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Full length research Paper

Phosphorus fertilization improves soybean growth under salt stress

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Soybean is one the most important crop in the world. This crop has expanded its cultivated area for regions with saline soils in several parts of the world. This fact occurs because of the large increase of the soybean productivity in recent decades, in parallel with an increasing demand for food. This work aimed to evaluate the salinity effects on the plant growth, and the interaction of phosphorus fertilization versus irrigation water salinity in soybean plants. The experiment was conducted in a greenhouse using recipients contained 6.0 dm^3 of mixture sand and commercial substrate (1:1 v/v). Five salinity levels in the irrigation water (0.8, 2.2, 3.6, 5.0 and 6.4 dS m^{-1}) and two levels of phosphorus fertilization (0 and 300 mg L^{-1}) were evaluated. After 36 days, the salt stress promoted reductions in the most of the growth variables, such as stem diameter, plant height, number and average length of branches, root length, shoot and root dry mass, and absolute growth rate. On the other hand, it increased the chlorophyll relative index, chlorophyll *a* and total content, and shoot/root dry mass ratio. The phosphorus-supplemented plants had higher stem diameter, number of branches, shoot dry mass and absolute growth rate. The salt stress-phosphorus interaction showed that the phosphorus attenuated the salt stress deleterious effects only on leaf area after 5 dS m^{-1} of saline water. Soybean plants can be irrigated using water with electrical conductivity up to 1.9 dS m^{-1} without disturbing its biomass components. The phosphorus fertilization improves the growth soybean subjected to salt stress but not reduce the salinity deleterious effects.

Key words: *Glycine max*, irrigation water, soybean, phosphorus, salinity stress.

INTRODUCTION

The inappropriate management of irrigation coupled with the fertilizers intensive use has contributed to increase the agricultural areas with salinity problems. This complication is particularly important in arid and semiarid regions due to low rainfall and high evaporative demand, hindering the lixiviation of the salts located in the soil arable layer. In Brazil, there are approximately 9,000,000 hectares with salinity problems, which are result from natural factors (primary salinity) and/or human activity (secondary salinity), being the most secondary salinization located in the irrigated areas of the Northeast Region (Carneiro et al., 2002).

Although the large majority of crops are severely affected by salt stress, some plant species are able to produce in an economically viable manner soils with high

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salinity levels (Correia et al., 2009). Moreover, some studies have shown that it is possible to use saline water in crop irrigation (Rhoades et al., 2000), such as in antelope grass (Morais Neto et al., 2012), sunflower (Morais et al., 2011), cucumber (Medeiros et al., 2009) and "Anão Verde" coconut (Marinho et al., 2006), which tolerate conductivity in the water irrigation up to 2.0, 3.53, 3.5 and 10 dS m^{-1}, respectively.

Several researches have evaluated the influence of phosphorus fertilization in crop salt stressed as strategy to reduce the adverse salinity effects (Sharpley et al., 1992; Shibli et al., 2001; Lacerda et al., 2006); however, various contradictory findings were found. Normally, the salinity reduces the plant tissue P contents through both the effects of ionic strength and the decreased in solubility of this mineral in response to increase of NaCl levels in the soil (Garcia et al., 2005).

In radish, the increase of P level in the growth medium was strongly correlated to the salt tolerance of this crop irrigated with saline water of electrical conductivity at 3.5 dS m^{-1} (Oliveira et al., 2010). Therefore, the interaction between salinity and phosphorus nutrition is complex and it depends on plant species or cultivar, stage of development, salt composition and concentration, and the P level in the medium growth (Grattan and Grieve, 1999).

Soybean (*Glycine max* L.) is one of the most important crop in the world, having doubled its productivity over the last two decades, increasing the worldwide pressure for more agricultural land because of the continuous increase for food demand (USDA, 2008). The high demand for cultivable land explains the actual reason of soybean not being cultivated only in traditional arable land, but also in marginal soils, that provide an expansion of this crop in saline soils from various parts of the world (Essa, 2002; Scanlon et al., 2005). According to Ayres and Westcot (1999), the soybean is moderately tolerant to salinity, showing reductions in growth only in the soils with electrical conductivity above 5.0 dS m^{-1}; however, other studies suggest lower values, around 2.0 dS m^{-1} (Katerji et al., 2000).

We hypothesized that phosphorus nutrition reduces the deleterious effects of salinity and improves the growth of soybean plants. Therefore, this study aimed to investigate the interaction between phosphorus fertilization and salinity in the irrigation water in soybean, and it evaluated the growth parameters, and the chlorophyll and phosphorus contents.

MATERIALS AND METHODS

Growth conditions and harvesting

The experiment was conducted in a greenhouse located in Fortaleza, Brazil (latitude 3°44'S, longitude 38°34'W), from September to November 2011. Soybean seeds (*Glycine max* L. Merril), cv. FT - 106 (Monsoy), were sown in plastic pots of 8 dm^3 with substrate prepared from the combination of sand and commercial substrate (1:1 v/v). Direct seeding was used as planting method, putting up 9 seeds in each pot, distributed in three planting pits of 1 cm depth.

The substrate used in the experiment had nutrient concentration of: 600.5, 437.6, 2875 475, 0.38, 4224.82, 297.33, 29.02, 779.69, 0.22, and 0.11 mg dm^{-3} of Ca, Mg, K, Na, P, Cl, N-NO_3, N-NH_4, S-SO_4, Fe, and Cu, respectively. The pH and electrical conductivity (EC) were respectively, 6.83 and 3.32 dS m^{-1}.

Five days after the emergence (DAE), the phosphorus levels of 0 and 300 mg dm^{-3} (superphosphate - P_2O_5), and the nitrogen (urea), potassium (potassium chloride) and micronutrients (FTE BR12) at 100, 150, and 50 mg L^{-1}, respectively, were applied. The fertilizers were diluted in water, and was added a volume of water sufficient to bring the soil up to field capacity. Eight DAE, we conducted a thinning to let one plant per pot; and after ten DAE, the saline treatments were started (irrigation with saline water).

Salt solutions were prepared by dissolving reagent NaCl and $CaCl_2.2H_2O$ (7:3 w/w) in tap water, and the values of water electrical conductivity (ECw), 0.8, 2.2, 3.6, 5.0 and 6.4 dS m^{-1}, were adjusted with conductivity meter with automatic correction of temperature. Sodium adsorption ratio (SAR) was 7.1, 19.44, 28.44, 35.22 and 40.81, respectively, for water with electrical conductivity of 0.8, 2.2, 3.6, 5.0 and 6.4 dS m^{-1}.

On day 36 of salt stress was measured the relative chlorophyll index (SPAD). Then, five plants from each treatment were individually harvested and determined plant height (H), stem diameter (ϕ stem), number of branches (NB), mean length of branches (MLB), root length (RL) and total leaf area (TLA) (LI - 3100, Area Meter, Li-Cor., Inc., Lincoln, Nebraska, USA). After that, the roots and shoot (stem + leaves) were separated and stored at -20ºC. The harvested material was dried by lyophilization and weighed to determine the shoot and root dry mass.

From the data of leaf area and shoots and roots dry mass, the indexes of shoot/root ratio, absolute growth rate (AGR), specific leaf area (SLA), leaf weight ratio (LWR) and leaf area ratio (LAR) were calculated according to the following equations (Benincasa, 2003):

Shoot/root ratio = (shoot dry mass) (root dry mass)$^{-1}$

AGR = $(DM_2 - DM_1) / (t_2 - t_1)$ (g day^{-1})

DM = dry mass total; t = time in days

SLA = LA/FM_T (dm^2 g^{-1})

LA = leaf area; FM = fresh mass of leaves

LWR = DM_L/DM_T

DM_L = dry mass of leaves; DM_T = total dry mass

Table 1. Mean square of variation sources and coefficient of variation for parameters SPAD index (SPAD), concentrations (µg cm^{-2}) of chlorophylls *a* (Chl *a*) and *b* (Chl *b*), chlorophyll *a/b* ratio (Chl *a/b*) and chlorophyll total (Chl *total*), and contents (µg $plant^{-1}$) of chlorophyll *a* (Chl a_C), *b* (Chl b_C) and total (Chl*total* $_C$) of soybean plants fertilized with phosphorus at 0 and 300 mg dm^{-3} after 36 days of salt stress

Parameters	Source				
	Saline (S)	Phosphorus (P)	S × P	Error	C.V. (%)
SPAD	10.866 ^{ns}	55.335 ^{ns}	32.66 ^{ns}	18.2359	11.57
Chl *a*	11.913**	4.173 ^{ns}	3.296 ^{ns}	3.7231	32.57
Chl *b*	9.957 ^{ns}	10.869 ^{ns}	4.924 ^{ns}	4.448	53.82
Chl a*/b*	0.108 ^{ns}	0.041 ^{ns}	0.036 ^{ns}	0.0743	17.41
Chl *total*	41.392*	28.492 ^{ns}	14.072 ^{ns}	13.1893	36.91
Chl a_C	14338392,7**	33786485,2 ^{ns}	34199301,3 ^{ns}	30317225,4	39,11
Chl b_C	51823376,6*	134746,94 ^{ns}	4984966,9 ^{ns}	14568385,8	42,46
Chl *total* $_C$	359057623,9**	38174521,9 ^{ns}	56258805 ^{ns}	76987774,2	38,05

* (p< 0.05), ** (p< 0.01) and ^{ns}(p> 0.05), for F test.

LAR = LA/DM_T (dm^2 g^{-1})
LA = leaf area; DM_T = total dry mass.

Determination of chlorophyll a, b and total

For determination of chlorophyll concentrations, five leaf discs (1.0 cm diameter) from fully expanded leaflets of each plant were collected and macerated in a mortar with 10 mL of 80% aqueous acetone (v/v). Then, the homogenate was centrifuged at 3,000 × *g* for 10 min, and the supernatant was collected and subjected to absorbance reading at 663 (A_{663}) and 645 nm (A_{645}). The chlorophyll *a* [Chl *a*], *b* [Chl *b*] and total [Chl *total*] concentrations were estimated using equations based on the specific absorption coefficients as reported by Arnon (1949). The chlorophyll concentrations were expressed as µg cm^{-2}.

Additionally, from the data of chlorophyll *a*, *b* and total concentrations and the leaf area, we calculated the chlorophyll total contents; being the values expressed as µg $plant^{-1}$.

Phosphorous concentrations

The phosphorus was extracted from 0.1 g of lyophilized samples from the shoot (Pshoot) and roots (Proot), subjecting to digestion with nitric acid. The phosphorus concentrations were estimated according to Braga and Defelipo (1974) and based on the absorbance reading at 660nm with potassium phosphate (KH_2PO_4) as a standard.

Experimental design and data analyses

The experimental design was completely randomized in a 5 x 2 factorial scheme, consisting of 5 salinity levels in the irrigation water - CEw (0.8, 2.2, 3.6, 5.0 and 6.4 dS m^{-1}) and 2 phosphorus levels (0 and 300 mg dm^{-3}) with five repetitions, being the experimental unit of one plant. F-test and its significance according to the ANOVA for salinity at 1 or 5% were used to implement significance analysis for regression. Statistical analyses were performed using the Sisvar software (Ferreira, 2011).

RESULTS AND DISCUSSION

After 36 days of salt stress, there were no significant changes (P > 0.05) in the SPAD index (SPAD), chlorophyll *b* concentration (Chl *b*) and chlorophyll *a*/*b* ratio (Chl *a*/*b*) (Table 1). On the other hand, the chlorophyll *a* (Chl *a*) and total concentration (Chl *total*), as well as the amount of chlorophyll *a* (Chl a_C), *b* (Chl b_C) and total (Chl*total* $_C$) were changed by the increase of salinity in the irrigation water (P < 0.05) (Table 1 and Figures 2a, b and c).

The Chl *a* and *total* concentrations were increased with increase of the salinity in the irrigation water from 0.8 to 4.5 dS m^{-1}, being the highest values achieved at high levels of salinity (Figures 1a and b). Above the 4.5 dS m^{-1} conductivity, theses parameters were reduced by salinity. However, the variables estimated from chlorophyll concentrations expressed as µg cm^{-2} do not express correctly the response of soybean plants to salt stress.

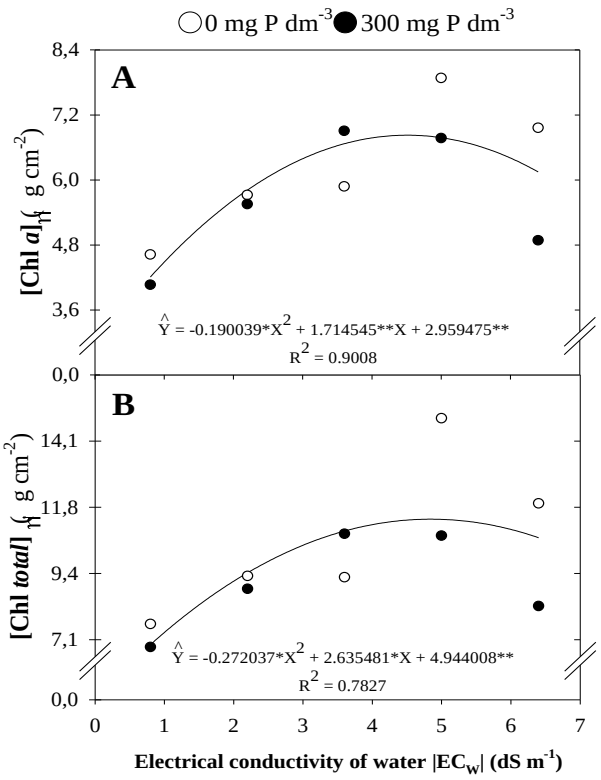

Figure 1. Regression curves for concentrations of chlorophyll *a* ([Chl *a*], **a**) and total ([Chl *total*], **b**) expressed as µg cm^{-2}, of soybean plants fertilized with phosphorus at 0 and 300 mg dm^{-3} after 36 days of salt stress.

As observed in the figures 2a, b and c, the total chlorophyll content (expressed as µg $plant^{-1}$) increased only until 2.7 dS m^{-1}; and above this value, the chlorophyll content was reduced with increase of salinity in the irrigation water.

Although the last majority of studies express the chlorophyll content in µg per cm^2 (Khawale et al., 2003; Heidari, 2011), this feature may bring false results. For example, in *Phaseolus vulgaris*, as reported by Seemann and Critchley (1985), low saline levels (25 mM NaCl) did not result in alterations in the chlorophyll concentrations, however, these compounds were reduced from 35 to 15 µg cm^{-2} in high saline levels. Thus, the reduction of chlorophyll content by salinity was around 57%. If the chlorophyll content were expressed as µg $plant^{-1}$, would not this reduction be greater?

The reductions of chlorophyll content induced by salinity may be due to an inhibition of chlorophyll synthesis or to an enhancement of chlorophyll degradation, by increasing of chlorophylls (EC: 3.1.1.14) enzyme activity (Stivesev et al., 1973). Several studies have shown that chlorophyll content is severely affected in response to increasing salt stress. In grape, basil and chickpea genotypes, the chlorophyll concentrations were reduced with increase of salt stress (Khawale et al., 2003; Garg and Singla, 2004; Heidari, 2011).

The saline stress altered negatively the stem diameter (ϕ stem), height (H), number of branches (NB), mean length of branches (MLB) and root length (RL), significantly at 1% (Table 2 and Figure 3). Phosphorus fertilization increased the ϕ stem and NB under salinity conditions. Moreover, there was interaction between salinity levels and phosphorus level on total leaf area (TLA).

Salt stress significantly reduced the stem diameter and plant height at a rate of 0.36 mm and 1.6 cm per increase of ECw unity, respectively; nevertheless, the phosphorus fertilization attenuated the reduction of the stem diameter to 0.34 mm per increase of ECw unity (Figures 3a, b). In soybean plants without phosphorus, the increase of 2.18

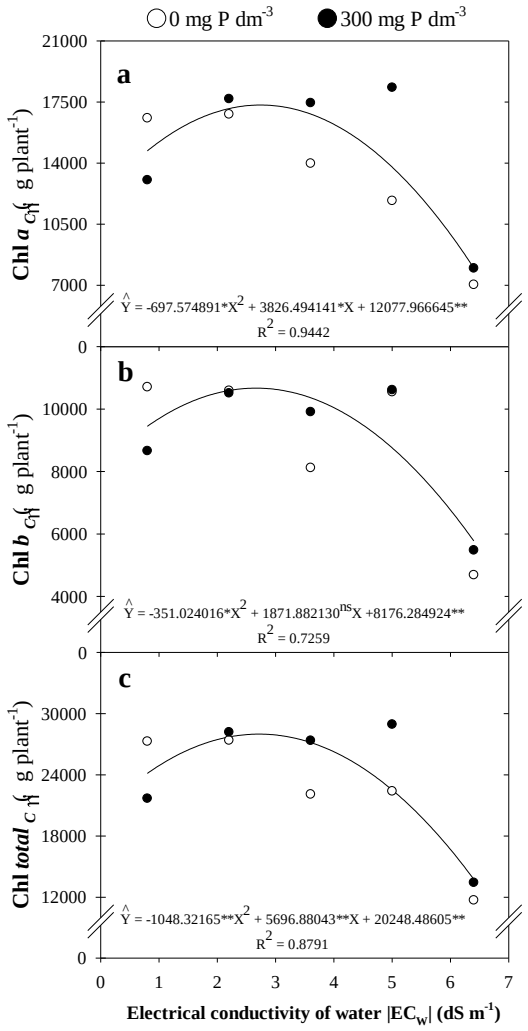

Figure 2. Regression curves for content of chlorophyll *a* (Chl *a* C, **a**), *b* (Chl *b* C, **b**) and total (Chl *total* C, **c**) expressed as µg plant^{-1}, of soybean plants fertilized with phosphorus at 0 and 300 mg dm^{-3} after 36 days of salt stress.

dS m^{-1} in the irrigation water reduced one branch unity; whereas in phosphorus-supplemented plants, this reduction only happened with increase of 3.68 dS m^{-1} (Figure 3c).

The parameters of mean length of branches and root length were reduced by salt stress at a rate of 1.6 and 3.95 cm per increase of ECw unity, respectively (Figures 3d, e). On the other hand, the interaction salinity × phosphorus showed that the phosphorus was able to reduce the salt deleterious effects on total leaf area by 42% as from ECw of 5 dS m^{-1} (Figure 3f).

After 36 days of salinity the variables shoot dry mass, roots dry mass, shoot/root ratio (shoot/root) and absolute growth rate (AGR) were significantly altered by salt stress ($p < 0.01$) (Table 3 and Figure 4). On the other hand, the phosphorus fertilization increased the shoot dry mass and AGR with increase of salinity in the irrigation water (Table 3 and Figures 4c, d).

Our results corroborate with very recent findings in *Phlomis purpurea*, reported by Alvarez et al. (2012), which showed that the irrigation with saline water of 4 dS m^{-1} decreased the plant height, total leaf area, and shoot

Table 2. Mean square of variation sources and coefficient of variation for parameters stem diameter (ϕstem), height (H), number of branches (NB), mean length of branches (MLB), root length (RL) and total leaf area (TLA) of soybean plants fertilized with phosphorus at 0 and 300 mg dm^{-3} after 36 days of salt stress

Parameters	Source				C.V. (%)
	Saline (S)	Phosphorus (P)	S X P	Error	
ϕstem	6,500**	3,920**	0,220 ^{ns}	0,53	11,38
H	164,770**	98,000 ^{ns}	20,850 ^{ns}	32,05	10,99
NB	8,130**	5,120*	0,970 ^{ns}	0,95	21,01
MLB	128,33**	13,520 ^{ns}	13,670 ^{ns}	8,57	18,96
RL	831,85**	64,980 ^{ns}	31,330 ^{ns}	46,17	12,07
TLA	63708552,57**	1292832*	618245,45*	209749	18,48

* (p< 0,05), ** (p< 0,01) and ^{ns}(p> 0,05), for F test.

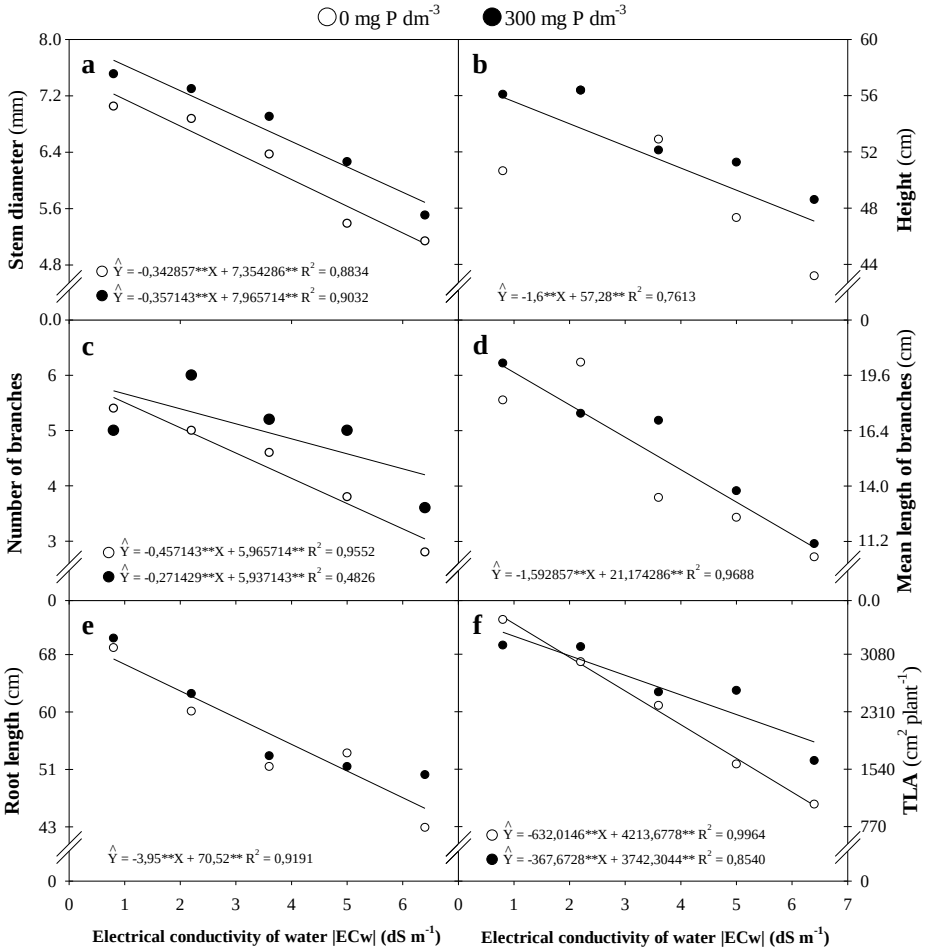

Figure 3. Regression curves for stem diameter (**a**), height (H, **b**), number of branches (NB, **c**), mean length of branches (MLB, **d**), root length (RL, **e**) and total leaf area (TLA, **f**) of soybean plants fertilized with phosphorus at 0 and 300 mg dm^{-3} after 36 days of salt stress.

and roots dry mass. However, our results for soybean plants did not confirm the findings in *Phlomis purpurea*, especially in the case of parameters stem diameter and shoot/root ratio, in which had no changes by salinity (Alvarez et al., 2012).

The dry mass of shoot and roots were reduced at a rate

Table 3. Mean square of variation sources and coefficient of variation for parameters shoot dry mass, roots dry mass, shoot/root dry mass ratio (Shoot/root ratio), absolute growth rate (AGR), specific leaf area (SLA), leaf weight ratio (LWR), leaf area ratio (LAR), shoot (P shoot) and roots (P root) phosphorus concentration of soybean plants fertilized with phosphorus at 0 and 300 mg dm^{-3} after 36 days of salt stress

Parameters	Source				C.V. (%)
	Saline (S)	Phosphorus (P)	S X P	Error	
Shoot dry mass	175,85**	54,08**	10,33 ^{ns}	4,68	14,42
Roots dry mass	7,9418**	0,4108 ^{ns}	0,1070 ^{ns}	0,2423	23,71
Shoot/root ratio	13,65**	3,4959 ^{ns}	3,5885 ^{ns}	1,6829	16,64
AGR	0,1210**	0,0285**	0,0058 ^{ns}	0,0029	14,72
SLA	2,203E^{-7} ^{ns}	3,362E^{-7} ^{ns}	2,507E^{-7} ^{ns}	2,19E^{-7}	17,36
LWR	9,3E^{-4} ^{ns}	13,55E^{-4} ^{ns}	10,07E^{-4} ^{ns}	1,84E^{-3}	11,36
LAR	0,0418 ^{ns}	0,0429 ^{ns}	0,0429 ^{ns}	0,0507	15,7
P shoot	6,34^{ns}	165,72**	16,81*	5,34	16,39
P root	50,44^{ns}	304,71*	49,49^{ns}	40,00	25,04

* (p< 0,05), ** (p< 0,01) and ^{ns}(p> 0,05), for F test.

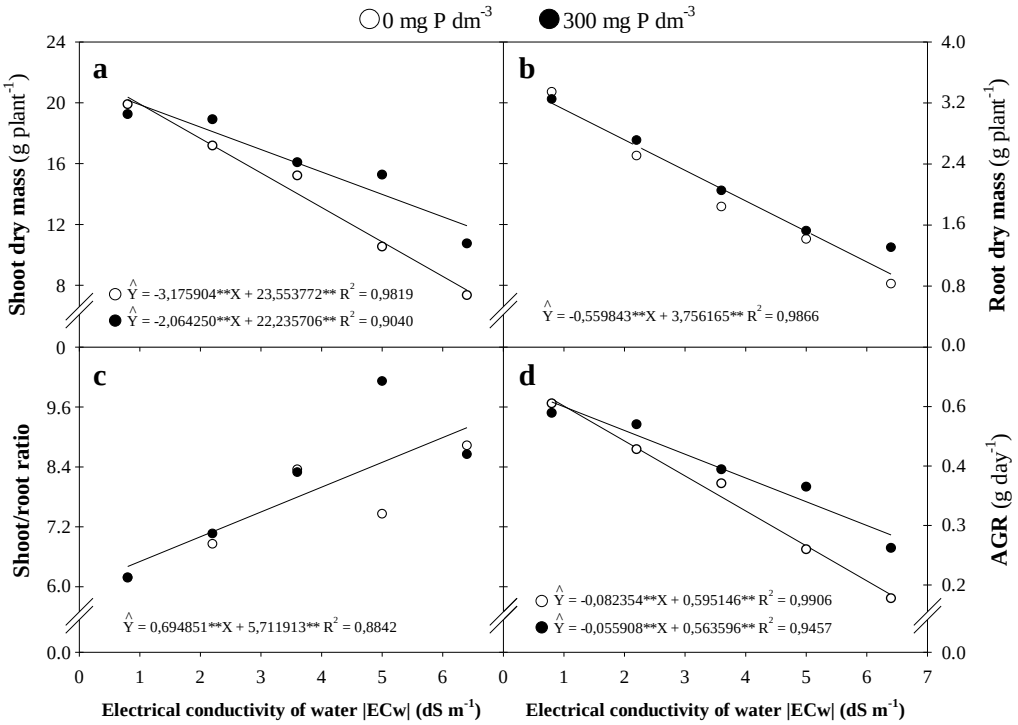

Figure 4. Regression curves for shoot (**a**) and roots dry mass (**b**), shoot/root ratio (**c**), absolute growth rate (AGR, **d**) of soybean plants fertilized with phosphorus at 0 and 300 mg dm^{-3} after 36 days of salt stress.

of 3.17 and 0.55 g per increase of ECw unity, respectively (Figures 4a, b). The relative reduction in dry mass per unit of ECw was higher in roots (94.8%) than shoots (84.7%). Thus, the shoot/root ratio was increased at a rate of 0.69 per increase of ECw unity (Figure 4c). On the other hand, the phosphorus supplementation promoted positive effects in plants under salt stress, reducing by 35% the damage of shoot dry mass (Figure 4a).

Highest shoot dry mass in phosphorus-fertilized plants were probably due to the large increase in the leaf area per plant, which increased the area available for photosynthesis, and thereby provided a higher biomass production (Figures 3f and 4a).

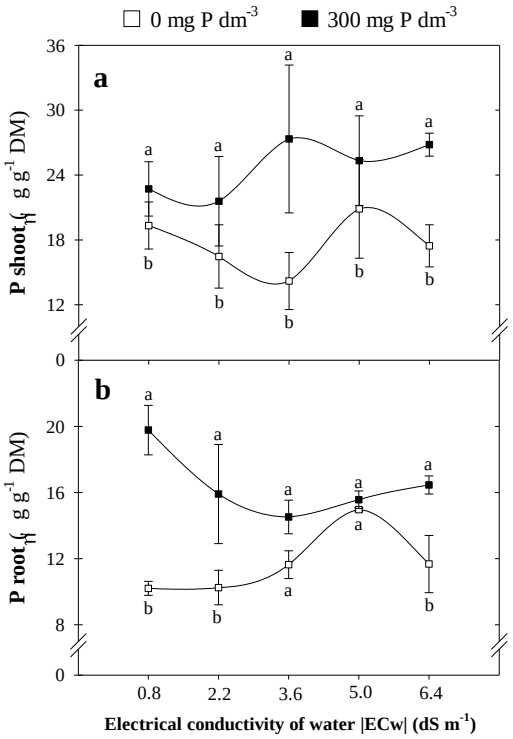

Figure 5. Phosphorus concentrations in the shoot (**a**) and roots (**b**) of soybean plants fertilized with phosphorus at 0 and 300 mg dm^{-3} after 36 days of salt stress. In the same salinity level, significant differences due to dose of phosphorus are indicated with different lowercase letters using F test.

There was a reduction in the AGR of 82.3 mg per day for each increase of ECw unity in the irrigation water; however, the phosphorus fertilization alleviated this reduction by up 32.11%, and the reduction of AGR in phosphorus-treated plants was 55.9 mg per day (Figure 4d).

The effects of salt stress in plants may be the result of two characteristic processes. Firstly, from hydric stress caused by osmotic effects of salts; and secondly, from specific effects of ions, mainly Na^+ and Cl^-, which may cause toxicity or change in the plant's ability to uptake, transport and utilize the ions necessary for growth (Munns and Tester, 2008). Besides reducing biomass production, the salinity may also change the partitioning of photo assimilates among plant parts (Ahmad et al., 2005). Therefore, the responses of plants to salinity stress in terms of growth are the ultimate expression of several interacting physiological and biochemical parameters.

The reduction on plant growth by salinity has been described in several crops species (Turan et al., 2010; Morais Neto et al., 2012; Yang et al., 2012). Unlike this study, in chickpea varieties, the effects of salinity to 4-6 dS m^{-1} in the irrigation water were less severe as reported by Garg and Singla (2004); and they added that the reductions by salinity in shoot and roots dry mass was to 3-7% and 5-14%, respectively; while in soybean these reductions were 58% and 74%, respectively in shoot and roots dry mass.

Whereas the shoot dry mass components of phosphorus-fertilized plants were not affected due to salinity by using water with electrical conductivity up to 2.7 dS m^{-1}; in unfertilized plants, yield components were not affected by using saline water up to 2.4 dS m^{-1}. On the other hand, the root biomass was reduced by salinity in the irrigation water up to 1.9 dS m^{-1}, regardless of phosphorus supplementation (Figure 4).

The salinity had no significant effect in phosphorus concentrations in soybean plants (Table 3). There was interaction between salinity levels and phosphorus fertilization ($P \leq 0.01$) for the shoot phosphorus concentrations (Table 3); the phosphorus fertilization increased the phosphorus concentrations only at the levels of ECw 0.8, 2.2 and 6.4 dS m^{-1} (Figure 5a).

Nevertheless, in the roots, the phosphorus supplementation increased the shoot phosphorus concentrations at all levels of salinity in the irrigation water (Figure 5b).

Several researches showed contradictory results about the phosphorus concentrations in plants under saline conditions. Whereas in maize plants the salinity increased the phosphorus concentrations in shoot and roots (Turan et al., 2010), in canola it reduced the shoot phosphorus concentrations (Farshidi et al., 2012). Unlike these studies, the salt stress did not affect the phosphorus concentration in soybean plants (Table 3 and Figure 5).

According to Sharpley et al. (1992), the phosphorus content can be reduced around 20 and 50% in saline environments without evidence of phosphorus deficiency in plants. Additionally, in salt stressed maize plants, Ferreira et al. (2007) observed linear reductions in leave P content by salinity at 90 and 120 days after sowing. On the other hand, Lacerda et al. (2006) evaluating the development of sorghum plants subjected to different phosphorus and salinity levels, verified the existence of interaction between salinity and phosphorus on the development and plant nutrition. In this study, the leaf phosphorus concentrations were increased in response to increase of phosphorus in solution, being the highest values in salt stressed plants.

CONCLUSION

Soybean plants can be irrigated using water with electrical conductivity up to 1.9 dS m^{-1}, without disturbing its biomass components. The phosphorus fertilization improves the growth of soybean plant subjected to salt stress but it does not reduce the salinity deleterious effects. The chlorophyll content in soybean and other crops should be preferentially expressed as µg $plant^{-1}$.

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