

Effect of Salt on the Growth and Metabolism of *Glycine max*

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ABSTRACT

Soybean plants cultivated with 50, 100 and 200 mM of NaCl, revealed that root growth was less affected by salinity than shoots. Salinity led to a reduction in leaf area and an increase in water content of the roots. These factors could contribute to the adaptation of the plant, improving its hydration. Although nitrate and free amino acid levels were reduced by salt treatment in roots, protein content of leaves was not altered. Salinity led to alterations in xylem amino acid composition, with increases in Ser, Ala, Gaba and Pro and a decrease in Asn. Similar changes were seen for Asn and Ser in roots together with a much stronger increase in Gaba. It is suggested that the decline in Asn reflects its conversion to Ala and Gaba (via Glu) in the roots while the increase in Pro and Gaba could be related to the adaptation of the plant to salinity.

Key words: soybean, stress, amino acid transport, asparagine, alanine, γ -aminobutyrate

INTRODUCTION

Salinization of soil affects approximately 20% of the world's agricultural land (FAO 2000). Salinity affects the dry and fresh mass (Tabatabaei 2006; Blanco et al. 2008; Demir and Mazi 2008) the development and the productivity of cultivated species (Neto and Nogueira 1999; Kaya et al. 2007; Zheng et al. 2008; Türkan and Demiral 2009, Galvan-Ampudia and Testerink 2011).

Reduction in the growth rate of the leaf can represent an adaptation to salt stress, since increased levels of salts in the soil impedes the uptake of water by the plant (Carillo et al. 2005) and the reduction in leaf area limits transpiration (Neumann 1997). Another adaptation to salinity is the increase in succulence, which reduces the concentration of salts in the protoplasm (Ghoulam et al. 2002). The greater growth of the roots in comparison with the aerial part is also considered

an adaptation to saline stress (Alshamary et al. 2004) since it results in an increased surface area for water uptake, thereby preventing dehydration (Okusanya 1977).

Salinity reduces the uptake and transport of nitrate (Carillo et al. 2005; Surabhi et al 2008; Maaroufi-Dguimi et al. 2011) and, consequently, the assimilation of nitrogen necessary for protein synthesis (Silveira et al. 2001; 2003; Qu et al. 2011).

Abiotic stresses alter the form and concentration of amino acids transported in plants (Souza and Sodek 2003; Renault et al. 2010). Under salt stress an accumulation of Asn (Ashraf and Harris 2004) and Gaba were observed (Bolarín et al. 1995)

Reactive oxygen species (ROS) are commonly produced in the cell and can act as signals in several transcription pathways, leading to altered patterns of gene expression which allow the plant to adjust adequately to the environment (Dutilleul

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et al. 2003; Foyer and Noctor 2005a; 2005b; Gratão et al. 2005; Noctor 2006; Mhamdi et al. 2010; Queval et al. 2011). However, stress can result in an increase in ROS, causing the oxidation of lipids, proteins and DNA, thereby compromising the growth of the plant (Duttilleul et al. 2003; Foyer and Noctor 2005a; Gratão et al. 2005; Gomes-Júnior et al. 2007). Some studies have indicated that Pro may act as a scavenger of ROS (Vicente et al. 2004) and can stabilize membranes during salt stress (Hasegawa et al. 2000). Its accumulation has been related to an increase in tolerance to salt stress (Kaya et al. 2007). Another role attributed to Pro is as an osmolyte, permitting the plant to adjust osmotically under salinity (Hasegawa et al. 2000). Nevertheless, its role in the osmotic adjustment of plants cultivated under salinity can vary from species to species (Silveira et al. 2003; Misra and Gupta 2005).

Brazil has salinized soils in 11% of its territory, which is equivalent to an estimated area of 90 million ha (FAO 2000). Brazil is one of the world's leading soybean producers. According to a USDA (2005) report, Brazilian soybean production was estimated at 60 million tons for the 2005/2006 crop. Soybean is considered to be moderately tolerant to salt stress (An et al. 2001). Nonetheless, there is a great diversity of responses among cultivars to salinity (Lee et al. 2004).

In this study soybean plants were used with the objective of determining the effects of salt on growth and metabolism together with an evaluation of the measured parameters as to their possible relationship with the adaptation of the plant to salinity.

MATERIALS AND METHODS

Plant material and growth conditions

Soybean plants, *Glycine max* (L.) Merr., cv. IAC 17, were cultivated without inoculation under natural conditions of light and temperature (23- 29 °C), for 45 days, in the greenhouse. The mean maximum and minimum relative humidity over the experimental period was 97.9% and 54.3%, respectively. The plants were grown in 1.5 L pots containing perlite and irrigated with tap water up to the V1 stage (Fehr et al. 1971). From then on they were given 250 mL of a complete nutrient solution (Hoagland and Arnon 1938) twice per week. In order to avoid osmotic shock,

NaCl was added to the nutrient solution in gradually increasing concentrations (Hasegawa et al. 2000), until the final concentrations of 50 mM, 100 mM and 200 mM were reached. The roots were assured a uniform distribution of the applied salt by placing the perforated pots with the plants inside similar non-perforated pots. This allowed the solution to rise by capillarity up the inner pot containing the plants, such that about 1 cm of nutrient solution remained in the outer pot. In order to avoid the accumulation of salt, immediately before the next salt application the substrate was washed and the external pot temporarily removed to allow complete drainage of the washed substrate.

Growth measurements

Measurements were taken of the length of the main root and shoot. Leaf area was determined using a LI-COR, model LI-3100. After fresh weight measurements, the roots, stems and leaves were dried in an oven at 80°C, for 72 hours, and weighed. The water content was calculated by subtracting the dry weight from the fresh weight value and then dividing by the dry weight.

Protein determination in leaves

Samples of fully expanded leaves were ground to a powder under liquid nitrogen and extracted with 0.1 N NaOH for 24 h. The extract was centrifuged at 1,200 x g, for five min and the supernatant used for the assay of proteins (Bradford 1976).

Extraction and assay of nitrate and amino acids from roots

The harvest, preparation and extraction of root material were carried out as described by Souza and Sodek (2003). Nitrate was determined by the method of Cataldo et al. (1975) and total free amino acids according to Yemm and Cocking (1955)

Separation and analysis of xylem sap amino acids by HPLC

Amino acids were separated by reverse phase HPLC, using *o*-phthaldialdehyde (OPA) derivatives, as described previously by Puiatti and Sodek (1999). Since proline does not form a derivative with OPA, this amino acid was analyzed by a different HPLC system using FMOC-Cl derivatives (Nasholm et al 1987) with minor modifications, as described by Marur et al. (1994).

Experimental design and data analysis

Treatments were completely randomized, using 10 replicates per treatment, where each pot with two plants represented a replicate. Data were submitted to an analysis of variance and, when *F* was significant, means were compared by the Tukey test at the 5% probability level.

RESULTS

Effect of salinity on growth

Salinity did not alter the length of the root of the soybean plants, but the height of the shoot was reduced by up to 34% for plants receiving 200 mM NaCl, compared to the control (Table 1). A

gradual reduction in leaf area was observed with the increase in salinity. This attained 57 % in the treatment with 200 mM of salt. The fresh weight of leaves and stems showed expressive reductions with increasing concentrations of NaCl in the medium but remained constant in the roots. The dry weights of leaf, stem and roots were also reduced in relation to the control with increasing salinity. The greatest reduction (62%) occurred in the stems, for the 200 mM treatment. No significant differences were observed in the water content of the aerial part of plants treated with salt compared to the control. However, the water content of the roots increased significantly in the presence of salt (Table 1).

Table 1 – Growth measurements of soybean plants, after receiving complete nutrient solution with different NaCl concentrations.

Analysis	NaCl concentration (mM)			
	0	50	100	200
Root length (cm)	31.40 a	28.60 a	24.10 a	26.40 a
Shoot length (cm)	83.60 a	70.60 ab	62.00 bc	55.20 c
Leaf area (cm ²)	574.64 a	426.46 b	327.59 bc	246.66 c
Leaf fresh mass (g)	5.00 a	3.67 ab	3.17 b	2.50 b
Shoot fresh mass (g)	4.95 a	3.76 ab	3.13 bc	2.55 c
Root fresh mass (g)	3.70 a	3.51 a	2.65 a	2.79 a
Leaf dry mass (g)	2.04 a	1.49 ab	1.37 ab	0.96 b
Shoot dry mass (g)	1.37 a	0.94 ab	0.81 bc	0.52 c
Root dry mass (g)	0.33 a	0.21 ab	0.19 b	0.18 b
Shoot water content (g g ⁻¹ DM)	2.44 a	3.12 a	2.62 a	3.14 a
Root water content (g g ⁻¹ DM)	9.51 c	13.76 b	14.35 ab	16.65 a

Means followed by different letters are significantly different by the Tukey test ($P \leq 0.05$). DM= dry mass

Effect of salinity on biochemical parameters

The level of protein of the soybean leaves was not altered significantly by salinity, compared to the control (Table 2). A strong decline in the nitrate content of the roots was found, in the presence of NaCl. At the lowest salt concentration, 50 mM, a

decrease of 72 % was recorded, followed by 80 % and 83 % for the treatments using higher concentrations of salt (100 mM and 200 mM, respectively).

The level of total free amino acids in the root decreased for all salt concentrations tested (Table 2).

Table 2 – Soluble protein in leaves, free amino acids and nitrate in roots of soybean, after receiving complete nutrient solution with different NaCl concentrations.

Analysis	NaCl concentration (mM)			
	0	50	100	200
Proteins (mg g ⁻¹ FM)	32.55 a	29.07 a	33.76 a	29.92 a
Nitrate (µg g ⁻¹ FM)	48.56 a	13.56 b	9.33 b	8.11 b
Amino acids (µmol g ⁻¹ FM)	2.58 a	1.06 b	0.75 b	0.98 b

Means followed by different letters are significantly different by the Tukey test ($P \leq 0.05$). FM= fresh mass.

The amino acid composition of both the xylem bleeding sap and root tissue of the soybean plants under the different salt treatments is shown in Tables 3 and 4, respectively. In the control, Asn was the most abundant amino acid (88%) in the xylem sap. Under the salt treatments there was a reduction in the proportion of Asn in the xylem sap and an increase in the other amino acids,

especially for Ser, Ala, Gaba and Pro. In the control these amino acids were close to 1% of the total, but under salt treatment Ser and Ala reached 9% or more (Table 3).

In the roots, Asn was again the most prominent amino acid (79%) and after treatment with salt it declined while Gaba showed the highest increase (Table 4).

Table 3 - Amino acid (AA) composition (mol %) of the xylem sap of soybean, after receiving complete nutrient solution with different NaCl concentrations.

AA (mol %)	NaCl concentration (mM)			
	0	50	100	200
Asp	1.12	2.25	3.62	1.49
Asn	88.84	60.41	57.53	60.26
Ser	0.87	9.21	8.96	5.12
Ala	0.70	9.20	9.40	11.25
Gaba	1.34	2.62	2.15	3.76
Pro**	0.74	3.52	7.72	4.68
Others*	6.39	12.79	10.62	13.44

*All other amino acids (Glu, Gln, His, Gly, Thr, Arg, Tyr, Met, Val, Phe, Ile, Leu and Lys) that individually were less than 3% in any treatment. Pro** was identified by derivatization with FMOC.

Table 4 - Amino acid (AA) composition of the roots of soybean, after receiving complete nutrient solution with different NaCl concentrations.

AA (mol %)	NaCl concentration (mM)			
	0	50	100	200
Asp	1.81	6.26	3.13	2.73
Glu	2.88	5.96	3.28	3.12
Asn	79.39	51.61	65.34	67.68
Ser	1.61	4.32	3.38	4.25
Gaba	4.76	16.51	11.92	8.58
Others*	9.55	15.34	12.95	13.64

*All other amino acids (Gln, His, Gly, Thr, Arg, Tyr, Ala, Met, Val, Phe, Ile, Leu and Lys) that individually were less than 3% in any treatment.

DISCUSSION

Although salt stress can provoke several metabolic alterations in plants such as the peroxidation of lipids, reduction in chlorophyll content, increase in ROS and anti-oxidative enzyme activity (Monteiro et al. 2011), all such alterations are accompanied by reduced plant growth. The evaluation of the sensitivity of plants to stress is, quite often, based on data for plant growth (Ahmad et al. 2007; Koca et al. 2007; Ulfat et al. 2007; Zheng et al. 2008; Karlidag et al. 2009; Campestre et al. 2011). Flowers and Hajibagheri (2001) and Qian et al. (2004; 2007) reported that the maintenance of normal root growth under salinity demonstrates that a plant is tolerant to salt. However, it is possible that the maintenance of root growth under

such conditions was due to the adaptation of the plant to salt stress rather than tolerance strictly speaking. In this study, an analysis of the growth parameters revealed that soybean roots were less affected by salt than the shoot. The length of the roots and the fresh weight were not reduced significantly by increasing concentrations of salt. Water content of the roots, based on dry weight, increased under salinity. The accumulation of water in the cell has been related to greater tolerance to salt stress (Greenway and Munns 1980; Costa et al. 2003), since, as a result, the plant would be able to diminish the concentration of salts in the cytoplasm (Ghoulam et al. 2002), thereby avoiding the interference of Na⁺ with cellular functions (Tester and Davenport 2003).

The reduction in leaf area found not only in this study but for other species subjected to salt stress (Silveira et al. 2003; Tabatabaei 2006, Zheng et al. 2008) could be seen as an adaptation to limit transpiration (Neumann 1997). This reduction could be the result of ion accumulation in the leaves, which accelerates their abscission (Tester and Davenport 2003; Tabatabaei 2006). It could also be caused by the reduction in leaf expansion, as a result of the fall in turgor pressure (Marcelis and Hooijdonk 1999), since under salt stress water uptake by the plant would be impaired (Umezawa et al. 2002; Carillo et al. 2005).

Salinity led to decreases in the dry weights of the soybean plants in this study which coincides with similar data reported for this (El-Samad and Shaddad 1997; Liu and Staden 2001) as well as several other species (Costa et al. 2003; Silveira et al. 2003; Kaya et al. 2007). The reduction in dry mass could be explained by reduced photosynthesis, in view of the diminished leaf area and availability of nutrients (Marcelis and Hooijdonk 1999), such as the reduction in nitrate uptake observed in this study.

The diminished fresh weight of the aerial part of the soybean plants could have arisen as a consequence of the lower water potential, due to elevated concentrations of salt in the external medium (Marcelis and Hooijdonk 1999).

The stability of proteins found in this study was also observed by Costa et al. (2003) in leaves of cowpea plants cultivated under salinity. Although proteolysis can increase under salt stress (Silveira et al. 2003), there are proteins that are specifically synthesized under stress conditions (Bouché et al. 2005; Parker et al. 2006; Younis et al. 2009). It is possible that protein levels did not alter due to a compensating increase in the synthesis of such stress-specific proteins. A comparative study of protein profiles in the presence and absence of salt should help to clarify this point.

The reduction in the concentration of nitrate observed in the roots was also reported for cashew (Silveira et al. 2003) and cowpea (Silveira et al. 2001). This reduction could be due to a reduced uptake of nitrate as a result of the interference of the Cl⁻ ion on certain systems of membrane transport (Silveira et al. 2001; Rubinigg et al. 2003). According to Carillo et al. (2005), the Cl⁻ ion competes with nitrate for the binding site of the transporter. The fall in root amino acid concentration found in this study has also been reported for cowpea (Costa et al. 2003) and may

well be related to the reduced uptake of nitrate by the roots (Rubinigg et al. 2003; Carillo et al., 2005) leading to lower assimilation of nitrogen and consequently synthesis of amino acids (Silveira et al. 2001; Flores et al. 2002).

According to Amarante and Sodek (2006) changes in the amino acid composition in the xylem appear to be useful indicators of changes in root N metabolism and may reflect changes in dynamic aspects of this metabolism not always seen when simply measuring the amino acid composition of the root. Asn is the most abundant amino acid found in the xylem sap of non-nodulated soybean (McClure and Israel 1979). The present data show a reduction in this amino acid under salt treatment. Puiatti and Sodek (1999), Souza and Sodek (2003) and Thomas and Sodek (2006) observed a reduction in the abundance of Asn in the xylem sap of soybean plants cultivated with nitrate and subjected to hypoxia and discussed the possibility that the reduction might involve conversion of this amino acid (or its precursor, Asp) to Ala (see Fig. 1). The reduction in Asn in function of stress occurs in several situations and appears to involve a decline in the activity of asparagine synthetase, responsible for the transformation of Asp to Asn (Lima and Sodek 2003; Antunes et al. 2008). Often, but certainly not always, the reduction in xylem Asn is accompanied by a concomitant increase in Asp (Lima and Sodek 2003). When an increase in Asp is not evident, as in the present study with salt stress, it is possible that Asp was rapidly metabolized to products other than Asn, as shown in Figure 1. According to that scheme, two such products are Ala and Gaba, the two amino acids that showed a substantial increase in the salt treatment, concomitant with the decline in Asn. The synthesis of Gaba occurs by α -decarboxylation of Glu, a reaction that consumes protons and consequently helps stabilize the pH of the cell (Crawford et al. 1994). Although Glu did not increase significantly under salinity, it may well be that its turnover increased but its concentration was maintained through dynamic equilibrium between synthesis from Asn/Asp and transformation to Gaba and Ala (Fig. 1). On the other hand, GABA might have accumulated through polyamine degradation, as observed in roots of the soybean cultivar Suxie-1, grown under salt stress (Xing et al. 2007). Further studies are required before any conclusion can be drawn regarding GABA accumulation.

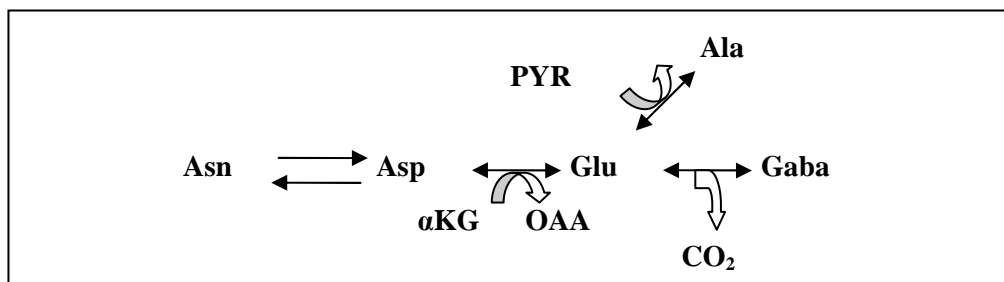


Figure 1 - Scheme showing alanine (Ala) and γ -aminobutyrate (Gaba) synthesis from asparagine. (Asn). Asp = aspartate; α KG = α -ketoglutarate; OAA = oxaloacetic acid; Glu = glutamate; Pyr = pyruvate.

Besides Gaba and Ala there was a perceptible increase in Pro and Ser in the xylem sap under salinity, which could be related to the observation that these amino acids are associated with abiotic stresses (Bouché et al. 2003; Souza and Sodek 2003; Ashraf and Harris 2004; Yazici et al. 2007). There is still controversy over the role of Pro in salt stress tolerance. While several authors regard Pro accumulation as a stress adaptation factor, others suggest it as a consequence of stress (see reviews by Ashraf and Harris 2004; Ashraf and Foolad 2007). When Pro content was measured in the leaves of two soybean cultivars with different sensitivities to salt stress, higher accumulation occurred in the most sensitive cultivar, leading to the conclusion that at least in that case the accumulation of Pro had no adaptive role towards salt stress (Moftah and Michel 1987). Another study with soybean and sorghum revealed that under saline stress the accumulation of Pro occurred only after the plants had become severely stressed (Waldren and Teare 1974). However, there are several reports of plant species showing that high concentrations of Pro are associated with considerable resistance to salinity (Misra and Gupta 2005; Ashraf and Orooj 2006; Ahmad et al. 2007; Koca et al. 2007; Yazici et al. 2007, Meloni et al. 2008). A recent review on the subject (Szabados and Savoré 2009) suggests that Pro accumulation is not an absolute requirement for adaptation to stress conditions but rather it is the metabolism of the amino acid that is important as a mechanism for plant homeostasis under stress. Since stress leads to a reduction in Calvin cycle activity, NADPH turnover will be slow which, under high light, will favor ROS accumulation. Pro biosynthesis in the chloroplast will consume NADPH and increase NADP^+ regeneration allowing increased electron chain activity and

thereby reduce photoinhibition and damage of the photosynthetic apparatus at higher light intensities. The authors also suggest that catabolism of the accumulated Pro after stress is important in providing energy for resumed growth through the generation of NADH and FADH_2 for mitochondrial respiration. Evidently more detailed studies of plant metabolism that go beyond the measurement of Pro levels are required to explain the role of Pro in salt stress.

CONCLUSION

The growth of roots of the soybean cultivar IAC 17 was less affected by salinity than the aerial part and the content of water in the roots increased under this condition. Leaf area diminished in the presence of salt. These factors together can contribute to the adaptation of the plant, improving its hydration. Salinity led to alterations in the composition of the amino acids transported in the xylem, with increases in amino acids characteristically related to stress, such as Pro and Gaba, which can contribute to the adaptation of the plant to salinity.

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