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# Alleviation of Salt Stress in Common Bean (*Phaseolus vulgaris*) by Exogenous Abscisic Acid Supply

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## Abstract

In this work the effect of abscisic acid (ABA) and 100 mM NaCl on common bean (*Phaseolus vulgaris* var. *Coco*) growth, nitrogenase activity, and nodule metabolism was studied. Experiments were carried out in a controlled environmental chamber and plants, at the vegetative growth stage (16 days old), were treated with ABA (1 μM and 10 μM) and 48 h later were exposed to saline treatment. Results revealed that plant dry weight, nodule dry weight, nitrogen fixation (acetylene reduction activity and ureides content), and most enzymes of ammonium and ureides metabolism were affected by both ABA and NaCl. The addition of 1 μM ABA to the nutrient solution before the exposure to salt stress reduced the negative effect of NaCl. Based on our results, we suggest that ABA application improves the response of *Phaseolus vulgaris* symbiosis under saline stress conditions, including the nitrogen fixation process and enzymes of ammonium assimilation and purine catabolism.

**Key words:** Abscisic acid; Ammonium assimilation; $N_2$ fixation; Nodule metabolism; *Phaseolus vulgaris*; Salt stress

## Introduction

Symbiotic nitrogen fixation in legume nodules is extremely sensitive to environmental stresses such as drought and salinity, which seriously limit legume yields in many arid and semiarid regions of the world. Nitrogen-fixing plants are more sensitive to salinity than N-fertilized plants (Cordovilla and others 1996; González and others 2001). Salt stress affects several physiological processes in root nodules. It affects the range of carbon substrate available to the bacteroids (Delgado and others 1993; Soussi and others 1998) and modifies the activity of enzymes related to carbon metabolism (Khadri and others 2001; Serraj 2003).

A critical question regarding $N_2$ fixation is whether the effect of the stress is first perceived in other physiological processes and the changes in $N_2$ fixation are a consequence of these other changes, or rather, the stress is directly and initially perceived by the $N_2$ fixation mechanism. Several studies have shown that $N_2$ fixation is more sensitive to salinity than to dry matter accumulation (Elsheikh and Wood 1990; Delgado and others 1994) and ammonium assimilation (Cordovilla and others 1994). Studies of Bekki and others (1987) and Soussi and

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others (1998) have revealed that nodulation was more affected by salinity than by nodule activity and suggested that the effect might be related to salinity inhibition of root-hair expansion and curling (Sprent and Zahran 1988). Serraj and others (1994) reported that the response of nitrogen fixation to salt showed a dramatic inhibition of nitrogenase activity due to the osmotic effect of NaCl. On the other hand, it has been suggested that proline accumulated in nodules under saline stress can contribute to osmotic adjustment (Delauney and Verma 1993) and also could be utilized as an energy source for $N_2$-fixation (Kohl and others 1994). However, commonly poor correlations between salt and proline concentrations have been found (Cordovilla and others 1996).

Salt apparently promotes the accumulation of ammonium, nitrate, and free amino acids in plants (Pessarakli and others 1989) and in nodules (Soussi and others 1999). In addition, Cordovilla and others (1994) found that NaCl tends to depress the activity of the enzymes involved in nodule ammonium assimilation like glutamine synthetase (GS) and NADH-glutamate synthase (NADH-GOGAT). Glutamate dehydrogenase (GDH), an enzyme originally thought to be involved in ammonium assimilation, seems to play a minor role in glutamate biosynthesis and is mainly implicated in its oxidation (Sawhney and others 1987). The resulting glutamate can be incorporated into aspartate and asparagine via the action of aspartate amino transferase (AAT) and asparagine synthetase (Vance and Grant 1992), although the main product of nitrogen transport from the nodules in some grain legumes like common bean and soybean are ureides such as allantoin and allantoic acid (Atkins 1991). Surprisingly, the ureide level has been found to increase as drought and $N_2$ fixation rates declined in soybean plants (Serraj and Sinclair 1996), although there is little evidence of the potential use (production and metabolism) of ureides as an indicator to asses $N_2$-fixation under salt stress conditions.

Levels of the plant hormone abscisic acid (ABA) increase as a result of osmotic stress, playing a central role in the plant response to drought (González and others 2001), salinity (La Rosa and others 1987), and cold (Chen and Gusta 1983); stresses that involve cellular water stress. Under salinity the quantity of ABA increases and that of cytokinins decreases (Downton and Loveys 1981). In addition, ABA seems to mediate osmotic adaptation of the plant, as well as in the synthesis of proline (Stewart and Voetberg 1985). Under these conditions, specific genes are expressed that can also be induced in unstressed tissues by the exogenous ABA application (Moons and others 1997; Chen and Plant 1999).

An exogenous ABA treatment reduces leaf abscission and increases salt tolerance in citrus plants (Gómez-Cardenas and others 2003), but it also decreases total biomass and increases the root to shoot ratio in poplar species (Yin and others 2004). In legumes grown under symbiotic $N_2$ fixation, ABA treatments produce different responses according to the species studied (Bano and Hillman 1986; González and others 2001). In *Vicia faba* the developed nodule receiving ABA treatment became elongated and developed a corklike texture and fragile vascular connections at their point of attachment to the host root (Bano and Hillman 1986). In contrast, in nodules of *Pisun sativum* ABA did not affect either the number or dry weight of nodules or the enzyme activities related to carbon and nitrogen metabolism (González and others 2001).

In the present study we investigated the effect of ABA treatments on common bean responses to salinity. The experiment involved measurements of growth, nodulation, nitrogen fixation, metabolite accumulation, and enzyme activities of ammonium and purine catabolism.

## Materials and Methods

### Plant, Bacteria, and Growth Conditions

Seeds of common bean (*Phaseolus vulgaris* L. cv. *Coco*) supplied from INRA (Montpellier, France), were surface-sterilized by immersion in 5% (v/v) $NaClO_4$ solution for 4 min, rinsed four times with sterile water, and germinated in moist sterile vermiculite at 28°C for 48 h. The young seedlings were sown in a modified Leonard jars (Caba and others 1990) with nutrient solution (Rigaud and Puppo 1975) containing 1 mM $KNO_3$, an N concentration that stimulates plant growth but does not inhibit nodule growth or nitrogenase activity (Streeter 1988). Each seedling, inoculated with 1 ml of a *Rhizobium tropici* CIAT899 suspension (c. $10^9$ cell $ml^{-1}$), was grown in a controlled environmental chamber with a 16/8 h light/dark cycle, 26°/18°C day/night temperature, relative humidity of 55%–75%, and photosynthetic photon flux density (400–700 nm) of 450 µmol $m^{-2}$ $s^{-1}$, supplied by combined fluorescent and incandescent lamps (Sylvania cool-white lifeline F96T12-CW-VHO, Sylvania Ltd Quebec, Canada) and incandescent lamps (30% fluorescent wattage).

### Treatments and Harvest

When plants were 16 days old (symbiosis established) they were treated with two ABA concentrations

(1 and 10 μM) added to the growth medium. These concentrations have been shown to be adequate as modulators of the response of legumes to salt stress (Fedina and others 1994). Two days after hormone treatment, NaCl (100 mM) was applied to the nutrient solution. Fresh nutrient solution replaced the old every 2 days, and the pH was kept at 7 ± 0.1. Plants were harvested at 3 and 6 days after the saline treatment (DAT). Six plants were included per treatment and harvest. A fresh sample of the root, containing nodules of each plant, was used for the nitrogenase assay, after which the nodules were removed, weighed, and dried at 70°C for 48 h to calculate dry weight. Other samples of nodules from each plant, leaves, and roots were pooled and stored at –80°C for the enzyme assays and analytical determinations. The fresh weight (FW) of roots (including the portion used for the nitrogenase assay), stems, and leaves were recorded, whereupon all the organs were dried at 70°C for 48 h and their dry weight (DW) calculated.

## Amino Acid, Proline, Protein, and Ureide Determination

Proline and free amino acids in nodules were extracted according to the procedure of Irigoyen and others (1992), using 1 g of nodules and 12 ml of extraction medium. Both proline (Irigoyen and others 1992) and free amino acids (Yemm and Cocking 1955) were determined using ninhydrine reagent. Standard curves, prepared with L-proline and L-asparagine, were used to estimate concentrations. The ureides content (allantoin and allantoic acid) in nodules was determined using 1 g of fresh sample and 4 ml of the extraction medium according to Vogels and van der Drift (1970). Protein content was measured at 660 nm by the Lowry method (Lowry and others 1951) with Folin-Ciocalteu reagent and bovine serum albumin as standard.

## Abscisic Acid Determination

For extraction of ABA, leaves, roots, or nodules (0.25 g) were homogenized with a mortar and pestle in extraction solution (80% methanol containing 2% glacial acetic acid). To remove plant pigments and other non-polar compounds that could interfere in the immunoassay, extracts were first passed through a polyvinylpyrrolidone column and C18 cartridges. The eluates were concentrated to dryness by vacuum evaporation and re-suspended in Tris-buffered saline before enzyme-linked immunosorbent assay (ELISA). Abscisic acid was quantified by ELISA (Walker-Simmons 1987). The ABA immunoassay detection kit (PGR-1) was specific for ± ABA (Sigma Chemical Co.).

## Nitrogen Fixation Assays

Nitrogenase (EC 1.7.9.92) activity was determined by incubating isolated nodule-bearing roots in 10% $C_2H_2$ for 10 min at room temperature in 100-ml bottles fitted with Suba-seals. The acetylene-reduction assay (ARA) was performed according to Hardy and others (1973) in a PerkinElmer 8600 gas chromatograph equipped with a Poropak-R column (Ligero and others 1986). Although the use of such a ''closed'' system for measuring acetylene reduction creates problems related to an acetylene-induced decline in nitrogenase activity (Minchin and others 1983), it is still useful for comparative purposes, especially when the assay time is short (Vessey 1994).

## Preparation of Extracts and Enzyme Assays

Cell-free extracts were prepared by homogenizing 0.5–1.0 g of fresh nodules, containing 10% (w/w) of polyvinylpolypyrrolidone, with 6 ml of 100 mM of maleic acid–KOH buffer pH 6.8 for the activities of GS, NADH-GOGAT, GDH, and AAT (Caba and others 1990) or 25 mM of TES-KOH buffer pH 7.5 for the activities of XDH and uricase (Schubert 1981). Homogenates for the enzyme activities of ammonium and purine metabolism were filtered through four layers of cheesecloth and further clarified at 30,000 × *g* for 30 min and 1100 × *g* for 15 min, respectively. All steps were carried out at 4°C.

Glutamine synthetase activity (GS, EC 6.3.1.2) was determined by the hydroxamate synthetase assay, measuring colorimetrically the amount of γ-glutamyl-hydroxamate (γ GH) produced (Kaiser and Lewis 1984). Assays were optimized for the amount of enzyme to give a linear reaction within at least 30 min. Two blanks without enzyme and without L-glutamate were also included.

NADH-glutamate synthase (GOGAT, EC 1.4.1.14) and glutamate dehydrogenase (GDH, EC 1.4.1.2) activities were determined spectrophotometrically at 30°C by monitoring the oxidation of NADH at 340 nm according to Groat and Vance (1981) and Singh and Srivastava (1986). For NADH-GOGAT, the assay medium consisted of 1 mM $Na_2$-EDTA, 2.5 mM 2-oxoglutarate, 1.0 mM aminooxyacetate, 0.15 mM NADH, 10.0 mM L-glutamine, and 0.1% (v/v) 2-mercaptoethanol in 50 mM potassium phosphate buffer, pH 7.5. For GDH, the assay

**Table 1.** Plant Dry Weight (PDW) and Nodule Dry Weight (NDW) in g plant$^{-1}$, Root to Shoot Ratio (RSR), and Acetylene Reduction Activity (ARA, μmol $C_2H_2$ g$^{-1}$ NDW h$^{-1}$) in *P. vulgaris* Inoculated with the *R. tropici* CIAT899 Strain and Treated with 1 μM and 10 μM of Abscisic Acid (ABA) at 48 h before the NaCl Treatment (0 and 100 mM).

| | | PDW | | RSR | | NDW | | ARA | |
|---|---|---|---|---|---|---|---|---|---|
| DAT | ABA (μM) | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 |
| | 0 | 1.28$^{b}$ | 0.88$^{a}$ | 0.34$^{ab}$ | 0.46$^{bc}$ | 0.062$^{b}$ | 0.019$^{a}$ | 121.4$^{b}$ | 35.1$^{a}$ |
| 3 | 1 | 1.09$^{a}$ | 1.09$^{bc}$ | 0.33$^{ab}$ | 0.35$^{a}$ | 0.034$^{a}$ | 0.042$^{b}$ | 46.0$^{a}$ | 47.5$^{a}$ |
| | 10 | 1.07$^{a}$ | 0.95$^{ab}$ | 0.44$^{c}$ | 0.44$^{ac}$ | 0.056$^{b}$ | 0.052$^{b}$ | 56.3$^{a}$ | 51.5$^{a}$ |
| | 0 | 1.76$^{d}$ | 1.15$^{c}$ | 0.27$^{a}$ | 0.38$^{ab}$ | 0.095$^{c}$ | 0.039$^{ab}$ | 146.7$^{b}$ | 37.6$^{a}$ |
| 6 | 1 | 1.20$^{ab}$ | 1.22$^{c}$ | 0.36$^{abc}$ | 0.36$^{a}$ | 0.057$^{b}$ | 0.053$^{b}$ | 54.2$^{a}$ | 49.6$^{a}$ |
| | 10 | 1.41$^{c}$ | 1.28$^{c}$ | 0.39$^{bc}$ | 0.49$^{c}$ | 0.075$^{bc}$ | 0.050$^{b}$ | 36.7$^{a}$ | 37.3$^{a}$ |
| LSD | ($P \le 0.05$) | 0.19 | | 0.09 | | 0.021 | | 30.9 | |

*Means followed by the same letter within a column are not significantly different (P ≤ 0.05) using the least significant difference (LSD) test.*
*DAT: days after saline treatment.*

medium consisted of 0.1 M $(NH_4)_2SO_4$, 2.5 mM 2-oxoglutarate, and 0.15 mM NADH in 50 mM potassium phosphate buffer, pH 7.5. Two controls (without 2-oxoglutarate or without glutamine) were used to correct endogenous NADH oxidation.

Aspartate aminotransferase (AAT, EC 2.6.1.1) activity was determined in an extraction medium that consisted of 4 mM MgCl2, 10 mM aspartic acid, 0.2 mM NADH, and 1 mM 2-oxoglutarate in 50 mM Tris-HCl buffer pH 8.0 (González and others 1995). Xanthine dehydrogenase (XDH, EC 1.2.1.37), and uricase (EC 1.7.3.3) activities were determined by the procedure of Schubert (1981). For the XDH activity, measured at 340 nm as hypoxanthine-dependent formation of NADH, the reaction medium consisted of 3.5 mM NAD+, and 0.5 mM hypoxanthine in 50 mM TES-KOH buffer pH 8.4. For the assay of uricase activity, measured at 293 nm due to the oxidation of uric acid, the reaction medium consisted of 50 μM uric acid in 85 mM glycine-KOH buffer pH 9.0.

### Statistical Design and Analyses

The experimental layout was a randomized complete block design. The growth values and parameters related to nitrogen fixation were means of six replicates per treatment. Four replicates were performed for the enzyme activity assays, and three replicates for amino acids, proline, and ABA content. The standard error (SE) was estimated and all results were subjected to a multifactor analysis of variance with a least significant difference (LSD) test between means. The simple correlation coefficients among the parameters studied were also calculated.

## Results

The effect of abscisic acid, added 48 h prior to the onset of salt stress, on plant growth and nitrogen fixation was monitored throughout the 6 days of the experimental period. Table 1 summarizes growth and nitrogen fixation parameters at 3 and 6 days after the saline treatment (DAT). Abscisic acid treatments decreased dry weight of control plants (NaCl-free treatment) about 15% in the first harvest (3 DAT), whereas in the second harvest (6 DAT) plant biomass was reduced 32% and 17% with 1 μM and 10 μM of ABA, respectively. In the absence of ABA, the salt treatment diminished plant biomass about 35%. However, with the ABA supply, NaCl did not affect plant dry weight in either harvest. On the other hand, the root to shoot ratio significantly increased with 10 μM of ABA in the growth medium and also in salt-treated plants grown in the absence of ABA.

Exogenous ABA, especially the lowest concentration, reduced nodule dry weight about 45% at 3 and 6 DAT. In addition, nodule biomass decreased about 75% with 100 mM of NaCl; however, the decrease produced by salt was not observed in the presence of the hormone. In non-salt-treated plants, ABA drastically reduced the nitrogenase activity (42%–54%) in the first harvest and more intensively (60%–75%) at 6 DAT. In the absence of ABA, the saline treatment strongly inhibited ARA (70%–75%) in both harvests; however, this parameter was not significantly affected in salinized plants treated with ABA. Regarding the ureide content in nodules (Table 2), our results show a similar behavior of NaCl and ABA with a tendency to inhibit ureide production, and even more severe at 6 DAT. The

**Table 2.** Content of Allantoin, Allantoic Acid, and Total Ureides (μmol $g^{-1}$ FW) in Root Nodules of *P. vulgaris* Inoculated with the *R. tropici* CIAT899 Strain and Treated with 1 μM and 10 μM of ABA at 48 h before NaCl Treatment (0 and 100 mM).

| | | Allantoin | | Allantoic acid | | Total ureides | |
|---|---|---|---|---|---|---|---|
| DAT | ABA (μM) | 0 | 100 | 0 | 100 | 0 | 100 |
| | 0 | 0.41[c] | 0.22[b] | 1.74[c] | 1.36[a] | 2.15[d] | 1.58[a] |
| 3 | 1 | 0.11[a] | 0.17[ab] | 1.68[b] | 1.93[e] | 1.79[b] | 2.10[d] |
| | 10 | 0.20[ab] | 0.09[a] | 1.37[a] | 1.58[c] | 1.56[a] | 1.67[ab] |
| | 0 | 0.46[c] | 0.37[c] | 2.15[d] | 1.35 [a] | 2.61[e] | 1.72[b] |
| 6 | 1 | 0.40[c] | 0.24[b] | 1.60[b] | 1.82[d] | 1.99[c] | 2.06[d] |
| | 10 | 0.27[b] | 0.37[c] | 1.64[bc] | 1.47[b] | 1.92[c] | 1.86[c] |
| LSD | ($p \leq 0.05$) | 0.09 | | 0.10 | | 0.09 | |

*Mean followed by the same letter within a column are not significantly different ($P \leq 0.05$) using the LSD test.*

increase of the total ureide content obtained with 1 μM of abscisic acid in salt-stressed plants at both 3 and 6 DAT is noticeable.

Nodule enzyme activities involved in ammonium metabolism (GS, GOGAT, GDH, and AAT) and ureide catabolism (XDH and uricase) decreased in ABA-treated plants, except AAT activity (Figure 1). Averaged GS activity was sixfold to ninefold higher than NADH-GOGAT activity. The presence of the hormone caused a decrease in GS activity in non-salinized plants, mainly in the first harvest; however, a greater reduction of this activity of about 70% was observed in plants exposed to saline stress in the absence of the hormone (3 DAT). This activity inhibition with the salt treatment disappeared or activity was even stimulated when ABA was added to the medium 48 h before the salt application, at least in the first harvest. NADH-glutamate synthase activity showed a trend similar to that of GS (3 DAT), decreasing with ABA treatment. However, in the second harvest, in salt-stressed plants, these enzymatic activities decreased significantly, even with the hormone addition. The connection of NADH-GOGAT and GS enzymes was highly correlated ($r = 0.74^{***}$; Figure 2B).

On the other hand, GDH and AAT activities were only slightly affected or unaffected by the ABA treatment, respectively. In salt-stressed plants 1 μM of ABA induced an increase of GDH activity of about 10% at 3 and 6 DAT; however, AAT was not altered by the saline conditions. Xanthine dehydrogenase and uricase activities showed similar behaviors, decreasing with the increase of ABA concentrations in the growth medium at 3 and 6 DAT (Figure 1). On the other hand, NaCl caused an inhibition of those activities of about 30%–35% in plants not previously exposed to the hormone treatment. However, at 3 DAT with 1 μM and 10 μM of abscisic acid both enzymes activities increased in nodules subjected to the saline stress. This result is in accordance with the increase of the total ureide content obtained in salinized plants treated with 1 μM of ABA, as mentioned above. The enzymes XDH and uricase were positively and significantly correlated ($r = 0.68^{**}$; Figure 2A). In addition, uricase and glutamine synthetase were also strongly correlated ( $r = 0.77^{***}$; Figure 2C).

The amino acid content in nodules decreased 20%–23% with abscisic acid treatments in non-salinized plants, at both harvests (Table 3). The effect of salt stress on amino acid accumulation was not clear in the first harvest, but in the second they increased in nodules of plants grown with 100 mM NaCl. Abscisic acid–treated plants (1 μM) showed a significant increase (50%–30%) of amino acid content under salt stress in both harvests, whereas with 10 μM ABA the increase was only about 10%. The proline content in nodules diminished with the ABA treatment (30%–40%). Conversely, NaCl caused an increase of proline content in the first harvest and a decrease in the second. In addition, it was observed that in stressed plants, proline showed a tendency to decrease with ABA treatment. On the other hand, during the experimental period, the soluble protein in nodules declined about 10% with ABA treatments compared to non ABA-treated plants. Nodule proteins also decreased slightly with the saline treatment, but with the addition of ABA, these differences between stressed and non-stressed plants disappeared. Besides, proline and amino acid contents in nodules showed a high and positive correlation ($r = 0.60^{**}$; Figure 2D), which is an indication of the connection between the function of these compatible solutes.

Changes in endogenous ABA in leaves, roots, and nodules of common bean plants treated with or

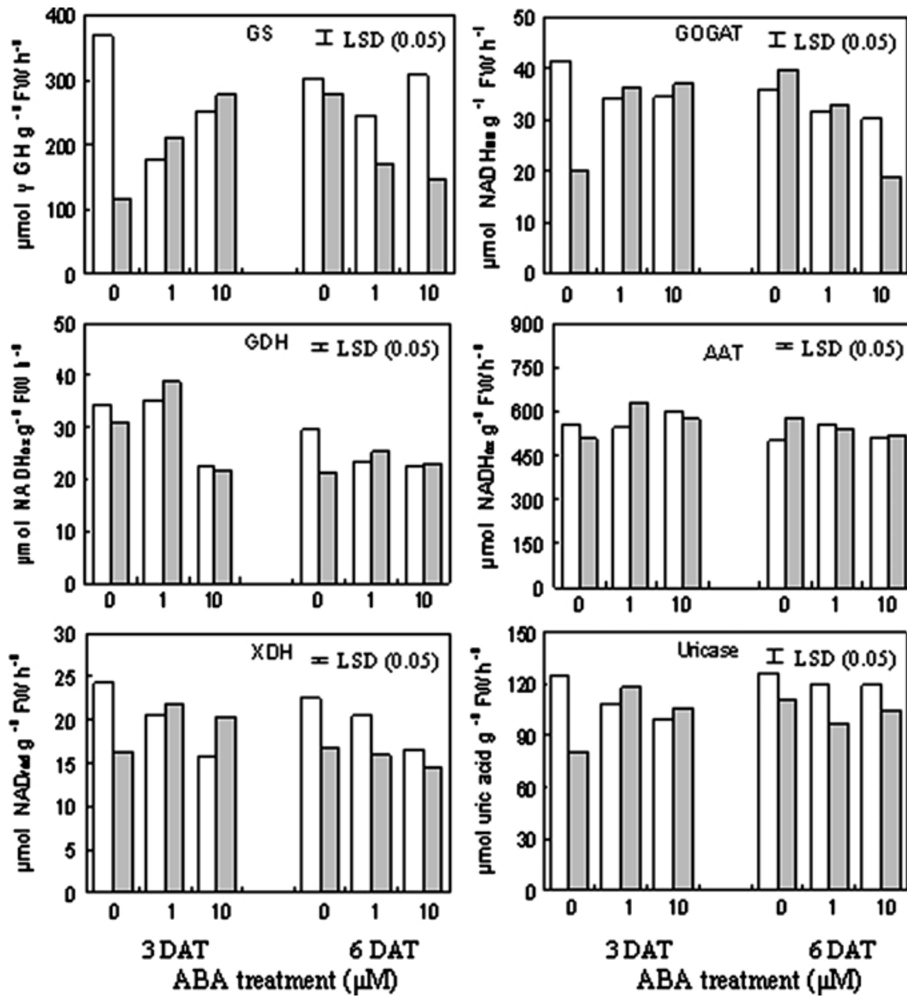


**Figure 1.** Effect of ABA on nodule glutamine synthetase (GS), NADH-glutamate synthase (GOGAT), glutamate dehydrogenase (GDH), aspartate aminotransferase (AAT), xanthine dehydrogenase (XDH), and uricase activities of *P. vulgaris* inoculated with the *R. tropici* CIAT899 strain and treated with 0 (open bars) and (shaded bars) 100 mM of NaCl. ABA (1 and 10 μM) was added to the nutrient solution 48 h prior to the onset of salt stress. DAT: days after saline treatment.

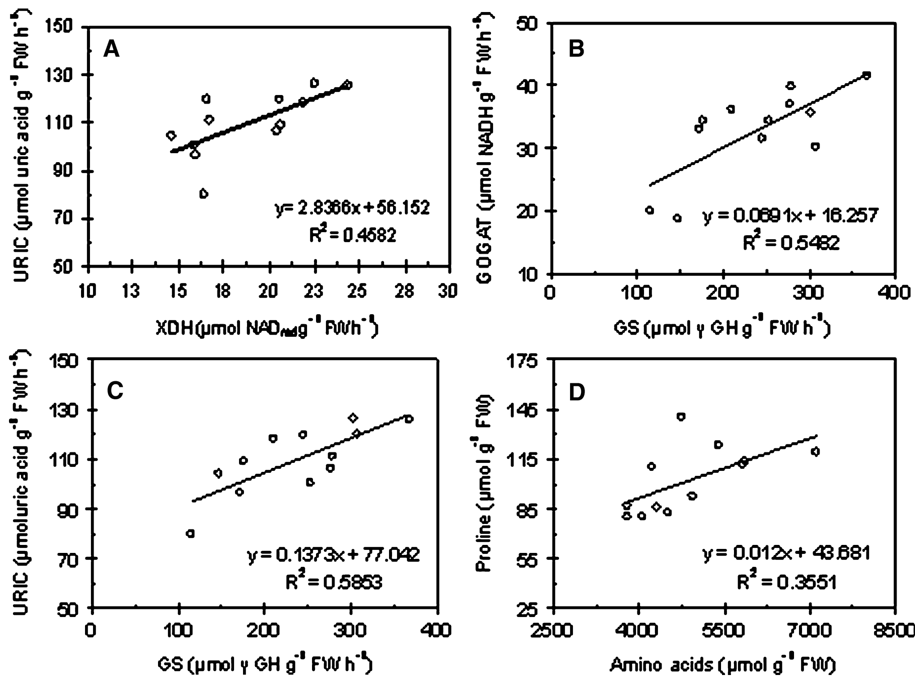


**Figure 2.** Correlation between enzyme activities (**A**) uricase (URIC) and xanthine dehydrogenase (XDH), (**B**) glutamate synthase (GOGAT) and glutamine synthetase (GS), (**C**) uricase and glutamine synthetase, or (**D**) proline and amino acid content in root nodules of *P. vulgaris* inoculated with the *R. tropici* CIAT899 strain and treated with 1 and 10 μM of ABA at 48 h prior to the onset of salt stress (100 mM).

**Table 3.** Content of Amino Acids and Proline (μmol $g^{-1}$ FW), and Proteins (mg $g^{-1}$ FW) in Root Nodules of *P. vulgaris* Inoculated with the *R. tropici* CIAT899 Strain and Treated with 1 μM and 10 μM of ABA at 48 h before the NaCl Treatment (0 and 100 mM)

| | ABA (μM) | Amino acids | | Proline | | Proteins | |
|---|---|---|---|---|---|---|---|
| DAT | | 0 | 100 | 0 | 100 | 0 | 100 |
| | 0 | 5871$^{c}$ | 5405$^{c}$ | 113.2$^{c}$ | 123.3$^{e}$ | 13.82$^{c}$ | 11.10$^{b}$ |
| 3 | 1 | 4514$^{b}$ | 7108$^{d}$ | 82.4$^{a}$ | 118.7$^{d}$ | 12.31$^{b}$ | 12.39$^{c}$ |
| | 10 | 4054$^{a}$ | 4324$^{a}$ | 80.4$^{a}$ | 85.7$^{a}$ | 12.22$^{b}$ | 12.35$^{c}$ |
| | 0 | 4742$^{b}$ | 5811$^{c}$ | 140.4$^{d}$ | 111.7$^{c}$ | 12.45$^{b}$ | 11.20$^{b}$ |
| 6 | 1 | 3811$^{a}$ | 4946$^{b}$ | 86.8$^{b}$ | 92.6$^{b}$ | 12.14$^{ab}$ | 9.25$^{a}$ |
| | 10 | 3811$^{a}$ | 4243$^{a}$ | 80.2$^{a}$ | 110.5$^{c}$ | 11.06$^{a}$ | 8.29$^{a}$ |
| LSD | ($P \leq 0.05$) | 433 | | 4.0 | | 1.08 | |

*Mean followed by the same letter within a column are not significantly different (P ≤ 0.05) using the LSD test.*

without exogenous ABA and NaCl are shown in Figure 3. Both the hormone and the saline treatment increased the endogenous ABA in all organs studied, but the magnitude and the behavior varied with the treatment and the organ studied. Higher increases were observed in leaves and nodules of plants treated with ABA (10 μM) 48 h before salt application. In this treatment, ABA content in leaves showed the maximum value at 19 days after sowing (DAS), whereas in nodules the maximum was observed at 21 DAS. In addition, endogenous ABA in vegetative organs decreased in the last harvest at 24 DAS; in roots, it continued to increase throughout plant growth. However, in non-ABA–treated plants there was not a substantial increase of endogenous ABA content after a few hours of salt stress.

## Discussion

The role of abscisic acid in stress physiology has received much attention, and there is now considerable experimental evidence that the physiological effects induced by salinity might be modulated by ABA. It has been shown that saline stress is accompanied by an increased in ABA content (Aspinall and Paleg 1981). In addition, *P. vulgaris* plants adapted to salinity had ABA concentrations substantially higher than those in non-adapted plants (Montero and others 1998). According to our results, ABA is also a growth inhibitor (Table 1); its tissue concentration has been previously found to be correlated with the growth inhibition of salt stressed plants (He and Cramer 1996; Montero and others 1998). In salt-stress conditions, we observed a positive effect of exogenous ABA (1 μM) added 48 h prior to the NaCl treatment on plant growth. Amzallag and others (1990) indicated that ABA induced enhancement of growth of sorghum plants during salinization, and suggested that application of exogenous ABA accelerated adaptation of salt in these plants. On the other hand, He and Cramer (1996) associated the tolerance to salinity in *Brassica* with low concentrations of ABA in plant tissues. Our study suggests that ABA appears to provide a protective role in conditions of high salinity.

The nitrogen-fixation process in nodules of *Phaseolus vulgaris* was inhibited by exogenous ABA, decreasing nodule biomass and nitrogenase activity (ARA). Results obtained by González and others (2001) with nodules of *Pisum sativum* showed that ABA supply did not affect nodule dry weight in agreement with the results of Bano and Hillman (1986). Nevertheless a significant reduction in apparent and total nitrogenase activity was described in ABA-treated plants of *Faba vulgaris* (Bano and Hillman 1986) and *Pisum sativum* (González and others 2001). Indeed, former authors suggested a lack of photosynthate supplied to *Faba vulgaris* nodules as the main cause of nitrogen fixation decline; however, other investigators demonstrated no limitation of photosynthate supply to *Pisum sativum* nodules based on the measure of total soluble sugar concentration. The interaction of ABA and salt treatments induced an increase in nodule biomass, also showing a positive and significant correlation between NDW and PDW (r = 0.88**). In addition, the interaction of ABA-NaCl was useful to protect or reduce the negative effect of salt stress on nitrogen fixation (ARA). Therefore, our results suggest that the inhibitory effect of NaCl in NDW and nitrogenase activity (ARA) is strongly attenuated when abscisic acid is supplied to the nutrient solution.

The GS/GOGAT pathway makes up a crucial function in the control of assimilation of reduced

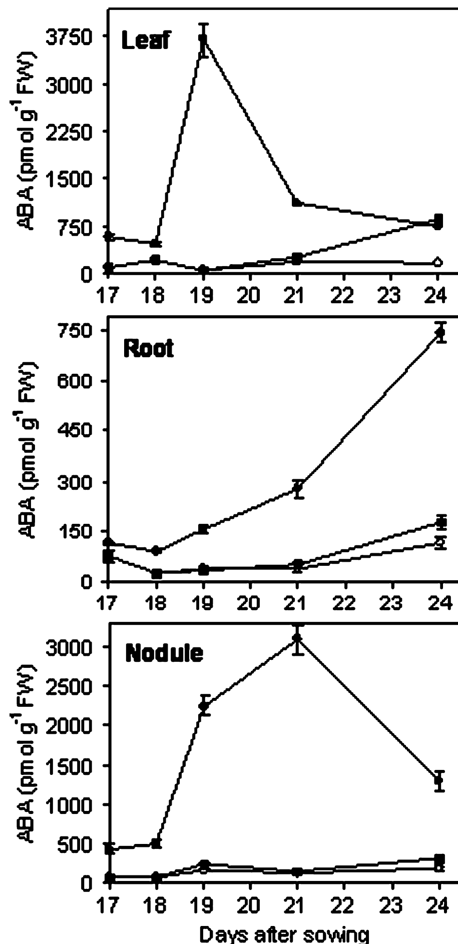


**Figure 3.** Changes in ABA content in leaves, roots, and nodules of *P. vulgaris* inoculated with the *R. tropici* CIAT899 strain. Control plants (○); non-ABA treated, salinized plants (■); ABA-treated, salinized plants (●). ABA (10 μM) and NaCl (100 mM) were added to the nutrient solution at 16 and 18 days after sowing, respectively. Data are means ± SE of three replicates.

nitrogen originated by the biological nitrogen fixation in nodules of legumes (Groat and Vance 1981). Inhibition of GS and GOGAT activity at high salt concentrations was reported by Cordovilla and others (1994) in *Vicia faba* nodules. In our experiment, GS activity had a positive and significant correlation with ARA (r = 0.60**), which supports the close relation between nitrogen fixation and the first enzyme of ammonium assimilation (Cullimore and Bennett 1988). However, the hormonal regulation of these enzyme activities has not yet been widely studied in legumes. Our data showed a decline in glutamine synthetase and glutamate synthase activities with the ABA treatment in nonstressed plants (Figure 1). A similar finding has also been reported by Chanda and others (1998), who also found an inhibition of cytosolic GS activity with ABA supply. In our salt-treated plants, GS and GOGAT activities increased with the ABA treatment in the first harvest. Indeed, without ABA supply, NaCl caused a decrease in GS and GOGAT activities in the first harvest, which was restored with the ABA treatment. Based on these results, we suggest that in parallel with the saline treatment, ABA had a beneficial effect in these enzymes.

Glutamate dehydrogenase and AAT activities were less affected by both ABA and NaCl treatments than the other enzymes studied here. It seems that, although GDH to glutamate synthase activities were comparable, GS-GOGAT is the main route for the entry of fixed N in *P. vulgaris* as in other legumes. Sawhney and others (1987) reported that GDH might be involved in glutamate detoxification. Nevertheless, in lentil nodules this enzyme was present in large amounts in the nodule cytosol (Chopra and other 2003). These authors suggested that this enzyme could play an important role under some nutritional and environmental conditions.

In nodules of bean plants, XDH and uricase make up an essential function in purine catabolism (Schubert 1981). In our study, salt and ABA treatments separately caused an inhibition of both enzymes (Figure 1). However, the ABA treatment (mainly 1 μM) induced a stimulation of XDH and uricase activities in stressed pants, similar to that obtained for GS and GOGAT activities. Therefore, the negative effect of NaCl was attenuated with ABA supply. In addition, ureide content of nodules (Table 2) showed the same trend as that XDH and uricase, increasing with 1 μM of ABA in salinized plants. This effect of the hormone could be considered a mechanism of adaptation to stress given that ABA intensified enzyme activities of purine catabolism, consequently increased ureide production and finally enhanced amino acid and protein synthesis (Table 3). Curiously, the positive response of ABA in all enzyme activities studied here in salt-stressed plants was mostly achieved in the short term (3 DAT). The variety of *Phaseolus vulgaris* used in our study is salt sensitive, thus presumably at 6 DAT NaCl (100 mM) causes greater damage to nodule metabolism and its functioning.

Regarding the effect of both ABA and salt treatments on the proline content of nodules, different results have been reported. The application of ABA to nonstressed plants provoked proline accumulation in pea (Fedina and others 1994) and in *Arabidopsis* (Savouré and others 1997); however, Thomas and others (1992) did not find significant differences in the proline content in untreated and ABA-treated plants. On the contrary, our results showed a decrease in the nodule proline content in

*P. vulgaris* treated with ABA (Table 3). On the other hand, NaCl caused an increase of proline content in the first harvest, but its tendency was to decrease with ABA application. Concerning the role of proline in stressed plants, although there are diverse reports, questions remain and the topic deserves more experimental analysis.

Concerning the effect of ABA and NaCl applications on endogenous ABA content, our results show an increase of this hormone in leaves, roots, and nodules (Figure 3). In addition, the ABA accumulation in common bean occurred preferentially in leaves and nodules in the short term. It seems that the combined treatments result in a synergistic effect of ABA content, which primarily augments leaves (one organ in which ABA synthesis takes place) and then nodules and roots. These results also suggest that this additional ABA is associated with the increase of endogenous ABA in response to the salt stress and therefore might be related to NaCl tolerance of common bean plants. This increase is in agreement with that found by Hsu and Kao (2003), who detected high ABA contents in leaves, shoots, and roots of rice seedlings after exogenous ABA application. The same authors found an increase of Cd tolerance in such conditions, and Gómez-Cardenas and others (2003) reported a protective role for ABA in citrus plants growing under salinity. We speculate that ABA may exert its regulatory effect on different physiological process such as transpiration, resulting in a decrease of plant water losses and increasing the tolerance to sodium chloride stress. According to our results, we suggest that ABA application may help to improve the response of *Phaseolus vulgaris* symbiosis under saline stress conditions, including the nitrogen fixation process and enzymes of ammonium assimilation and purine catabolism.

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