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Salt tolerance of a common bean (*Phaseolus vulgaris* L.) cultivar as affected by rhizobia

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Abstract
In this work, we analyzed the effect of rhizobia on salt-tolerance in common bean (*Phaseolus vulgaris* L.). For this purpose, a salt-sensitive cultivar (cv. Coco blanc) was inoculated separately with three rhizobia strains (Ar2, B155, and KH28) under glasshouse conditions. Symbiotic parameters (nodule number and nodule mass), shoot nitrogen content, plant growth, root to shoot ratio (RSR) and efficiency in utilization of the rhizobial symbiosis (EURS) were recorded in plants grown in absence (control) and presence of salt (30 mM NaCl). Nodule number and mass were greatly reduced by NaCl in plants inoculated either with Ar2 or B155. In contrast, the two symbiotic parameters were less affected by salt in plants inoculated with KH28. Salt-stressed plants showed a decrease in shoot nitrogen content and growth; but, in plants inoculated with KH28, the decrease was significantly lower. Without salt, RSR was not different between the three symbiotic associations but it tended to increase with salt, being significantly higher with KH28. In addition, nodule efficiency of KH28 was the highest under saline conditions. We concluded that rhizobia strains with superior symbiotic efficiency under saline conditions may significantly improve salt-tolerance in common bean (*P. vulgaris* L.).

Keywords: Salinity, rhizobia, symbiosis, *Phaseolus vulgaris* L.

1. Introduction

Legumes play an important role in agriculture by furnishing much of the available nitrogen in cultivated areas (Iselay, 1982) through symbiotic interaction with rhizobia. Thereby, these plants are becoming interesting candidates for improving saline soil fertility (Crespi and Galvez, 2000). However, the legume-rhizobia symbiosis can be specifically affected in saline soils, as shown for several legumes including *Phaseolus vulgaris* L. (Zahran, 1999). Soil salinity is a major environmental stress and a substantial constraint to crop production for both dry-land and irrigated agriculture (Epstein and al., 1980). Salinity inhibits the initial steps of the legume-rhizobia symbiosis (Zahran, 1999). Bacterial colonization and root hair curling of *V. faba* were reduced in the presence of 50 to 100 mM NaCl (Zahran, 1986; Zahran and Sprent, 1986) and the proportion of root hairs containing infection threads was reduced by 30%.

In the soybean-*Bradyrhizobium japonicum* symbiosis, nodulation was completely suppressed by 210 mM NaCl (Tu, 1981). Nodulation capacity and nodule function are adversely affected even by mild stress conditions which have no adverse effect on plant growth depending on combined nitrogen (Merchan et al., 2003). The effect of salt stress on nodulation and nitrogen fixation in legumes is well documented (Abdel-Wahab and Zahran, 1981; Delgado et al., 1994; Velagaleti et al., 1990; Zahran, 1986). The reduction of N_2-fixing activity by salt is usually attributed to a reduction in respiration of nodules (Delgado et al., 1994) and a reduction in cytosolic protein production, specifically leghemoglobin, by nodules (Delgado et al., 1993; Delgado et al., 1994). The depressive effect of salt on N_2-fixation in legumes is directly related to the salt-induced decline in dry weight and nitrogen content in the shoot (Cordovilla et al., 1995). The salt-induced distortions in nodule structure could also be reasons for the decline in the N_2-fixation rate by legumes subject to salt stress (Zahran, 1986; Zahran and Abu-Gharbia, 1995). Reduction in photosynthetic activity may also affect growth and N_2-

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fixation by legumes under salt stress (Georgiev and Atkias, 1993).

A large number of rhizobia strains can tolerate high levels of salt, but they have usually showed significantly decreased symbiotic efficiency under salt stress (Nair et al., 1993). Recent reports support the finding that some strains of salt-tolerant rhizobia have the potential to form successful N_2-fixing symbiosis with legumes under saline conditions up to 130 mM NaCl (El-Mokadem et al., 1991; El-Sheikh and Wood, 1995). Inoculation of clover and alfalfa with salt-tolerant strains of *R. leguminosarum* bv. *trifolii* and *R. meliloti* enhanced nodulation and nitrogen content of plants under salt stress up to 1% NaCl (El-Mokadem et al., 1991). El-Sheikh and Wood (1995) reported that under saline conditions, the salt-tolerant strains of rhizobia formed more effective N_2-fixing symbiosis with soybean than did the salt-sensitive strains. Although the strains of rhizobia that are best able to form effective symbiosis with their host legumes at high salinity levels are salt-tolerant, they are not necessarily derived from saline soils (Subba Rao et al., 1990).

Common bean (*Phaseolus vulgaris* L.) is sensitive to salinity, like many other leguminous crops, and suffers reduced yield even if it is grown at soil salinity less than 2 dS m^{-1} (Mass and Hoffman, 1977). Therefore, the improvement of salt-tolerance in this important crop using the rhizobial symbiosis may be useful. However, reports on such tolerance as influenced by rhizobia in *Phaseolus vulgaris* L. appear to be rare. Finally, it is important to identify differences in salt-tolerance among rhizobial symbioses with legumes as a possible selection strategy for the cultivated areas affected by salt. In our study, we analyzed the variation of salt-tolerance in a common bean cultivar inoculated with three rhizobia strains. Our objective was to determine if the ability to grow under saline conditions can be improved in *Phaseolus vulgaris* L. using rhizobial symbiosis.

2. Materials and Methods

Biological material and growth conditions

To evaluate the effect of rhizobia on salt-tolerance in common bean (*Phaseolus vulgaris* L.), a salt-sensitive, white seeded bean cultivar Coco blanc (Saadallah et al., 2004; Bouhmouch et al., 2005) was inoculated with three rhizobia strains kindly provided by INRAT-Tunisia, Ar2 and B155 were isolated in the northern Tunisia and KH28 is from France. In a previous preliminary test using growth media culture technique, Ar2 was found to be salt-sensitive and the two other rhizobia salt-tolerant. Plants were grown under glasshouse conditions at day/night temperatures of 22±5°C/15±2°C and 60±10% relative humidity during the day. Seeds were surface-sterilized with calcium hypochlorite (35 $g.l^{-1}$) then washed thoroughly in 10 changes of sterile distilled water. Thereafter, seeds were germinated for 3 days in Petri dishes containing sterile distilled water. The seedlings were transplanted individually into 250 cm^3 plastic pots containing sterile sand and irrigated with nutrient solution (Vadez et al., 1996). During the first 2 weeks (i.e. before nodule function), the nutrient solution was complemented with 2 mM urea, and thereafter, was supplied without urea.

Rhizobial inoculants, as a liquid culture in YEM medium, were applied by soaking the seedlings in the inoculants 30 minutes prior to transplantation. Salt treatment was initiated when plants were 10 days old by supplying the nutrient solution with 30 mM NaCl versus 0 mM as control.

Measurements and data analysis

Plants were harvested at early flowering (R6) in order to measure nodule number and nodule mass. Nodules were removed from roots, their number recorded and the plants were separated into shoots and roots. The separated plant organs and nodules were placed in a 70°C oven and dried prior to weighing. Thereafter, samples of shoots were ground and total nitrogen content was measured by the Kjeldahl procedure.

The efficiency in utilization of the rhizobial symbiosis (EURS) was estimated by the slope of the regression model of plant biomass as a function of nodule biomass. For a linear adjustment-curve, i.e. $y = ax + b$, b corresponds to the plant biomass production without nodules (g pDW_0), and a corresponds to the EURS as (g pDW – g pDW_0) g^{-1} nDW. (pDW = plant dry weight; nDW = nodule dry weight).

The analysis of data was performed with ANOVA using AGROBASE 99 software that was kindly provided by ICARDA (Aleppo). Fisher's least significant difference (LSD) test was used to compare means.

3. Results

Symbiotic parameters and shoot nitrogen content

Results in Fig. 1 show variation of the symbiotic parameters, with and without 30 mM NaCl, among the three symbiotic associations. Without salt, nodule number was not different in plants inoculated with either Ar2 or B155 (Fig. 1a). With the later rhizobia this parameter was significantly higher than that with KH28. With salt, there was no significant difference whatever the rhizobia, although there was a trend of higher number of nodules with KH28 in comparison to that of the two other rhizobia. Nodule mass did not show important variation among the three symbiotic associations under control conditions but the mass recorded with Ar2 was higher than that with KH28 (Fig. 1b). Under saline conditions, nodule dry weight was

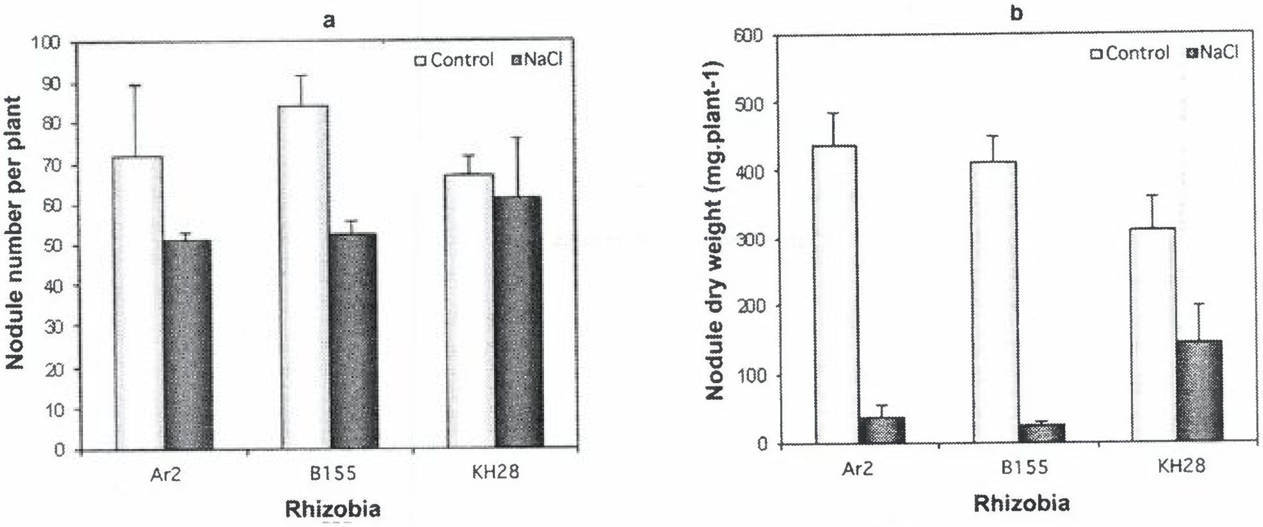

Figure 1. Variation of a) nodule number and b) nodule mass (mg.plant−1) in *P. vulgaris* plants cv. Coco blanc inoculated with three rhizobia (Ar2, B155, KH28) and grown without salt (control) or with 30 mM NaCl. Plants were grown in glasshouse and harvested at early flowering (R6). (For nodule number LSD=16.15; CV=16.83 and for nodule mass LSD=109.66; CV=32.37). Results are means and SD of 5 replicates.

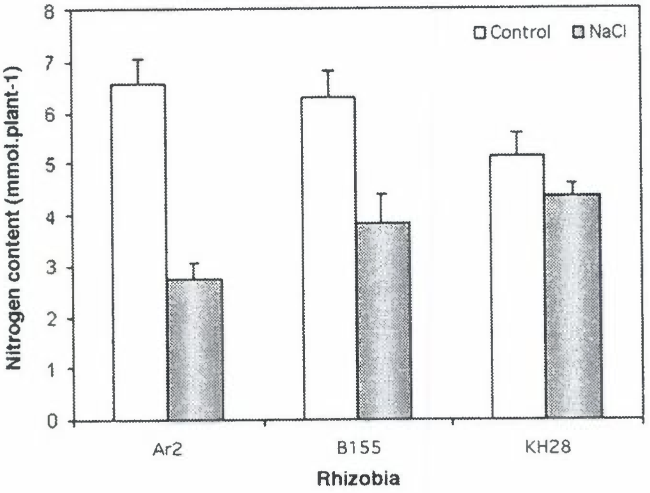

Figure 2. Variation of shoot nitrogen content (mmol.plant−1) in *P. vulgaris* plants cv. Coco blanc inoculated with three rhizobia (Ar2, B155, KH28) and grown without salt (control) or with 30 mM NaCl. LSD=0.96; CV=13.43. Results are means and SD of 4 replicates.

significantly reduced in plants inoculated with Ar2 and B155 with respect to the decrease observed for KH28. With the later, nodule mass was three and five-fold higher than respectively that with Ar2 and B155.

Concerning shoot nitrogen content, without salt, the lowest nitrogen fixation was recorded in plants inoculated with KH28 (Fig. 2). Under saline conditions, an important variation of salt effect was recorded among the three symbiotic associations. Ar2 presented the lowest shoot nitrogen content with respect to the two remaining rhizobia. In addition, this parameter was highly reduced by salt when plants were inoculated with Ar2 or B155; the decrease being more than, respectively, 50% and 40% of that in control plants. By contrast, no significant effect of salt was observed in plants inoculated with KH28.

Plant growth

Plant biomass production was studied in the three symbiotic associations, under control and saline conditions. Without salt, the symbiotic potential as expressed by shoot growth was lower with KH28 than that with the two remaining rhizobia (Table 1). By contrast, root dry weight and root to shoot ratio did not show any significant difference between the three symbiotic associations (Table 1). Under saline conditions, a significant effect of salt that varied with rhizobia, was observed on plants growth (Table 1). The symbiotic potential of KH28 was significantly higher than that of Ar2. Salinity decreased the shoot growth in plants inoculated with Ar2 and B155 by more than 69% and 61%, respectively versus less than 35% for those inoculated with KH28. In the same way, root dry weight was highly reduced by salt with Ar2 and B155 (more than 58% and 48% of control, respectively) while being lightly affected with KH28. RSR tended to increase with salt and was significantly higher with KH28 in salt-stressed plants than in control plants inoculated with Ar2 and B155.

Efficiency in utilization of the rhizobial symbiosis (EURS)

In order to assess whether the efficiency in utilization of the symbiosis varied with rhizobia, the plant dry weight of each individual plant was plotted as a function of its nodule mass (Fig. 3). The EURS was found to vary with both rhizobia and treatment. Without salt, a significant difference

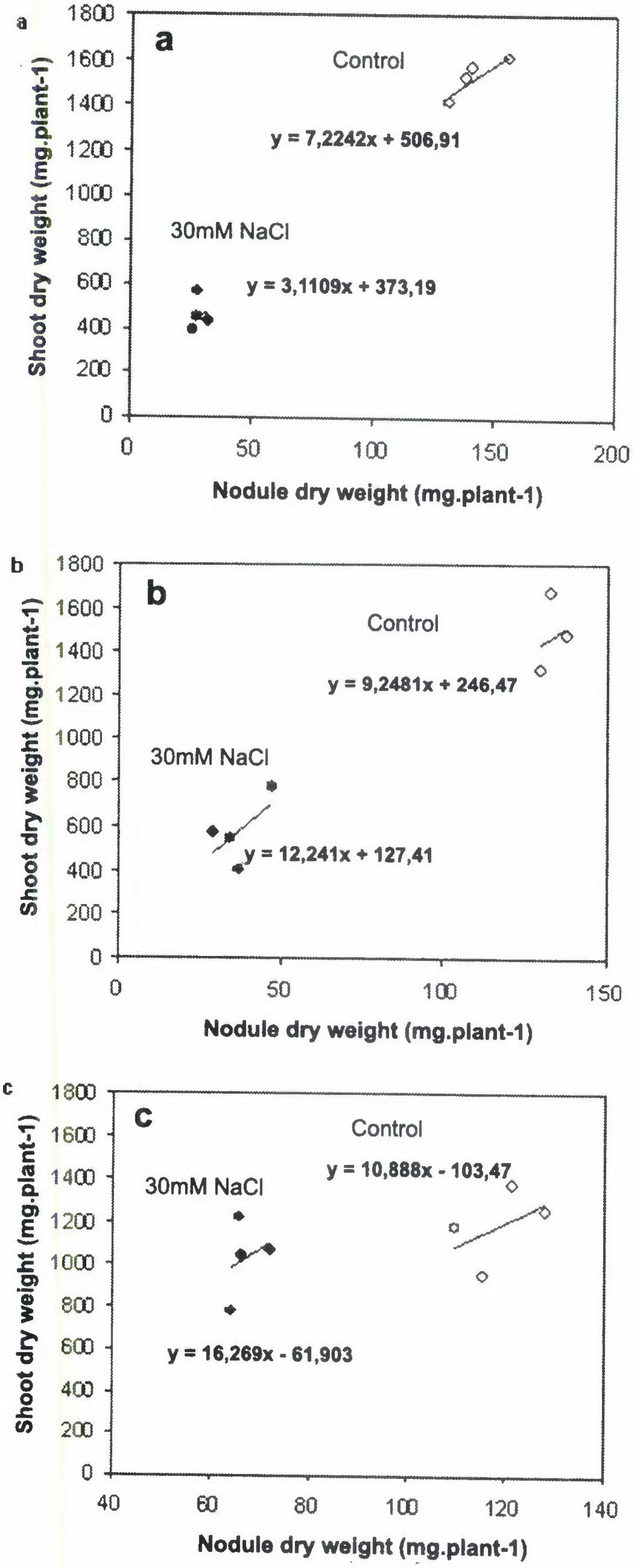

Figure 3. Variation of efficiency in utilization of the rhizobial symbiosis (EURS) in a salt sensitive common bean cultivar (Coco blanc) inoculated with rhizobia strains a) Ar2, b) B155 and c) KH28. Plants were grown in glasshouse and harvested at R6.

in this efficiency parameter was apparent among rhizobia. Nodule efficiency of KH28 was more than 1.5-fold higher than that of Ar2. Nodules of B155 showing an intermediate value (9.24). With salt, the EURS was largely decreased for Ar2 from 7.22 to 3.11. By contrast, a significant increase of this parameter was recorded with B155 and to a larger extent with KH28. The later rhizobia strain presented the highest nodule efficiency (16.26).

4. Discussion

In the legume-rhizobia symbiosis, the adverse effects of salinity on nodulation, nodule functioning, N_2-fixation and growth vary in intensity according to plant species, duration and conditions of exposure to the saline condition but also to rhizobia strain. Reduction of nodulation, plant growth and nitrogen content has been reported in several important grain legumes including *Phaseolus* (Delgado et al., 1994); although an increase of nodule mass with increasing salinity was observed in chickpea (Soussi et al., 1998) and in faba bean (Cordovilla et al., 1999). Our results indicate that the nodule mass decreased largely due to salt stress (Fig. 1b) as described earlier (Delgado et al., 1994).

However, the decrease was found to vary with rhizobia. Plants inoculated with Ar2 or B155 were poorly nodulated under saline conditions and salt reduced their nodule mass to less than 50% of that in control plants (Fig. 1b). By contrast, with KH28, plants presented a good nodulation under salt stress, indicating a better infectivity potential of this strain. These results provide evidence that some rhizobia are able to nodulate common bean plants under saline conditions, and are in agreement with El-Mokadem et al. (1991) who report that inoculation of legumes by salt-tolerant strains enhanced nodulation. However, Bouhmouch et al. (2005) observed that osmotolerant rhizobia failed to nodulate a salt-tolerant common bean cultivar while the same strains were able to nodulate the salt-sensitive Coco blanc under saline conditions.

Nitrogen-fixing activity is highly sensitive to environmental stresses like salinity and slight variations in the stress conditions applied may produce great changes in nodule activity. Important variations of shoot nitrogen content were observed in the present study (Fig. 2). As for symbiotic parameters, the differences in nitrogen content varied with rhizobia. Symbiosis with Ar2 proved to be the most salt-sensitive concerning this parameter. Indeed, salinity greatly decreased the nitrogen content of plants inoculated with Ar2 while with the two other rhizobia, the decrease was significantly lower (Fig. 2). Our results are in harmony with those reported in clover and alfalfa by El-Mokadem et al. (1994) using salt-tolerant strains of *R. leguminosarum* bv. *trifolii* and *R. meliloti*.

However, fluctuations of this parameter may occur as a consequence of dilution or a concentration effect depending on the relative severity of salt stress on growth or nitrogen

Table 1. Variation of shoot dry weight (g.plant−1), root dry weight (g.plant−1) and root to shoot ratio (RSR) in *P. vulgaris* plants cv. Coco blanc inoculated with three rhizobia (Ar2, B155, KH28) and grown without salt (control) or with 30 mM NaCl. Plants were harvested at R6. Results are mean and SD of 6 replicates.

Rhizobia	Treatment	Shoot dry weight	Root dry weight	Root to shoot ratio
Ar2	Control	1.531±0.084	0.424±0.042	0.277±0.02
	30 mM NaCl	0.463±0.075	0.177±0.019	0.393±0.09
B155	Control	1.490±0.271	0.437±0.062	0.302±0.07
	30 mM NaCl	0.577±0.152	0.223±0.057	0.405±0.13
KH28	Control	1.189±0.273	0.449±0.032	0.388±0.06
	30 mM NaCl	0.779±0.071	0.386±0.070	0.503±0.13
LSD		0.263	0.074	0.139
C.V.		17.65	14.41	24.77

uptake (Frota and Tucker, 1978; Pessarakli and Tucker, 1985, 1988). The decrease in nitrogen fixation is usually a consequence of both the lower number of nodules and the decrease in the specific nitrogenase activity per gram of nodule. The results on nitrogen content corroborated those recorded on nodulation and demonstrate that KH28 is the most efficient strain under saline conditions. Similar results suggesting that under salinity, the salt-tolerant strains of rhizobia formed more effective N_2-fixing symbiosis than did the salt-sensitive strains have been reported in soybean by El-Sheikh and Wood (1995).

The effect of salt on plant growth was significant and also varied with rhizobia. These results are in agreement with reports supporting that some rhizobia have the potential to form a successful symbiosis with legumes under salt stress (Zahran, 1999; Abdel-Wahab and Zahran, 1981; Cordovilla et al., 1995a,b). Symbiosis with KH28 led to the least decrease of plant growth in response to salt stress (Table 1). This may be attributed to the highest symbiotic performance of this rhizobia under saline conditions. Results on the efficiency in utilization of the rhizobial symbiosis (EURS) corroborated this trend, showing an important increase of nodule efficiency due to salt with KH28 (Fig. 3).

From the present work, it appears that rhizobia KH28 has the symbiotic potential to improve salt tolerance in common bean (*Phaseolus vulgaris* L.). Because of the large genetic diversity in efficiency of rhizobia nodulating common bean under various environmental stresses, research for strains with superior symbiotic performance under saline conditions may lead to a more significant improvement of salt-tolerance in this crop. To a certain extent, inoculation of other legumes with salt-tolerant strains of rhizobia will probably be potential for improvement of N_2-fixation and soil fertility in saline environments including Mediterranean cultivated areas.

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