracts) for every treatment.

	Electrical conductivity (dS m^{-1})			
Treatment	**SEEDING**	**R1-R2**	**R3-R4**	**R8**
0 dSm^{-1}	0.325±0.005	0.447±0.073	0.344±0.069	0.415±0.059
2 dSm^{-1}	0.382±0.019	1.737±0.352	1.596±0.244	2.048±0.398
4 dSm^{-1}	0.421±0.026	2.543±0.603	2.916±0.551	3.173±0.250

Table 2. Mean values for soybean pod and grain production and standard deviation. Different letters indicate differences between treatments for each variable (*P*<0.05).

	Salinity irrigation treatment					
	0		**2**		**4**	
Pod number	38.50 ± 1.26	a	29.00 ± 1.00	ab	26.50 ± 3.11	b
Pod weight (g plant^{-1})	8.94 ± 0.62	a	7.78 ± 0.71	a	7.24 ± 1.74	a
Grain number	53.50 ± 1.89	a	36.67 ± 3.71	b	23.00 ± 4.00	c
Grain weight (g plant^{-1})	10.68 ± 2.30	a	7.85 ± 0.64	b	1.22 ± 0.45	c

Table 3. Mean values for soybean leaf Na^+. K^+ and Cl^- tissue concentration (mg g^{-1} dry matter) and standard deviation for each sampling date. Different letters indicate differences between treatments for each variable and stage (*P* <0.05).

		Salinity irrigation treatment					
		0		**2**		**4**	
R1-R2	**Na^+**	1.40 ± 0.14	a	2.36 ± 0.32	a	4.31 ± 0.35	b
	K^+	3.09 ± 0.61	a	3.93 ± 0.01	a	3.40 ± 0.21	a
	Cl^-	1.76 ± 0.48	a	5.01 ± 0.75	b	6.09 ± 0.06	b
R3-R4	**Na^+**	1.75 ± 0.04	a	3.31 ± 0.26	b	4.99 ± 0.15	c
	K^+	3.17 ± 0.17	a	2.60 ± 0.35	a	3.00 ± 0.77	a
	Cl^-	1.38 ± 0.32	a	3.37 ± 0.18	b	5.33 ± 0.43	c
R8	**Na^+**	1.28 ± 0.08	a	3.59 ± 0.91	b	5.08 ± 0.70	b
	K^+	2.09 ± 0.31	a	2.16 ± 0.37	a	3.18 ± 0.03	a
	Cl^-	1.03 ± 0.05	a	3.51 ± 0.86	b	6.01 ± 0.85	c

Table 4. Linear regression models for all sampling dates. Y: soybean grain yield (g dry matter plant^{-1}); CL: leaf chloride concentration (mg g^{-1} dry matter); K/NA: Leaf K^+/Na^+ concentration ratio.

Regression models	R^2	MSE	P value

R3-R4	Y= 13.138 - 2.278 CL	0.97	83.835	<0.001
	Y= 5.180 K/NA - 0.5437	0.95	81.886	0.001
R8	Y= 11.86 - 1.81 CL	0.97	83.329	< 0.001
	Y=2.173 K/NA - 2.324	0.94	80.702	0.0016

MSE is the mean square error

Figure 1. Soybean plant height for different water irrigation salinity levels (0,2, and 4 dS m^{-1}), at different reproductive developmental stages. Different letters indicate significant differences between treatments for each growth stage ($P < 0.05$).

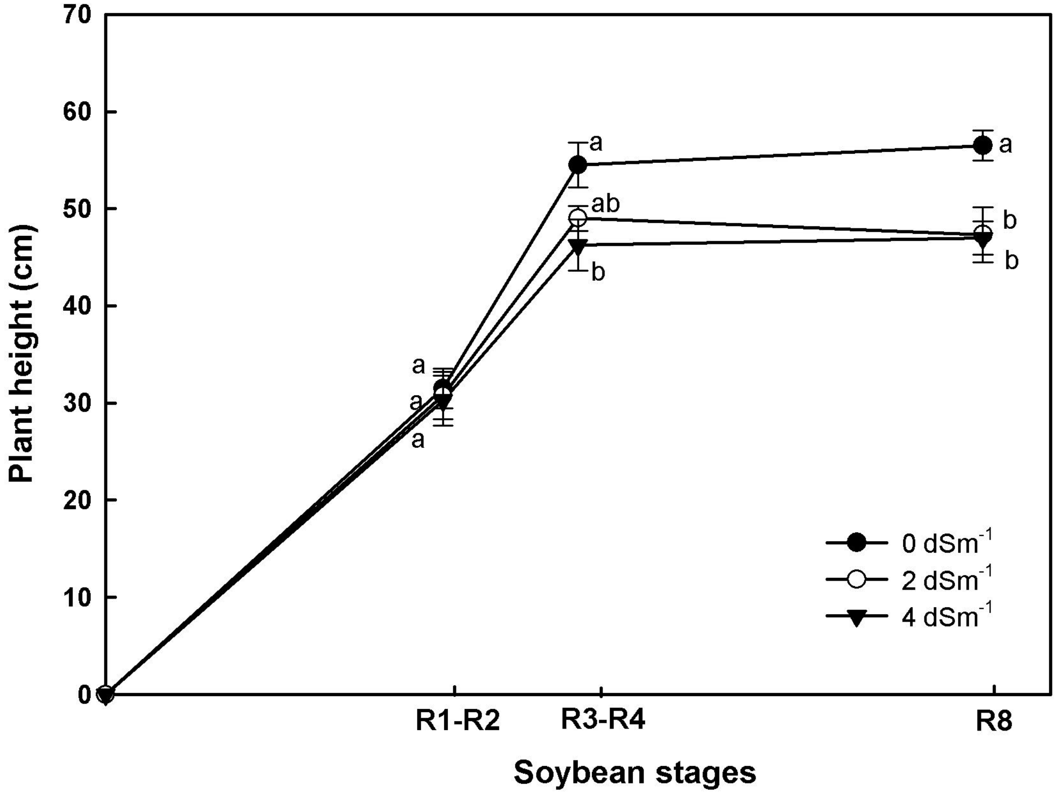

Figure 2. Soybean biomass production for each growth stage. Different letters indicate significant differences between treatments for each growth stage (*P* <0.05).

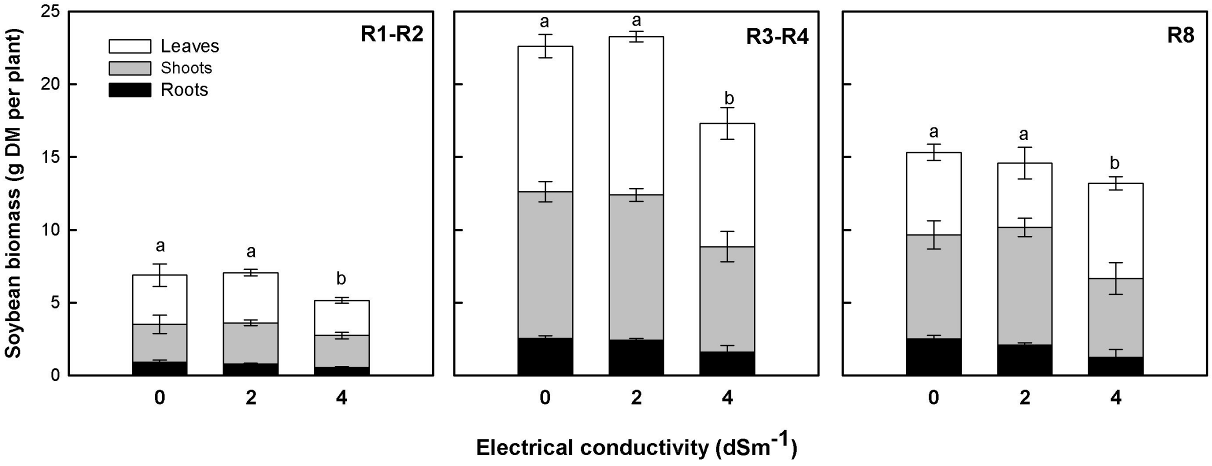

Figure 3. Relationship between A) soybean leaf Cl^- concentration at R1-R2 and grain yield, and B) soybean leaf K^+/Na^+ ratio at R1-R2 and grain yield. (**)$P < 0.01$; (***)$P < 0.001$.