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Original article

Effects of hydroponic culture system and NaCl on interactions between common bean lines and native rhizobia from Tunisian soils

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Abstract – Common bean (*Phaseolus vulgaris* L.) lines, namely BAT477, CocoT, Flamingo, DOR364 and NAG310, were inoculated with reference strain *Rhizobium tropici* CIAT899 or native rhizobia, namely *Sinorhizobium fredii* 1a6 and *R. etli* 12a3 and then grown in two hydroponic semi-sterile systems: (i) gravel in pots, (ii) aerated nutrient solution in bottles. In the aerated solution system, shoot plant growth was 5 to 9 times higher, depending on the symbiosis, than on gravel. A strain × line interaction was observed in the gravel system. However, such an interaction was not significant in the aerated system because of large intra-treatment variability. In the aerated system and under NaCl treatment, BAT477 inoculated with local strains (1a6 and 12a3) appeared to be a highly tolerant line. In contrast, NAG310 was the least tolerant line when inoculated by CIAT899. However, the tolerance of NAG310 to NaCl increased when it was inoculated with 12a3, although this strain conferred low nodule growth to Flamingo under NaCl. It is concluded that strain × line interactions are important and should be considered for selecting the legume most adapted to such an environmental constraint as salinity, and that the aerated solution system is efficient for selecting highly efficient rhizobial symbioses.

common bean / culture system / NaCl / *Rhizobium* / symbiotic interactions

Résumé – Effets du système de culture hydroponique et de NaCl sur les interactions entre lignées de haricot et des rhizobia natifs des sols de Tunisie. Les lignées de haricot (*Phaseolus vulgaris* L.) BAT477, CocoT, Flamingo, DOR364 et NAG310 ont été inoculées avec la souche de référence CIAT899 (*Rhizobium tropici*) et celles locales 1a6 (*R. fredii*) et 12a3 (*R. etli*), et cultivées sur deux systèmes hydroponiques semi-stériles : (i) en pots sur gravier et (ii) en flacons sur solution nutritive aérée. La croissance des parties aériennes est 5 à 9 fois supérieure dans le milieu hydroponique aéré par rapport à celui utilisant le gravier comme support. Une interaction souche × lignée a été observée sur gravier mais non sur milieu aéré où elle semble être masquée par une importante variabilité. Sous traitement salin en milieu aéré, la lignée BAT477 inoculée avec les souches locales (1a6 et 12a3) se montre particulièrement tolérante. La lignée NAG310 est la moins tolérante quand elle est inoculée avec la souche CIAT899. Par contre, la souche 12a3 lui confère une tolérance au sel pour la production de la biomasse nodulaire contrairement à sa symbiose avec la lignée Flamingo. Il est conclu qu'il est important de prendre en considération les interactions souche × lignées pour la sélection d'une légumineuse adaptée à une contrainte comme la salinité, et que la culture sur solution aérée est une bonne méthode pour la sélection de symbioses rhizobiennes à fort potentiel de fixation d'azote.

haricot / interactions symbiotiques / NaCl / *Rhizobium* / système de culture

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1. INTRODUCTION

In the common bean-rhizobia symbiosis, lines of *Phaseolus vulgaris* L. as well as rhizobial strains can affect the nodulation, the nitrogenase activity and the nitrogen accumulation in reproductive and vegetative tissues [12]. Thus, for the selection of genotypes with high yield via an increase of their symbiotic nitrogen fixation (SNF), it is necessary to understand determinant factors of plant or bacterial growth [7] which can be assessed via contrasting symbioses. Variability between common bean-nodulating rhizobia on one common bean line and between common bean lines inoculated with one strain have been shown by many authors [1, 10, 18]. Moreover, interaction between legume and rhizobial factors influencing the establishment and the issue of the symbioses under various growth conditions have already been emphasised [4, 7].

However, under field conditions common bean × rhizobia symbiosis is particularly sensitive to various environmental factors. In semi-arid and arid regions of the Mediterranean basin, where osmotic constraints due to water deficiency or salinity are major limitations to leguminous plants, growth depends particularly on SNF [1, 3, 13].

The aim of this work was to analyse common bean lines × rhizobia strains interaction and its modulation by culture systems and salt application.

2. MATERIAL AND METHODS

2.1. Biological material

The common bean lines BAT477, Flamingo, DOR364 and NAG310 were provided by V Voysest (CIAT Cali, Colombia) and chosen according to the results of Trabelsi (ESA Mateur, Tunisia) within the FYSAME project who multiplied them together with the CocoT line largely cultivated in Tunisia. In addition, the DOR364 line has been tested because it is widely grown in Central America and in the Caribbean, and can be grown under Mediterranean conditions [13].

They were inoculated with either *Rhizobium tropici* CIAT899, as reference strain, or with *Sinorhizobium fredii* 1a6 or *R. etli* 12a3, both isolated from the Cap Bon region in Tunisia, which were previously characterised at the phenotypic and molecular levels by Mhamdi et al. [11].

2.2. Culture systems

For the culture in pots on gravel, bean seeds were sterilised, germinated on 0.9% agar and transferred to plastic pots of 0.5 l capacity filled with gravel previously sterilised for 48 h at 180 °C. Three days after sowing in the pots the cotyledons were gently passed through holes fitted in the pot lids, and 1 ml of rhizobial suspension containing approximately 10^9 cells·ml^{-1} was applied to the plant roots.

For the liquid-culture aerated nutritive-solution, each seven-day-old seedling root was passed carefully through the pierced rubber stopper of a 1 l serum-bottle. Plants were fixed with cotton fitted around the hypocotyl, and the nutritional solution was permanently aerated by compressed air at a flow of 400 ml·min^{-1}, as described previously [5].

The nutrient solution [18] was supplied regularly by distilled water to the gravel culture and changed weekly, whereas it was completely renewed every two weeks in the hydroaeroponic culture. The salt treatment was studied exclusively with the latter system. It consisted of 45 mM NaCl at each change of nutrient solution. This NaCl concentration had a significantly inhibitory effect on studied lines and it was comparable to NaCl concentration in experimental soils of the Medjerda valley [15].

Plants were harvested at the appearance of the first pod. Nodule, shoot and root weights were measured after desiccation at 75 °C for 48 h.

3. RESULTS

3.1. Nodulation and growth of plants on gravel

The nodule dry weight varied with *Rhizobium* strains ($p < 0.01$) and *P. vulgaris* line ($p < 0.01$) (Fig. 1A). The native rhizobia 1a6 and 12a3 induced more nodular tissues than CIAT899 and the line NAG310 nodulated significantly less than the other lines. The rhizobia × line interaction was not statistically significant. However, Strain 1a6 induced much more nodular dry matter than 12a3 with BAT477, but it was the opposite with Flamingo.

The shoot growth varied with *Rhizobium* strain ($p < 0.01$) and *P. vulgaris* line ($p < 0.01$) (Fig. 1B). Again, the symbiosis BAT477-1a6 showed the highest shoot dry matter. Whatever the rhizobia, growth of NAG310 was about 50% of that of other lines. Interaction between both partners of symbiosis was significant ($p < 0.01$). While 1a6 and CIAT899 conferred to BAT477 or Flamingo a higher shoot growth than CocoT, an opposite result was obtained with strain 12a3.

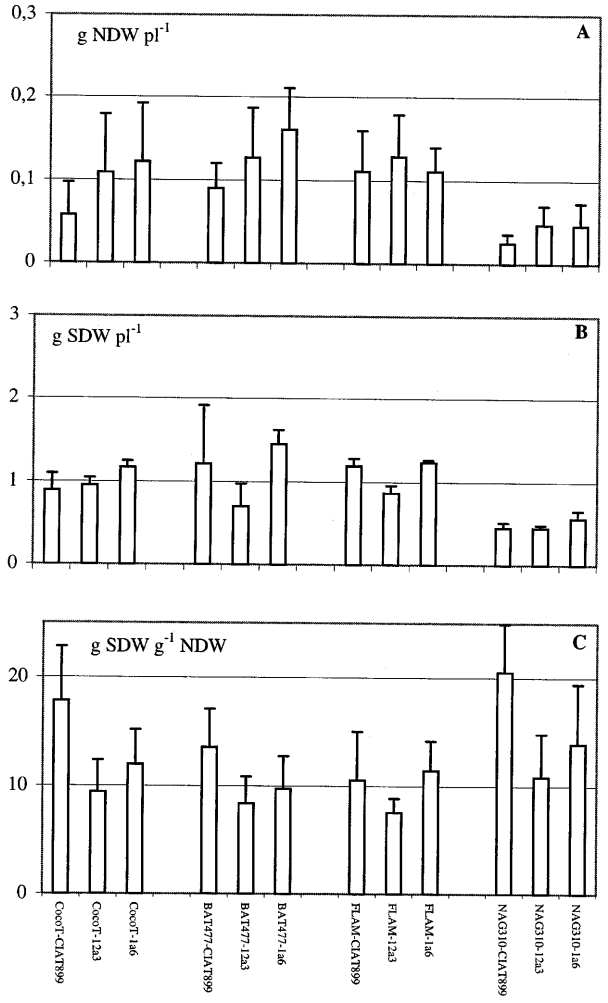

Figure 1. Production of nodule (A) shoot (B) dry matter and nodular efficiency (C) of BAT477, Flamingo, CocoT, and NAG310 lines cultivated on gravel and inoculated with 1a6, CIAT899 and 12a3 strains.

The nodular efficiency assessed by g SDW·g^{-1} NDW varied significantly according to rhizobia ($p < 0.01$) and lines ($p < 0.04$) (Fig. 1C). The symbioses CIAT899-NAG310 and CIAT899-CocoT had the highest average values with 20.5 and 17.8 g SDW·g^{-1} NDW, respectively. In contrast, nodular efficiency of Flamingo and BAT477 symbiosis with 12a3 was particularly low, with 7.6 and 8.4 g SDW·g^{-1} NDW, respectively.

3.2. Nodulation and growth of plants in hydroaeroponics

Figure 2A shows that depending on symbiosis nodular dry matter was 9 times higher on aerated solution than on gravel. However, the variance analysis did not show any significant difference between rhizobia or lines for the nodule dry matter parameter, due to a large intra-symbiosis variability. Strain 1a6 nodulated generally more than 12a3 and CIAT 899, as observed for the gravel (Fig. 1A). It was of interest that NAG310 nodulation was similar to that of other lines. No difference between rhizobia for BAT477 nodulation (Fig. 2A) contrasted with differences observed in the gravel system (Fig. 1A).

Figure 2B shows that depending on the symbiosis, shoot dry matter was between 5 and 9 times higher on aerated solution than on gravel (Fig. 2A). A significantly higher shoot growth was observed with CIAT899 than with 1a6 for BAT477, Flamingo, DOR364 and NAG310, in contrast with CocoT, that showed a better shoot growth with 12a3 and 1a6 than CIAT899 (Fig. 2B).

Whatever the rhizobia, the nodular efficiency was significantly higher with DOR364 than with Flamingo, despite a large intra-symbiosis variability (Fig. 2C). Particularly DOR364-CIAT899 and NAG310-12a3 symbioses showed the highest values with 19.2 and 18.0 g SDW·g^{-1} NDW respectively, and Flamingo-1a6 and NAG310-1a6 the lowest ones with 4.9 and 5.7 g SDW·g^{-1} NDW. Averages of nodular efficiency for the symbioses varied in a 4.9–19.2 interval, comparable to that of 7.6–20.5 on gravel. However, nodular efficiency was differently affected by growth system according to symbioses. In fact CIAT899-CocoT was 2 times higher in the gravel system than in the aerated one, whereas it was the opposite for Flamingo-1a6. On the other hand, symbioses with strain 12a3 showed a generally lower efficiency in the gravel than in hydroaeroponics.

3.3. Effect of salt

In aerated solution, whatever the rhizobia the presence of salt limited CocoT nodulation much more than that of all the other lines (Fig. 2A). Under salt treatment Flamingo-1a6 symbiosis was the most nodulated and CocoT-CIAT899 the lowest one and also the most sensitive symbiosis. However NAG310-12a3 appeared to be the most tolerant symbiosis. A significant line-rhizobia interaction for NaCl tolerance was found. In particular, with rhizobial strain 12a3, the nodulation of Flamingo and DOR364 was about 70% of the control, compared to 98 and 86%, respectively, for BAT477 and NAG310. In

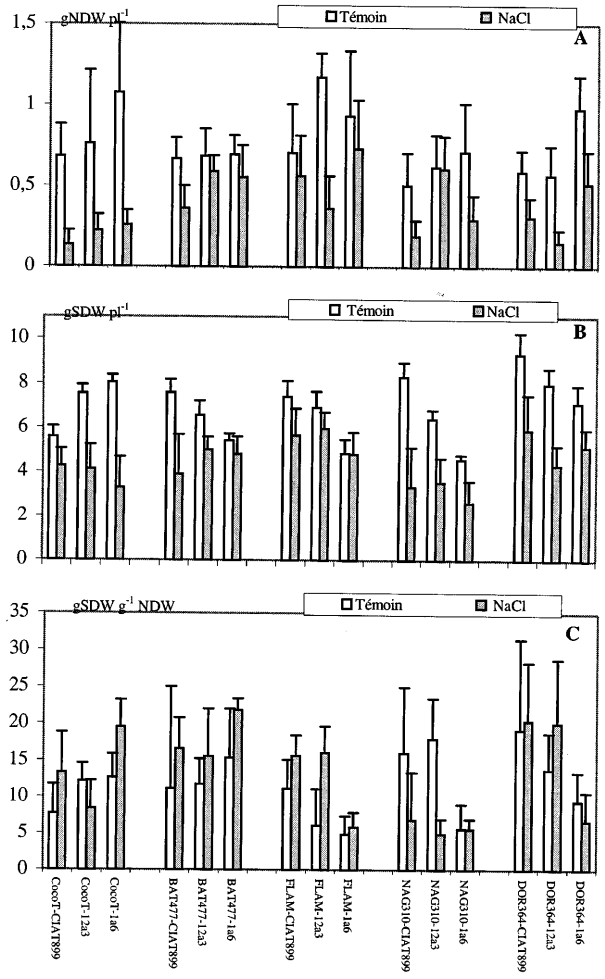

Figure 2. Production of nodule (A) shoot (B) dry matter and nodular efficiency (C) of BAT477, Flamingo, CocoT, NAG310 and DOR364 lines cultivated on aerated medium and inoculated with 1a6, CIAT899 and 12a3 strains under control conditions and 45 mM NaCl.

contrast, with CIAT899, NAG310 nodulation was more inhibited than that of Flamingo (Fig. 2A).

For shoot dry matter the variance analysis of data obtained under 45 mM of NaCl did not show significant effect and no line × strain interaction was revealed. The Flamingo and BAT477 lines appeared to be the most tolerant with respectively 87 and 72% of control values. By contrast NAG310 and CocoT, particularly with native rhizobia, appeared to be the most sensitive with 50 and 57%, respectively (Fig. 2B). The Flamingo-12a3 and DOR364-CIAT899 symbiosis expressed the highest growth under NaCl whereas NAG310-1a6 was the lowest.

Nodular efficiency under NaCl was higher for BAT477 and lower for NAG310 whatever the rhizobia (Fig. 2C). The BAT477-1a6 symbiosis showed a higher nodular efficiency under NaCl, whereas NAG310-12a3 was the least efficient symbiosis. This parameter was modified by salt treatment from a highly inhibitory effect for NAG310-12a3 (to 27% of control) to a highly enhancing effect for Flamingo-12a3 (to 263% of control).

4. DISCUSSION

The level of growth we have observed with the hydroaeroponic system is comparable with previous observations [17]. This growth system leads to plant nodulation and growth of at least nine and five times higher, respectively, than those on gravel. Thus, the aerated solution appears to be an ideal growth condition for symbiosis since all plants expressed their development potential. In contrast, the gravel system appeared to be a constraint to plants which subsequently expressed their tolerance potential. This conclusion agrees with other work [2, 7].

From the overall results, plant growth appeared to be differently affected by the culture system according to the symbiotic partners. For example, growth on gravel limited the nodulation potential of BAT477 less than that of NAG310. This latter line on gravel expressed low N_2-fixing potential as shown before [13], while on aerated solution, it expressed a comparable potential to the other lines. It benefited from the CIAT899 and 12a3 interaction in spite of its low nodular mass. Thus, the sensitivity of the NAG310 line on gravel was closely related to its genotypic behaviour and intrinsic sensitivity to a limitation of root environment rather than a drought stress. There would be, therefore, an effect of the aerated solution on the nodular functioning which would vary according to the lines. In addition, a strain × culture-system interaction is illustrated with 1a6 supporting the highest growth of plants on gravel but the lowest in hydroaeroponics. However, strain × line interactions were significant only with the gravel system.

The line × strains interaction varied with the analysed parameters. We could not observe the higher infectiveness of BAT477 with the *R. etli* 12a3 strain, by contrast with observations by Martinez et al. with another *R. etli* strain [10]. For nodular efficiency, on the contrary, CIAT899 (*R. tropici*) expressed its higher symbiotic potentialities with CocoT, BAT477 and NAG310 on gravel where pH varied from 7 to 5 through the

experiment. This agrees with the adaptation of CIAT899 to acidic soil [10].

Salinity was considered as a major limiting constraint for SNF [1, 3, 13]. The analysis of its effect showed that nodular dry weight was generally more affected than shoot dry weight. However nodular efficiency, both in gravel and the hydroaeroponic system without salt, ranged between 5 and 20 g SDW·g^{-1} NDW. The NAG310 line, in contrast with Flamingo and DOR364, in symbiosis with 12a3 was the most affected by the salt for nodular efficiency, in spite of its high tolerance for nodule growth. These data seemed to emphasise the particular involvement of line genotype in the plant growth under symbiotic conditions. This observation was confirmed by Serraj and Drevon's work on alfalfa [14]. Strain variability and interaction with different lines was also obtained for the 12a3 and CIAT899 strains in relation to NAG310 and Flamingo in nodule growth. The involvement of strain was explained by difference in early or late nodulation which was considered to affect efficiency [9]. The intrinsic rhizobia strain potential also appeared to be involved in the adaptation of symbiosis to NaCl stress via maintaining the nodular efficacy. The influence of the intrinsic tolerance of microsymbiont to salt was shown to be related to the nitrogen fixation ability of the symbiosis [8].

Finally, the line BAT477, known for its high potential for N_2 fixation [6, 10], displayed here a high NaCl tolerance particularly with local strains. This line could be used for more detailed studies. Nevertheless, selection in controlled conditions can give restricted indication of the symbiosis behaviour. Only field trials permit us to know the real effect of constraints and involvement of native rhizobia which are generally more competitive than introduced strains [16].

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