Symbiosis (2013) 60:115–122
DOI 10.1007/s13199-013-0247-x

Genotypic variation of nodules' enzymatic activities in symbiotic nitrogen fixation among common bean (*Phaseolus vulgaris* L.) genotypes grown under salinity constraint

Mustapha Faghire · Farissi Mohamed · Khadijattou Taoufiq · Rachid Fghire · Adnane Bargaz · Btissam Mandri · Khalid Oufdou · Amenc Laury · Jean-Jacques Drevon · Cherki Ghoulam

Received: 31 August 2012 / Accepted: 31 May 2013 / Published online: 22 June 2013

Abstract The effect of salt stress, under glasshouse conditions, was studied on plant biomass, nodulation, and activities of acid phosphatases (APase, EC 3.1.3.2) and trehalose 6-phosphate phosphatase (TPP, EC 3.1.3.12) in the symbiosis common bean (*Phaseolus vulgaris* L.)-rhizobia nodules. Four common bean recombinant inbred lines (147, 115, 104 and 83) were separately inoculated, with CIAT 899 or RhM11 strains and grown in hydroaeroponic culture. Two NaCl levels (0 and 25 mM NaCl plant^{-1} week^{-1} corresponding, respectively, to the control and the salt treatment) were applied and the culture was assessed during 42 days after their transplantation. The results showed that the nodulation of these lines was not affected by salinity except for the line 83 inoculated with CIAT 899, whose nodule dry weight decreased by 48.24 % compared with the corresponding controls. For the other symbiotic combinations, shoot and root biomasses were not significantly affected by salt constraint. Salinity stress generally reduced acid phosphatise and trehalose phosphate phosphatase activities in nodules that were less affected in plants inoculated with RhM11. Based on our data, it appears that nodule phosphatase activity may be involved in salinity tolerance in common beans and the levels of salt tolerance depend principally on specific combination of the rhizobial strain and the host cultivar.

Keywords Common bean · Nodulation · Salinity constraint · Acid phosphatase · Trehalose phosphate phosphatase · RT-PCR in situ

M. Faghire
Department of Biology, Faculty of Sciences, Ibn Zohr University, B.P 8106, Agadir, Morocco

F. Mohamed · B. Mandri · C. Ghoulam
Faculté des Sciences et Techniques Guéliz-Marrakech, Equipe de Biotechnologie Végétale et Agrophysiologie des Symbioses, BP 549, 40000, Marrakech, Morocco

M. Faghire (✉) · K. Taoufiq · R. Fghire
Centre des Études et Recherches sur l'Espace Marocain (CEREM), Province Tahanaoute, Morocco
e-mail: mustaphafaghire@yahoo.fr

M. Faghire
e-mail: m.faghire@uiz.ac.ma

K. Oufdou
Faculté des Sciences-Semlalia, Laboratoire de Biologie et de Biotechnologie des Microorganismes, B.P.2390, Marrakech, Morocco

A. Laury · J.-J. Drevon
INRA-Montpellier-SupAgro, UMR1222, Rhizosphère et Symbioses, 2 Place Viala, 34060 Montpellier, France

A. Bargaz
Department of Biosystems and Technology, Swedish University of Agricultural Sciences, Box 103, 230 53 Alnarp, Sweden

1 Introduction

During the last decade, the importance of grain legumes for human nutrition increased considerably in the Mediterranean basin. These legumes fix nitrogen by symbiosis with rhizobia, and supply the necessary of nitrogen for plant growth and development, and contribute to the improvement of the soil nitrogen status. Consequently, rhizobia–legume symbiosis, will allow farmers to economise the cost of chemical fertilizers and to avoid nitrate pollution. The low and unstable yields of common bean (*Phaseolus vulgaris*) production is, particularly, due to the effects of biotic and

abiotic factors, especially when its growth depends on biological nitrogen fixation (BNF) (Jonas et al. 2010; Faghire et al. 2011; Mandri et al. 2012). Common bean cultivars are salt-sensitive species (Lauchli 1984; Soussi et al. 1999; Faghire et al. 2011) and the limitation of their productivity is associated with a low growth of the host plant, less symbiotic development of the root-nodule bacteria (Georgiev and Atkins 1993) and, consequently, a reduction capacity of nitrogen-fixation (Delgado et al. 1993). Under stress conditions, many physiological and biochemical mechanisms such as, the accumulation of compatible osmolytes like proline, glycine betaine, sucrose and mannitol (Zhu 2002) were switched on to protect major processes like cell respiration, photosynthetic activity, nutrient transport, nitrogen and carbon metabolism.

In salt conditions, the acid phosphatase (APase, EC 3.1.3.2) activity is known to contribute to salt tolerance by maintaining an inorganic phosphate level which can be co-transported with H^+ along a gradient of proton motive force (Olmos and Hellin 1997). Protective roles of the antioxidant enzymes, under salt constraint, have been reported for a number of plants such as wheat (Almeselmani et al. 2006; Esfandiari et al. 2007), and *Catharanthus roseus* (Jaleel et al. 2007). Intracellular acid phosphatases (APase, EC 3.1.3.2) are ubiquitous in plant organs, they were implicated in providing P during seed germination from stored phytate, hydrolysing of orthophosphate monoesters into more mobile orthophosphate anions, synthesis glycolate from P-glycolate and glycerate from 3-PGA during photorespiration (Vance et al. 2003). Moreover, the characteristics of some acid phosphatases (APase, EC 3.1.3.2) purified from nodules of soybean (*Glycine max L.*) showed that these enzymes were involved in the conversion of purines to ureides during symbiotic N fixation (Penheiter et al. 1997).

Trehalose 6-phosphate phosphatase (TPP, EC 3.1.3.12) is an enzyme that catalyzes the chemical reaction (alpha, alpha-trehalose 6-phosphate + H_2O ⇌ alpha,alpha-trehalose + phosphate) (Cabib and Leloir 1958). The trehalose (D-glucopyranosyl-1,1-D-glucopyranoside) is a non-reducing disaccharide that has been found in a wide variety of organisms such as yeasts, fungus, bacteria, plants, insects and other invertebrates (Crowe et al. 1984; Wiemken 1990). This molecule plays an important role in the protection from abiotic stress by stabilizing dehydrated enzymes and membranes as well as protecting biological structures from desiccation damage (Lopez et al. 2008; Lopez and Lluch 2012).

Therefore, the overall aim of this study was to use biochemical and molecular approaches to evaluate the effect of salt stress on (i) The activities of acid phosphatase (APase, EC 3.1.3.2) and trehalose 6-phosphate phosphatase (TPP, EC 3.1.3.12) and (ii) The abundance of APase gene transcripts in common bean-rhizobia root nodules.

2 Materials and methods

2.1 Plant material and experimental treatments

The experiments were conducted in a glasshouse under natural light with day/night temperatures of 28/20 °C and 16 h photoperiod consists of 16 h of light and 8 h of darkness with an additional illumination of 400 μmol photons $m^{-2}{\cdot}_S{}^{-1}$. The relative humidity during the day was 70 %. The study was carried out using four common bean inbred lines 147, 115, 104 and 83 originating from the International Centre of Tropical Agriculture. Seeds were surface sterilized with 3 % calcium hypochlorite and then washed thoroughly in ten successive baths of sterile distilled water. Thereafter, they were germinated for 4 days at 28 °C in paper roll germination soaked with sterile distilled water. Seedlings with uniform roots were inoculated with the reference *Rhizobium* strain, *Rhizobium tropici* CIAT899, or with the local *Rhizobium* strain, *Rhizobium gallicum* RhM11 (Faghire et al. 2011) grown in a liquid of yeast extract mannitol medium (YEM) at 28 °C for 2 days. Thereafter, seedlings were transferred to 40 l (40,000 cm^3) plastic containers with a nutrient solution, where the seedlings were aerated intensely and arranged in a fully randomized block design. Roots of selected seedlings were passed through the hole of a rubber stopper on the vat cover. Wool cotton was fitted at the hypocotyl level to maintain root system suspended in the nutrient solution (Vadez et al. 1999). Urea was supplied at 2 mM per plant into the nutrient solution during the initial week of growth to avoid N deficiency during nodule development. Thereafter, the plants were grown in N-free nutrient solution with NaCl concentrations of 0 and 25 mM added to the nutrient solution at 7th day after their transplantation (appearance of the first true leaves). The pH nutrient solution was adjusted, every week, to around pH 7 by the addition of 0.2 $g{\cdot}l^{-1}$ of $CaCO_3$ and the medium was aerated during the experiment by an ambient air flow of 400 $ml{\cdot}min^{-1}$.

Plants were harvested 42 days after their transplantation, which coincide with the flowering stage and the optimum of functional activities of nodules. At harvest, root systems were superficially washed with deionized water and plants were separated into shoots, roots and nodules. Nodule samples were frozen at −80 °C for the essay of the enzymes activities. Thereafter, each plant organs and nodules samples were oven-dried at 75 °C for 48 h to determine their dry weight (DW).

2.2 Preparation of extracts and enzyme assays

2.2.1 Acid phosphatase (APase) activity

The extracts were prepared by homogenizing 0.2 g of fresh nodules in a mortar with 33 % (w/w) polyvinyl-polypyrrolidone and 500 μl of acetate-buffer (0.1 M pH 5),

and 5 μl β-mercapto-ethanol. The extracts were centrifuged at 13,000 g for 30 min at 4 °C, and three aliquots of 100 μl of the supernatant were used for enzyme assays. All the operations were carried out at 4 °C.

For the determination of APase activity, 100 μl of nodular extracts were incubated during 30 min at 37 °C with a mixture 200 μl of acetate-buffer and 200 μl of p-nitrophenyl phosphate (p-NPP). The reaction was stopped by addition of 1.0 ml NaOH 0.5 N. Then the optical density (OD) was measured by spectrophotometer at 410 nm. APase activity was defined as the amount of the protein hydrolysing 1 nmol of p-NPP per min per g of fresh nodules.

2.2.2 Trehalose 6-phosphate phosphatase (TPP, EC 3.1.3.12) activity

The extracts were prepared by homogenizing 0.2 g of fresh nodules in a mortar with 33 % (w/w) polyvinyl-polypyrrolidone and 2 ml of 50 mM Tris–HCl buffer (pH 7.5) containing 2.5 mM MgCl2, 100 mM NaCl and 10 mM β-mercaptoethanol. The extracts were centrifuged at 13,000 g for 20 min and the supernatants were used to determine the enzyme activities. All operations were carried out at 4 °C.

The TPP activity was assayed by monitoring the phosphate released from trehalose 6-phosphate (Padilla et al. 2004). The reaction was carried out in a supernatant volume of 0.25 ml that contained 25 mM Tris–HCl (pH 7.0), 10 mM $MgCl_2$ and 1 mM trehalose-6-phosphate. Samples were assayed for phosphate activity by malachite green method (Ohno and Zibilske 1991).

2.2.3 Sample preparation and fixation for in situ RT-PCR

Sample preparation and fixation for in situ RT-PCR were carried out according to the protocol described by Molina et al. (2011) and Bargaz et al. (2012). The method involves in situ the amplification of specific nucleic acid sequences on nodules sections, followed by fluorescence detection (Van Aarle et al. 2007) of the localized PCR product using epifluorescence microscopy. In brief, 5 mm of the diameter of root nodules of the two combinations 115-CIAT899, 147-CIAT899 and their corresponding salt treatment, were immediately and separately harvested from plants 42 days after their transplantation, and thoroughly washed with diethyl pyrocarbonate (DEPC) treated water, and then fixed in 4 % (v/v) paraformaldehyde, 45 % (v/v) ethanol and 5 % (v/v) acetic acid, and kept for 2 h under vacuum, and then stored overnight at 4 °C. Fixed nodules were included in 9 % (m/v) low-melting-point-agarose and cut into 50 μm thick sections using a microtome (Micro-cut H1200 Vibrating Microtome; Bio-Rad, Marnes la Coquette, France). The obtained sections were collected into a small tubes containing 0.5 ml of DEPC-treated water and freed from residual agarose by three washes with DEPC-treated water heated to 60 °C.

For reverse transcription, the first cDNA strand was synthesized from the total of RNA of 50 μm of nodules slices using MMLV reverse transcriptase (following the manufacturer's recommendations). Nodules slices were incubated in 40 μl reverse transcriptase mix containing the APase gene-specific reverse primer APrev. Negative controls were prepared by omitting the reverse transcriptase. Then the reverse transcriptase mix was removed and 40 μl of PCR mix was added including 0.25 μM of each constituent, APdir specific-gene (5′-CTGCTTACGAATGACGACGGC-3′) and APrev (5′-CCAGTTTCAAAGGCGTTACCG-3′) primers. The thermocycling was performed using 30 cycles of 95° for 30 s, 60 °C for 30 s, and 72 °C for 45 s, with an extension at 72 °C for 2 min.

Then amplified cDNA in the fixed tissues was detected after removing the PCR mix. The samples were washed and incubated in 100 μl blocking solution under gentle agitation in the dark at 37 °C. The blocking solution was replaced by 100 μl of phosphatase-conjugated anti-digoxigenin Fab fragment (Roche Diagnostics, Basel Switzerland) diluted at 1:1,000 in 2 % BSA. Afterwards, the samples were incubated at room temperature for 90 min and then washed three times to remove the excess of antibody. Detection of phosphatase was carried out using tELF-97® endogenous phosphatase detection kit (Molecular Probes, Leiden, The Netherlands). The observations were made with an Olympus BX61® microscope equipped with an epifluorescence condenser, Hoechst/DAPI long-pass filter set configured at an excitation filter of 360–370 nm, a dichroic mirror of 400 nm and an emission of 420 nm. The images were photographed with grey View II® camera (ORCA AG; Hamamastu) using Analysis® software (Soft Imaging System, Munster, Germany). The analysis image was performed using ImageJ software as an image analysis program.

2.3 Statistical analysis

The experimental layout was a randomized block design. The values representing dry biomass are means of seven replicates per treatment. Three replicates were performed for the other studied parameters. All results were subjected to a two-way analysis of variance, with a Least Significant Difference test (LSD) for the comparison of means using Costat software.

3 Results

In the plant experiment, one set of pots with different combinations of bean lines and *Rhizobium* strains that did not receive any salt treatments was used as controls. The

symbiotic combinations 83-CIAT899, 104-CIAT899 and 104-RhM11, showed high SDW that reached 2.4 g per plant whereas 115-CIAT899 symbiotic combination presented the lowest SDW that was less than 2 g per plant (Fig. 1a). The addition of 25 mM NaCl to the nutrient solution reduced significantly the SDW in the combinations 83-CIAT899, 104-RhM11 and 147-RhM11 respectively to 57, 15 and 40 % comparison with their corresponding controls. The other symbiotic combinations did not show, under salinity treatment, any significant variation in the growth except for 83-RhM11 that showed a significant increase in SDW (Fig. 1a).

For the RDW (Fig. 1b) and NDW (Fig. 1c) measured, the effect of salinity treatment on these parameters did not show any regular or significant variation in all of the tested symbiotic combinations, except for 83-CIAT899 which caused a significant reduction on both RDW (47 %) and NDW (52 %), whereas this constraint caused a significant increase of these parameters for 104-RhM11 combination (Fig. 1b and c).

3.1 Effect of NaCl on nodule enzyme activities

In the plant experiment, one set of pots with different combinations of bean lines and *Rhizobium* strains that did not

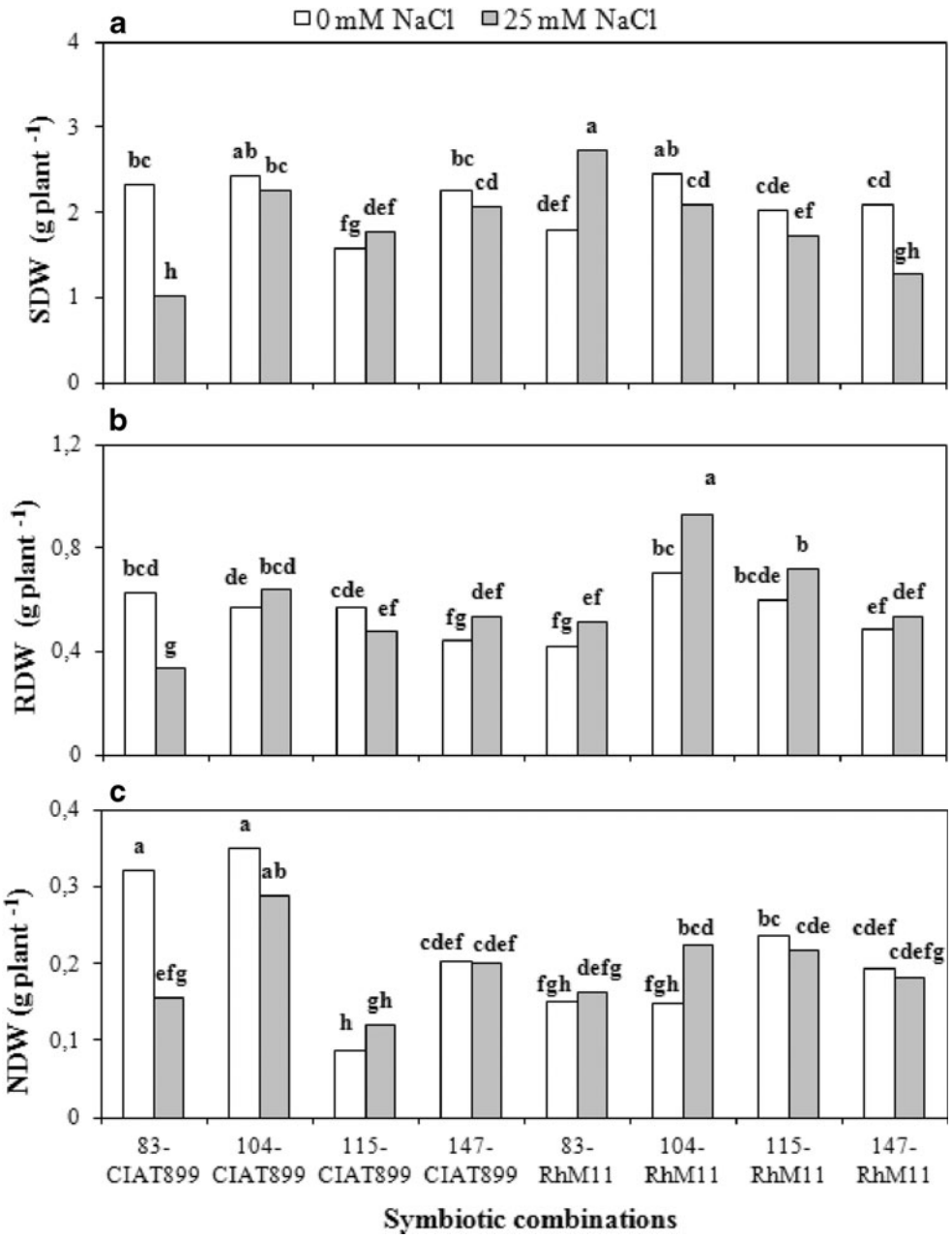

Fig. 1 Effects of salinity on growth parameters: **a** shoot dry weight (SDW), **b** RDW, root dry weight **c** NDW, nodule dry weight. *Means followed by the same letter* within a column are not significantly different ($P<0.05$) using the LSD test

receive any salt treatments was used as controls. The nodules of the majority of common bean–rhizobia symbiotic combinations showed high APase activity that exceeded 1,000-nmol min^{-1} g^{-1} FM (Fig. 2a), while salinity treatment induced a significant decrease of APase activity in the nodules of plants inoculated with CIAT899 in all four inbred lines of common bean. This decrease was not significant when plants were inoculated with RhM11 except for nodules of 147-RhM11 symbiotic combination. Also, salinity affected nodules APase activity of 115-CIAT899 and 147-CIAT899 symbiotic combinations and reductions of 24 % and 34 % were respectively noted comparatively to their corresponding controls (Fig. 2a).

For TPP activity and in absence of salt treatment, the nodules of 147-RhM11 presented the highest value followed by 83-CIAT899 or 83-RhM11, while 104-RhM11 symbiotic combination presented the lowest nodular TPP activity which did not exceed 100 nmol·min^{-1}·g FM^{-1} (Fig. 2b). However, the presence of salt in rooting medium caused generally a decrease of TPP activity in nodules of the tested symbiotic combinations that was significant for the nodules of 83-CIAT899, 83-RhM11 and 147-RhM11 symbiotic combinations (Fig. 2b).

3.2 Localisation of acid phosphatase (APase) transcripts

To test the transferability of the information between Uni-Tag profiles and other platforms, selected nodule-metabolism-associated transcripts were localized in common bean nodules. For this purpose, PCRs of acid phosphatase-annotated Uni Tags, in situ, were performed in nodules slices. Main features of the anatomy of common bean nodules are depicted in Fig. 3.

Under salt treatment, acid phosphatase gene transcripts were mostly detected in the outer cortex and less extended in the inner cortex. These transcripts were also detected in the infected zone for both symbiotic combinations 115-CIAT899 and 147-CIAT899 (Fig. 4b and c). We noted that negative controls did not show any fluorescent signal (Fig. 4a). In salinity conditions, APase transcripts showed less fluorescent signal for both combinations compared with their corresponding controls, also APase transcripts were more abundant in

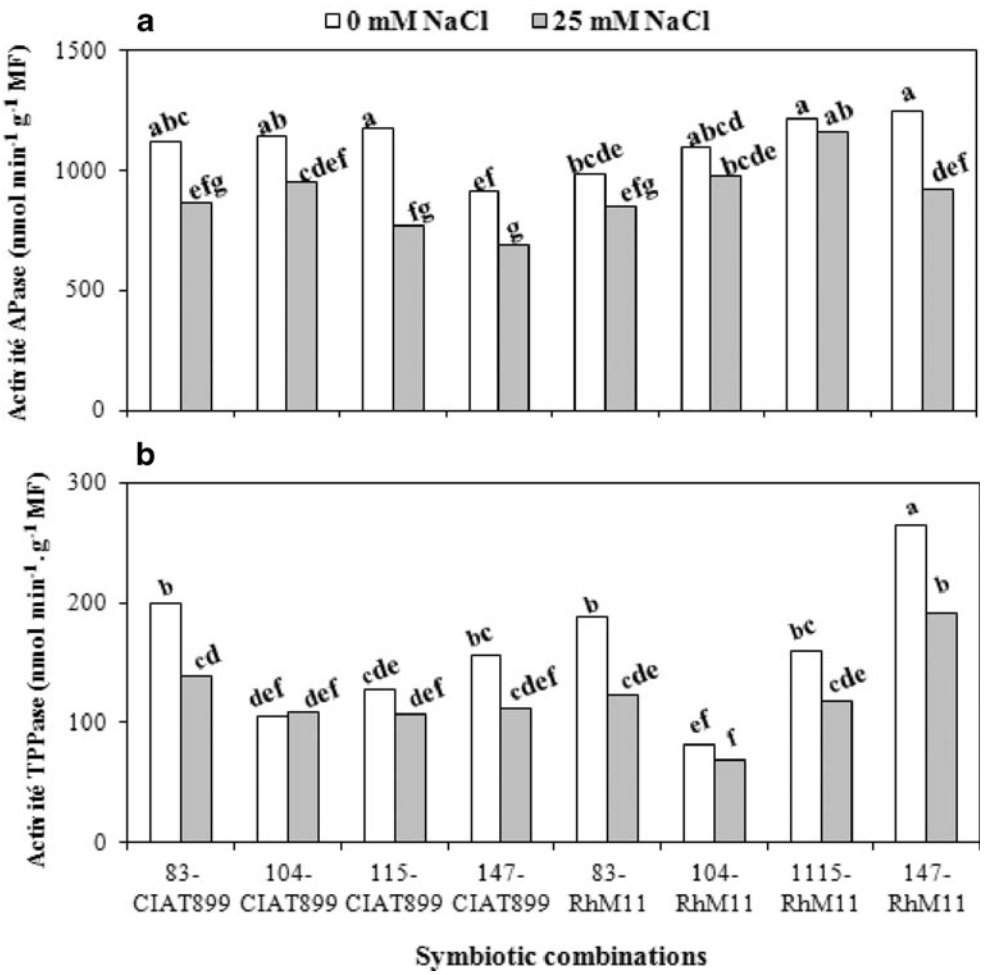

Fig. 2 Effects of salinity on nodules enzymes activities: **a** Phosphatase acid (APase) and **b** Trehalose phosphate-6P phosphatase (TPP). *Means followed by the same letter* within a column are not significantly different ($P<0.05$) using the LSD test

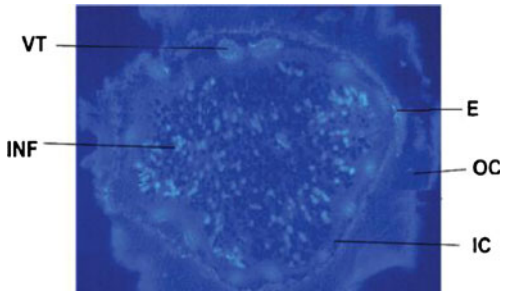

Fig. 3 Cross section through a common bean nodule. Abbreviations: (*INF*) Infected zone, (*E*) Endodermis, (*VT*) Vascular trace, (*IC*) Inner cortex, (*OC*) Outer cortex

147-CIAT899 nodules than in 115-CIAT899 nodules of the symbiotic combination (Fig. 4c). In contrast, nodules of the symbiotic combination 115-CIAT899 did not show any difference of fluorescent signal compared with the control of 147-CIAT899 symbiotic combination.

4 Discussion

Many previous studies have shown that salt stress conditions induced a decrease of plant growth and its development processes (Sairam and Tyagi 2004; Faghire et al. 2011). Nevertheless, it is known that plants have many morphological and physiological strategies that allow them to survive under salt stress conditions. Because of the importance of legume–*Rhizobium* symbiosis in agriculture, we evaluated the symbiotic interaction involving the Moroccan native strain RhM11 and CIAT899 with four lines of common bean under salt stress conditions.

Our results showed that under salinity and symbiotic conditions, the reaction of host plant depends on the plant line and also on the rhizobial strain used for inoculation (RhM11 or CIAT 899). In this regard, salt conditions affected the nodulation of common bean except for the plants of the line 83 inoculated with RhM11. Under salinity treatment, the nodule dry weight of the line 104 plants, inoculated with RhM11, increased significantly compared to the control treatment. In our work, the mean biomass of nodules represented approximately 8 % of the whole plant biomass. These results are consistent with the findings of several researchers (Tang et al. 2001; Kouas et al. 2005; Faghire et al. 2011). We noted that SDW plant was not affected by salinity in the majority of the tested symbiosis combinations except for 83-CIAT899 and 147-RhM11 (Fig. 1a). Our results showed also that the proportion of the allocated mass decreased in shoot from 74 % (control assay) to 69 % (stressed assay) and it increased in roots from 19 % to 22 % (data not shown). Based on this observation, we noted that the shoot was more sensitive to salinity than the root (Fig. 1a, b). Similar results were also found in other grain legumes such as *Cicerarietinum*

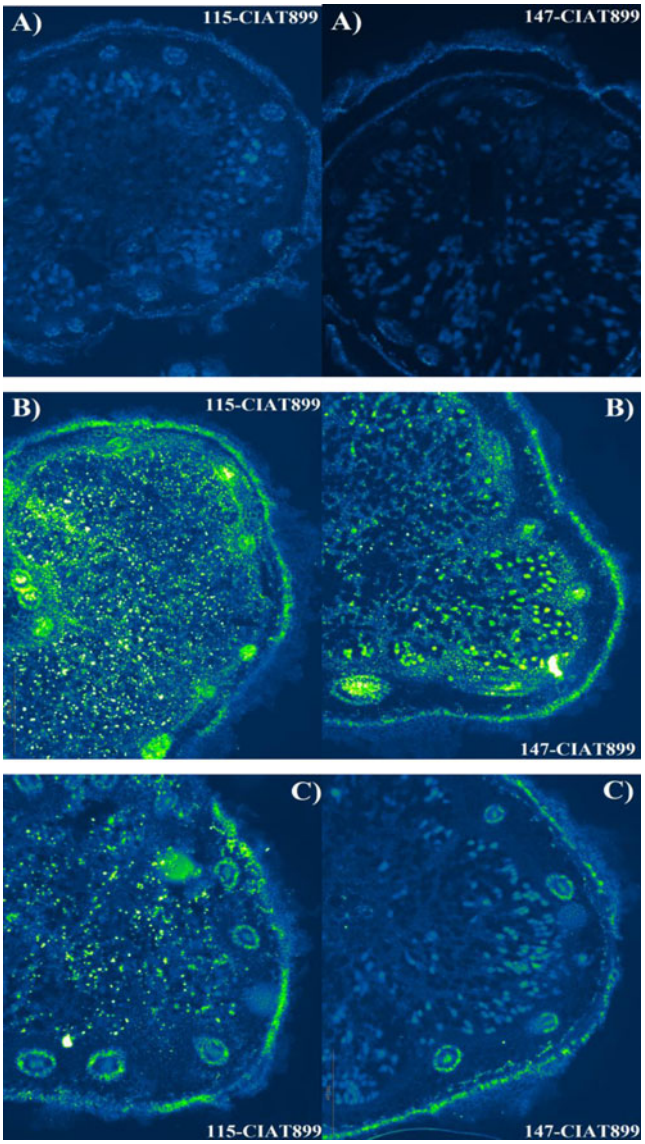

Fig. 4 In situ localization of phosphatase acid transcript isoforms in common bean root nodules (115-CIAT899 and 147-CIAT899). **a** Control without reverse transcription **b** Nodules from non-stressed plants **c** Nodules from stressed plants. *Bars*, 500 μm (this figure is available in colour at *Symbiosis* online)

(Soussi et al. 1998) and *P. vulgaris* (Tejera et al. 2004; Lopez et al. 2006).

In order to understand the effect of salt stress and its relationship with nodulation, we evaluated the nodule enzyme activities under salt conditions. Indeed, this constraint implies many enzymatic processes including phosphatase (Tejera et al. 2004; Adelson et al. 2008; Mandri et al. 2012). Generally, our results under control treatment (0 mM NaCl), can be compared to similar values measured by Adelson et al. (2008); Bargaz et al. (2012) and Mandri et al. (2012) in nodules of common bean plants. The results of biochemical assays of APase activity are consistent with the results in situ of the Acid Phosphatase (APase) transcripts visualisation. Our study,

as well as previous studies of Penheiter et al. (1997); Tejera et al. (2004); Adelson et al. (2008); and Mandri et al. (2012) did not allow to distinguish whether this enzyme activity, specified in crude extracts of ground nodules, derived from tissue fractions of nodules or rhizobia. The our results of nodules TPP activity have agreed with those reported by Lopez et al. (2006) in nodules of common bean plants grown with or without NaCl. Lopez et al. (2006) have also observed that TPP activity was less affected by salinity treatment in common bean nodules.

Nodules APase activity is known to contribute to resistance under salt stress, by maintaining a certain level of inorganic phosphate that can be co-transported with H^+ along a gradient of proton motive force (Olmos and Hellin 1997). Our results showed a positive correlation (up to r=0.6) between nodules APase activity and nodules TPP activity, which suggests high contribution of these enzymes in salt stress. This observation has been reported for a number of plants (Almeselmani et al. 2006; Esfandiari et al. 2007; Jaleel et al. 2007). Based on these observations, we conclude that the inoculation with CIAT899 strain can allow common bean to acquire stress tolerance, whereas inoculation with the RhM11 local strain had the most remarkable capacity to improve tolerance of bean genotypes under salt conditions.

Acknowledgments This work was financially supported by PRAD project N° 06-08. The authors wish to thank Hélène Vailhe (INRA, Montpellier) for her technical assistance.

References

Adelson PA, Claude P, Drevon JJ (2008) Phosphatase and phytase activities in nodules of common bean genotypes at different levels of phosphorus supply. Plant Soil 312:129–138

Almeselmani M, Deshmukh PS, Sairam RK, Kushwaha SR, Singh TP (2006) Protective role of antioxidant enzymes under high temperature stress. Plant Sci 171:382–388

Bargaz A, Ghoulam C, Amenc L, Lazali M, Faghire M, Abadie J, Drevon JJ (2012) A phosphoenol pyruvate phosphatase transcript is induced in the root nodule cortex of *Phaseolus vulgaris* under phosphorus deficiency. J Exp Bot. doi:10.1093/jxb/err313

Cabib E, Leloir L (1958) The biosynthesis of trehalose-6-phosphate. J Biol Chem 231:259–275

Crowe J, Crowe L, Chapman D (1984) Preservation of membranes in anhydrobiotic organisms: the role of trehalose. Science 223:701–703

Delgado MJ, Garrido JM, Ligero F, Lluch C (1993) Nitrogen fixation and carbon metabolism by nodules and bacteroids of pea plants under sodium chloride. Physiol Plant 89:824–829

Esfandiari E, Shekari F, Shekari F, Esfandiari M (2007) The effect of salt stress on antioxidant enzymes activity and lipid peroxidation on the wheat seedling. Not Bot Hort Agrobot Cluj 35:48–56

Faghire M, Bargaz A, Farissi M, Palma F, Mandri B, Lluch C, Tejera García NA, Herrera-Cervera JA, Oufdou K, Ghoulam C (2011) Effect of salinity on nodulation, nitrogen fixation and growth of common bean (*Phaseolus vulgaris*) inoculated with rhizobial strains isolated from the Haouz region of Morocco. Symbiosis 55:69–75

Georgiev GI, Atkins CA (1993) Effects of salinity on N_2 fixation, nitrogen metabolism and export and diffusive conductance of cowpea root nodules. Symbiosis 15:239–255

Jaleel CA, Gopi R, Manivannan P, Panneerselvam R (2007) Antioxidative potentials as a protective mechanism in *Catharanthusroseus* (L.) G. Don. Plants under salinity stress. Turk J Bot 31: 245–251

Jonas NC, Nkonya EM, Mairura FS, Justina NC, Akinnifesi FK (2010) Biological nitrogen fixation and socioeconomic factors for legume production in sub-Saharan Africa. Agron Sustain Dev. doi: 10.1051/agro/2010004

Kouas S, Labidine N, Debez A, Abdelly C (2005) Effect of P on nodule formation and N fixation in bean. Agron Sustain Dev 25:389–393

Lauchli A (1984) Salt exclusion: an adaptation of legume for crops and pastures under saline conditions. In: Staples RC, Toenniessen GH (eds) Salinity tolerance in plants. Strategies for crop improvement. John Wiley and Sons, New York, pp 171–187

Lopez M, Lluch C (2012) Trehalose and abiotic stress tolerance. Abiotic stress responses in plants. 253–265. doi:10.1007/978-1-4614-0634-1_14

Lopez M, Herrera CJA, Iribarne C, Tejera NA, Lluch C (2006) Growth and nitrogen fixation in *Lotus japonicus* and *Medicagotruncatula* under NaCl stress: nodule carbon metabolism. J Plant Physiol 165:641–650

Lopez M, Tejera NA, Iribarne C, Lluch C, Herrera CJA (2008) Trehalose and trehalase in root nodules of *Medicagotruncatula* and *Phaseolus vulgaris* in response to salt stress. Physiol Plant 134:575–582

Mandri B, Drevon JJ, Bargaz A, Oufdou K, Faghire M, Plassard C, Payre H, Ghoulam C (2012) Interactions between common bean genotypes and rhizobia strains isolated from moroccan soils for growth, phosphatase and phytase activities under phosphorus deficiency conditions. J Plant Nutr 35:1477–1490

Molina C, Zaman-Allah M, Khan F et al (2011) The salt-responsive transcriptome of chickpea roots and nodules via deepSuperSAGE. BMC Plant Biol. doi:10.1186/1471-2229-11-31

Ohno T, Zibilske LM (1991) Determination of low concentrations of phosphorus in soil extracts using malachite green. Soil Sci Soc Am J 55:892–895

Olmos E, Hellin E (1997) Cytochemical localization of ATPase plasma membrane and acid phosphatase by cerium based in a salt-adapted cell line of *Pisumsativum*. J Exp Bot 48:1529–1535

Padilla L, Kramer R, Stephanopoulos G, Agosin E (2004) Overproduction of trehalose: heterologous expression of Escherichia coli trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase in *Corynebacteriumglutamicum*. Appl Environ Microbiol 70: 370–376

Penheiter AR, Duff SMG, Sarath G (1997) Soybean root nodule acid phosphatase. Plant Physiol 114:597–604

Sairam RK, Tyagi A (2004) Physiological and molecular biology of salinity stress tolerance in plants. Curr Sci 86:407–420

Soussi M, Ocana A, Lluch C (1998) Effect of salt stress on growth, photosynthesis and nitrogen fixation in chickpea (*Cicerarietinum*L.). J Exp Bot 325:1329–1337

Soussi M, Lluch C, Ocana A (1999) Comparative study of nitrogen fixation and carbon metabolism in two chick-pea (*Cicerarietinum* L.) cultivars under salt stress. J Exp Bot 50:1701–1708

Tang C, Hinsinger PJ, Jaillard B, Rengelz Z, Drevon JJ (2001) Effect of phosphorus deficiency on the growth, symbiotic N_2 fixation and proton release by two bean (*Phaseolus vulgaris*) genotypes. Agronomie 21:683–689

Tejera GNA, Olivera M, Iribarne C, Lluch C (2004) Partial purification and characterization of a non-specific acid phosphatase in leaves and root nodules of *Phaseolus vulgaris*. Plant Physiol Biochem 42:585–591

Vadez V, Lasso JH, Beck DP, Drevon JJ (1999) Variability of N_2-fixation in common bean (*Phaseolus vulgaris* L.) under P-deficiency is related to P use efficiency. Euphytica 106:231–242

Van Aarle IM, Viennois G, Amenc LK, Tatry MV, Luu DT, Plassard C (2007) Fluorescent in situ RT-PCR to visualise the expression of a phosphate transporter gene from an ectomycorrhizal fungus. Mycorrhiza 17:487–494

Vance CP, Uhde-Stone C, Allan DL (2003) Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytol 157:427–447

Wiemken A (1990) Trehalose in yeast, stress protectant rather than reserve carbohydrate. Anton Leeuw J Gen Microbiol 58:209–217

Zhu JK (2002) Salt and drought stress signal transduction in plants. Annu Rev Plant Biol 53:247–273