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Rhizobium Tropici vs. Rhizobium Etli: A comparative analysis of nitrogen fixation in common bean under water stress

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Drought stress is one of the most important factors limiting plant growth; in particular, water is regarded as a major problem in symbiotic nitrogen fixation for legume growth. The adverse effects of water stress on the rhizobial symbiosis of common bean genotypes CocoT and Flamingo were investigated in field conditions. Common bean plants were inoculated with rhizobia strains: *Rhizobium tropici* CIAT899 or *Rhizobium etli* 12a3, and grown under field conditions, plants were spray irrigated until the first flower was observed on set. Irrigation was stopped for 20 days in plants submitted to water stress. The enzyme-linked immunosorbent assay (ELISA) technique was used with polyclonal antibody to assess the occupancy in nodule of the rhizobia used for inoculation. Under water stress, a significant decrease in nodule population, nodules biomass, shoot content of nitrogen, growth and chlorophyll with all symbioses, whereas proline accumulation increased. The interaction between symbiosis and water stress for all parameters was significant. Overall, 12a3 was more efficient than CIAT899 for Flamingo, though not for CocoT. It is concluded that symbiosis between Flamingo and 12a3 has potential for application in field under water stress in desert soil.

Key words: Enzyme-linked immunosorbent assay (ELISA), *Phaseolus vulgaris*, rhizobia, symbiosis, symbiotic nitrogen fixation, water stress.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is considered one of the most important grains for human alimentation due to the fact that this plant is rich in elements like potassium, calcium, iron and phosphor and it contains 18 to 32% of protein in every grain averagely (Numan and Nuri, 2005). In Tunisia, symbiotic N_2 fixation in Common bean is constrained by many factors, including the paucity of suitable soil rhizobia, salinity and water stresses and low concentrations of nutrients in the soil such as phosphorus. Under field conditions as biotic and abiotic factors can influence introduced strain performance in the field, especially when in competition with indigenous soil rhizobia. These native strains often out-compete introduced rhizobia for nodule formation in the host plant, leading to poor legume response to inoculation (Okogun and Sanginga, 2003). It is therefore important that the competitiveness of selected rhizobial strains is tested in the field prior to recommending their use as commercial inoculants. In order to estimate the occupancy and identify strain inside nodules, Spriggs and Dakora (2009) reported that the ELISA technique is highly specific, reproducible, and commonly used to detect rhizobial strains directly from nodules. Additionally,

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the method is sensitive, can detect antigens in small nodules, uses small quantities of reagents, is relatively quick, and permits the rapid screening of large nodule samples. It can also detect double strain occupancy of nodule. However, cross-reaction with native strains in field soils can lead to false positive results, thus limiting its application. That is no false positive results for non-appropriate antigen × antibody combinations.

Nodulation and symbiotic nitrogen fixation depend on the nodulating strain in addition to the plant cultivar. Water stress affects root hair infection, bacteroid differentiation, nodule structure, and the functioning of the legume root nodule (Daniel et al., 2007). Zahran (1999) reported that symbiotic N_2 fixation of legumes is also highly sensitive to soil water deficiency. A number of temperate and tropical legumes, for example, *Medicago sativa*, *Pisum sativum*), *Arachis hypogaea*, *Vicia faba*, *Glycine max*, *Vigna* sp., *Aeschynomene*, and the shrub legume *Adenocarpus decorticand* exhibit a reduction in nitrogen fixation when subject to soil moisture deficit. The same author reported that soil moisture deficiency has a pronounced effect on N_2 fixation because nodule initiation, growth, and activity are all more sensitive to water stress than are general root and shoot metabolism.

Therefore, Daniel et al. (2007) reported that several mechanisms have been proposed to explain nitrogen fixation inhibition under abiotic stresses. Oxygen permeability appears to be a limiting factor for nodule functioning, A reduction in nodule carbon flux has also been related to the inhibition of nitrogen fixation under water stress, In these conditions, nodule Suc synthase (SS) activity sharply declines (Gordon et al., 1997), thus limiting the carbon flux required for bacteroid respiration. On the other hand, Emam et al. (2010) reported that the response of nodulation and N_2 fixation to water stress depends on the growth stage of the plants. It was found that water stress imposed during vegetative growth was more detrimental to nodulation and nitrogen fixation than that imposed during the reproduction stage. There was little chance for recovery from water stress in the reproductive stage. Nodule P concentrations and P use efficiency declined linearly with soil and root water content during the harvest period of soybean-*Bradyrhizobium* symbiosis (Franson et al., 1991). Sellstedt et al. (1993) found that N derived from N_2 fixation was decreased by about 26% as a result of water deficiency when measured by the acetylene reduction assay. Therefore, the accumulation of specific organic solutes (osmotica) is a characteristic response of plants subject to prolonged severe water stress. One of these solutes is proline, which accumulates in different legumes, for example, *Glycine max* (Fukutoku and Yamada, 1982) and *Phaseolus vulgaris* (Kapuya et al., 1995). In these plants, positive correlations were found between proline accumulation and drought tolerance.

The most important strategies employed to reduce the effect of water stress on legume production have been focused on a selection of host genotypes that are tolerant to high drought stress conditions (Zlatev and Stoyanov, 2005). Thus, an increase of tolerance to water stress of rhizobial bacteria might constitute another approach to improve plant productivity under symbiosis, or adaptation to various environmental limitations (Saleh Al-Garni, 2006).

The aim of the present study was to assess the effect of water stress on common bean-rhizobia symbiosis through growth, nodulation, N percentage, chlorophyll concentration and proline accumulation. In order to test whether it is more rational to inoculate with introduced rhizobia, the nodules occupancy was assessed with enzyme-linked immunosorbent assay (ELISA) technique for rhizobial identification.

MATERIALS AND METHODS

Biological material

Two common-bean cultivars were used in this work: CocoT, selected as a pure line from the local cultivar Coco; Flamingo, selected on the basis of it's adaptability to Mediterranean conditions (Trabelsi, 1997) among a collection initially supplied by B. Voyesset from CIAT (Colombia). Inoculation was performed with *R. tropici* CIAT899 as reference rhizobia initially isolated in an acid soil of Colombia, kindly supplied by CIAT, or with the native rhizobia *R. etli* 12a3, that was previously isolated from the Cap Bon region in Tunisia and characterized at the phenotypic and molecular levels by Mhamdi et al. (1999).

Field conditions

Experiment was conducted in a field of the experimental station of the ESAM in the north of Tunisia (region of Mateur) which lies in the bioclimatic wet, the average temperature was 25°C and the main soil characteristics of the field are listed in Table 1.

A randomized complete block design was used with sub-plots and three replications. The plots constituted treatments with and without water stress.

Each block contained three treatments: non-inoculated, or inoculated with CIAT899, or 12a3. Each treatment was subdivided into two subplots cultivated with the two cultivars of common bean, CocoT and Flamingo. The subplots were 5×4 m^2 in size and contained ten rows at a sowing density of 20 seeds m^{-2}. A non-cultivated margin of 3 m separated the treatments. The inoculant rhizobia was grown to late exponential phase in YEM (Vincent, 1970) and diluted to 1/20 with well water, thus containing 10^8 ml^{-1}. This suspension was used to inoculate the emerging seedlings using a watering can.

All plots were spray irrigated until the first flower was observed on set. Irrigation was stopped for 20 days in plots submitted to water stress. Plots were protected from rain with shelter made of glass (not interfering with photosynthesis) and to protect the rain test.

Dry mass determination and nitrogen percentage

The plants were harvested after 60 days of growth. Shoot, nodules and roots were separated and dried at 70°C for 2 d. Dry mass of each fraction was measured. The non–inoculated treatment

(without water stress) was used only in order to estimate the rate of indigenous rhizobia (nodulation and ELISA test).

The estimation of leaf area was carried out using a planimeter (Paton electronic Pty Ltd, Adelaide, SA) to measure directly the leaf area (cm^2) of detached leaves treated as screens to light rays.

Total nitrogen (TN) was measured by Kjeldahl procedure on shoots for inoculated plants with and without water stress.

Chlorophyll content

Chlorophyll concentration (Chl) was measured using the method as described by Moran and Porath (1980). For inoculated plants with and without water stress, three replicates of the third foliole of each leaf was excised and the fresh weight was measured. Thereafter the folioles of the plants were mixed with the N, N-dimethylformamide solvent (10 w/w) in darkened and hermetic flask, and transferred in a cold room. When the folioles became completely white after 48 h, the concentration of Chl a and Chl b in the solution was measured by spectrophotometer, and was calculated in mg chl g^{-1} fresh folioles, as (d E_{652} V) $(36 W)^{-1}$ with d = 4, coefficient of dilution of chlorophyll in the solvent; E_{652}, absorbance of Chl a and Chl b at 652 nm; V, volume of the solution of chlorophyll; W, fresh weight of the sample of folioles immersed in the solvent; 36, coefficient of specific merger of the solvent.

Proline determination

Proline concentration was determined according to the method described by khedr et al. (2003) for inoculated plants with and without water stress treatment. Approximately 0.5 g of fresh leaf material was homogenized in 10 ml of 3% aqueous sulfo-salicyclic acid, filtered through Whatman's No. 2 filter paper and 2 ml of solution was mixed with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at 100°C. The reaction mixture was extracted with 4 ml toluene and cooled to room temperature and the absorbance was measured at 520 nm.

ELISA analysis

After nodule numeration of the plants, a subsample of 30 nodules was prepared by mixing 15 nodules from Flamingo and 15 nodules from CocoT for the non-inoculated plants, and similarly 30 nodules for the inoculation treatment with 12a3, and 30 nodules for the inoculation treatment with CIAT899. They were preserved in tubes with anhydrous $CaCl_2$ until indirect ELISA analysis, as previously described by Aouani et al. (1997). In details the following steps of Beck et al. (1993) were performed: (a) each individual nodule was crushed in 1 ml of distilled water, adjusted to the similar turbidity (A600 = 0.4), diluted 50-fold; (b) nodule extracts were added to the wells (100 µl/well) of disposable plastic trays with 96 wells each, specifically for adsorption of proteins in ELISA tests (Immulon II), and incubated for 90 min at 37°C ; (c) plates were then washed ten times with PBS containing 0.1% Tween 20 (100 µl/well); (d) an amount of 100 µl of antisera diluted in PBS was added to the wells with titers of 1/5000 for CIAT899 and 12a3, and plates were incubated for 90 min at 37°C followed by 30 min at 4°C; (e) step (c) was repeated; (f) goat anti-rabbit IgG conjugated with alkaline phosphatase was diluted 1000 fold in PBS and added (100 µl) to the wells, and plates were incubated for 90 min at 37°C followed by 30 min at 4°C; (g) Step (c) was repeated; (h) Excess liquid was removed from the plates, then 100 µl ELISA buffer substrate was added (1 mg p-nitrophenyl phosphate ml^{-1} diethanolamine buffer, pH = 9.8); (i) the enzyme reaction was stopped after 15 min by adding 100 µl of 2 M NaOH; (j) positive results were indicated by a yellow color, read by using a visible color scale of 0 to 4 (0 = no color, 4 = strong yellow).

Statistical analysis

The SAS software (1997) was used to perform the ANOVA of results and the comparison of means was achieved by the Duncan's multiple range test (P < 0.05). The regressions were performed using the general linear model procedure of the 2-D graphing analysis system package (File Version: 1.27).

RESULTS

Nodule occupancy by inoculated rhizobia

The indirect ELISA technique was very successful in identifying the rhizobia strains. Thus, results show that both inoculated rhizobia were more frequently found than native rhizobia in nodules for both genotypes. Indeed, the nodule occupancy by inoculated rhizobia was 95% for 12a3 and 93% for CIAT899.

On the other hand, wide serological differences were observed between CIAT899 and 12a3, since no cross-reaction was found among these two strains, nor with native rhizobia. Indeed, none of the antisera raised against the two strains reacted significantly with extracts from nodules formed by indigenous rhizobia.

Effect of water stress on nodulation

Water suppression during the beginning of the flowering stage reduced most of the characteristics assessed. Figure 1A presents the mean number of nodules under various treatments in common bean genotypes. Thus, the nodule number varied significantly with rhizobia (P < 0.01), plant genotype (P < 0.05), and the interaction with water stress treatment (P < 0.001). The combination of Flamingo and 12a3 without or with stress showed the highest values for number of nodules. By contrast, the lowest number of nodules was observed for non-inoculated plants in both genotypes. No significant differences between both rhizobia in symbiosis with CocoT with or without water stress treatment. Water stress induced a significant decrease in number of nodules of 60 and 47% with CIAT899 and 51 and 55% with 12a3 for CocoT and Flamingo, respectively, in comparison with control plants (Figure 1A).

Stress treatment induced a significant decrease (P < 0.01) in the nodule mass per plant with both genotypes and both rhizobia (Figure 1B). The stress effect induced a significant decrease in nodule dry mass of 47 and 59% with CIAT899 and 52 and 49% with 12a3 for CocoT and Flamingo, respectively, in comparison with control plants (Figure 1B). On the other hand, Flamingo inoculated with 12a3 showed the highest nodule dry mass in plants without or with water stress, respectively, in comparison to other treatment, and the non-inoculated plants showed

Table 1. Chemicals and physical properties of experimental soil.

Clay %	Silt %	Sand %	pH (1/2.5)	EC mhom/cm (1/5)	Organic matter %	Total N %	Olsen P_2O_5 ppm
23	49	28	8.1	0.4	1.3	1.68	129

Electrical Resistivity (mohm/cm).
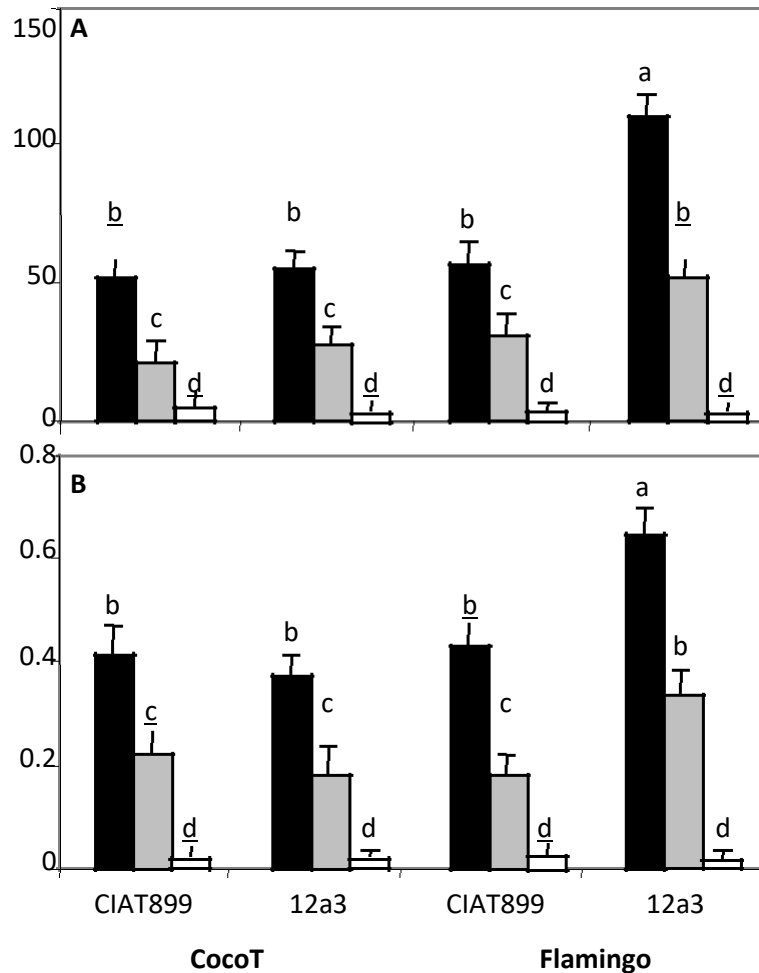
Figure 1. Effect of water stress on number (A) and dry mass (B) of nodules of common bean genotypes CocoT and Flamingo inoculated with *R. tropici* CIAT899 and *R. etli* 12a3: control plants (black bars), plants with stress (grey bars) and non-inoculated plants (open bars). Data are means ± SD of 15 replicates harvested at 60 days after sowing; different letters indicate significant differences between treatment means according to Duncan's multiple range test ($P < 0.05$).

the lowest nodule dry mass whatever the genotypes (Figure 1B).

Nitrogen percentage and correlation with nodulation

Total nitrogen (TN) accumulated in common bean shoot is presented in Figure 2. Generally, N_2 percentage decreased significantly ($P < 0.001$) with the water stress for all symbioses since for both genotypes, stress effect induced about 50% decrease in N content (Figure 2). Nevertheless, without drought stress, Flamingo inoculated with 12a3 show the highest N content. By contrast, in stress condition, total nitrogen percentage was decreased in the range of 49 and 53% with CIAT899 and 61 and 47% with 12a3 for CocoT and Flamingo, respectively in comparison with control plants (Figure 2).
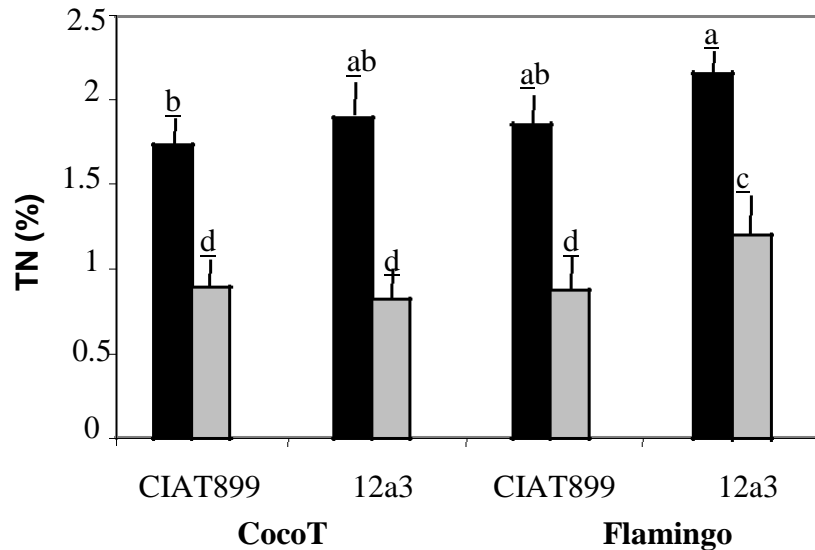
Consequently, a significant correlation between nodule

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Figure 2. Effect of water stress on shoot nitrogen percentage of common bean genotypes CocoT and Flamingo inoculated with *R. tropici* CIAT899 and *R. etli* 12a3: control plants (black bars) and plants with stress (grey bars). Data are means ± SD of 15 replicates harvested at 60 days after sowing; different letters indicate significant differences between treatment means according to Duncan's multiple range test (P < 0.05).

dry mass and nitrogen percentage was observed for all symbioses with or without stress (Figure 3). The slope of the linear regression was found to vary with rhizobia, genotypes and the effect of drought stress treatment. Thus, the slopes of the curves were significantly higher for plants without drought stress whatever the genotype and the rhizobia strain. Without water stress, Flamingo inoculated with 12a3 was characterized by the significantly highest nitrogen percentage/nodule biomass ratio of 2.76%N g^{-1} NDW (Figure 3D). Another difference between symbioses was that the slope of the curve was significantly higher with 12a3 (2.03 and 2.76%N g^{-1} NDW) than with CIAT899 (1.55 and 2.28 %N g^{-1} NDW) for CocoT and Flamingo, respectively (Figure 3). Also, with water stress and for CocoT, the slope of the curve was significantly higher with 12a3 (1.87%N g^{-1} NDW) than with CIAT899 (1.07%N g^{-1} NDW), but for Flamingo the slope of the curve was weakly increased with CIAT899 (1.91%N g^{-1} NDW) than with 12a3 (1.84 %N g^{-1} NDW) (Figure 3).

Shoot, root growth and leaf area

Figure 4 shows that plant-growth was strongly decreased by water stress treatment (P < 0.01). Thus, stress effect induced a significant decrease in shoot dry mass about of 41% in CocoT with both rhizobia and 39 and 33% in Flamingo with CIAT899 and 12a3, respectively, in comparison to control plants (Figure 4A). On the other hand and whatever the rhizobia strain, the highest shoot

growth was observed in Flamingo with or without water stress, in comparison to that in CocoT (Figure 4A).

Root dry mass was also affected by water stress (P < 0.05) (Figure 4B). In comparison to control plants, stress effect induced a significant decrease in root dry mass of 48% in CocoT with both rhizobia, and 47 and 39% in Flamingo with CIAT899 and 12a3 respectively (Figure 4B). No significant difference for root dry mass between both genotypes inoculated with both rhizobia, in control plants or under drought stress treatment.

Regarding leaf area, generally, data in Figure 5 showed that leaf area decreased significantly (P < 0.05) with water stress in all symbioses. However, Flamingo showed the highest leaf area in control plants with both rhizobia, in comparison to that in CocoT. Also, under water stress the highest leaf area was observed in Flamingo inoculated with 12a3. Stress effect induced a significant decrease of leaf area of 40 and 38% with CIAT899 and 37 and 25% with 12a3 in CocoT and Flamingo, respectively, in comparison to that in control plants (Figure 5).

Leaf chlorophyll and proline concentration

Figure 6A shows chlorophyll concentration of common bean genotypes inoculated with both rhizobia with or without water stress. Generally, data showed that chlorophyll concentration decreased significantly (P < 0.001) with stress treatment in all symbioses. However, without stress, inoculation with 12a3 induced the highest
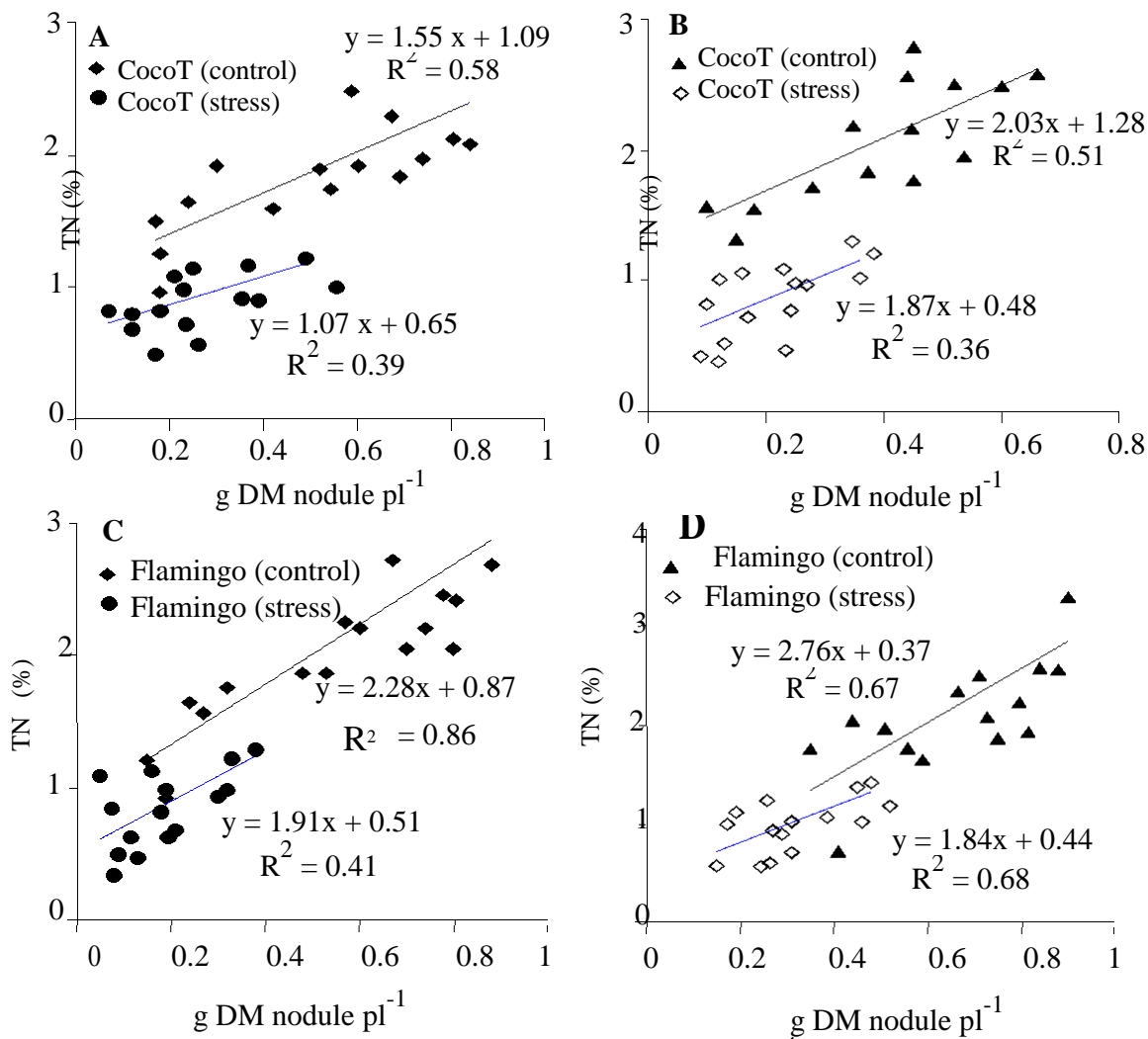
Figure 3. Relation between nitrogen percentage and nodule dry mass (g plant^{-1}) in common bean genotypes
CocoT and Flamingo inoculated with *R. tropici* CIAT899 (A and C) and *R. etli* 12a3 (B and D). Data are means of 15 replicates harvested at 60 days after sowing.

quantity of chlorophyll in both genotypes. Also, under stress, the highest value for chlorophyll content was observed in Flamingo inoculated with 12a3. In comparison to control plants, stress treatments induced a significant decrease of 67 and 69% in CocoT and 57 and 43% in Flamingo with CIAT899 and 12a3, respectively (Figure 6A).

Figure 6B showes proline concentration of common bean genotypes inoculated with rhizobia with or without water stress. Generally, proline accumulation varied with symbiosis in response to water deficit (P < 0.05). Under drought stress, the highest proline accumulation was found in CocoT plants inoculated with CIAT899, while the lowest was obtained in Flamingo with 12a3 (Figure 6B). Thus, except for this last symbiosis, drought effect increased proline accumulation more than 50% for other symbioses.

DISCUSSION

In Tunisia the distribution of native (indigenous) rhizobia was found by Mhamdi et al. (2002) to vary among regions, with *R. leguminosarum* being found exclusively in Bizerte, in contrast with *R. etli* found exclusively in Cap Bon. In our work, native rhizobia existed in low number for non-inoculated plants (Figure 1A), suggest that these rhizobia are in limited number or have a low potential of infectivity. The wide serological differences between *R. etli* 12a3 and *R. tropici* CIAT899 and the absence of cross-reaction between these strains and the indigenous rhizobia suggest that local rhizobia might have specific antigenic components. The higher competitiveness of both strains CIAT899 and 12a3 towards native rhizobia as shown by ELISA methodology which resulted the higher percentage of nodule occupancy by inoculated
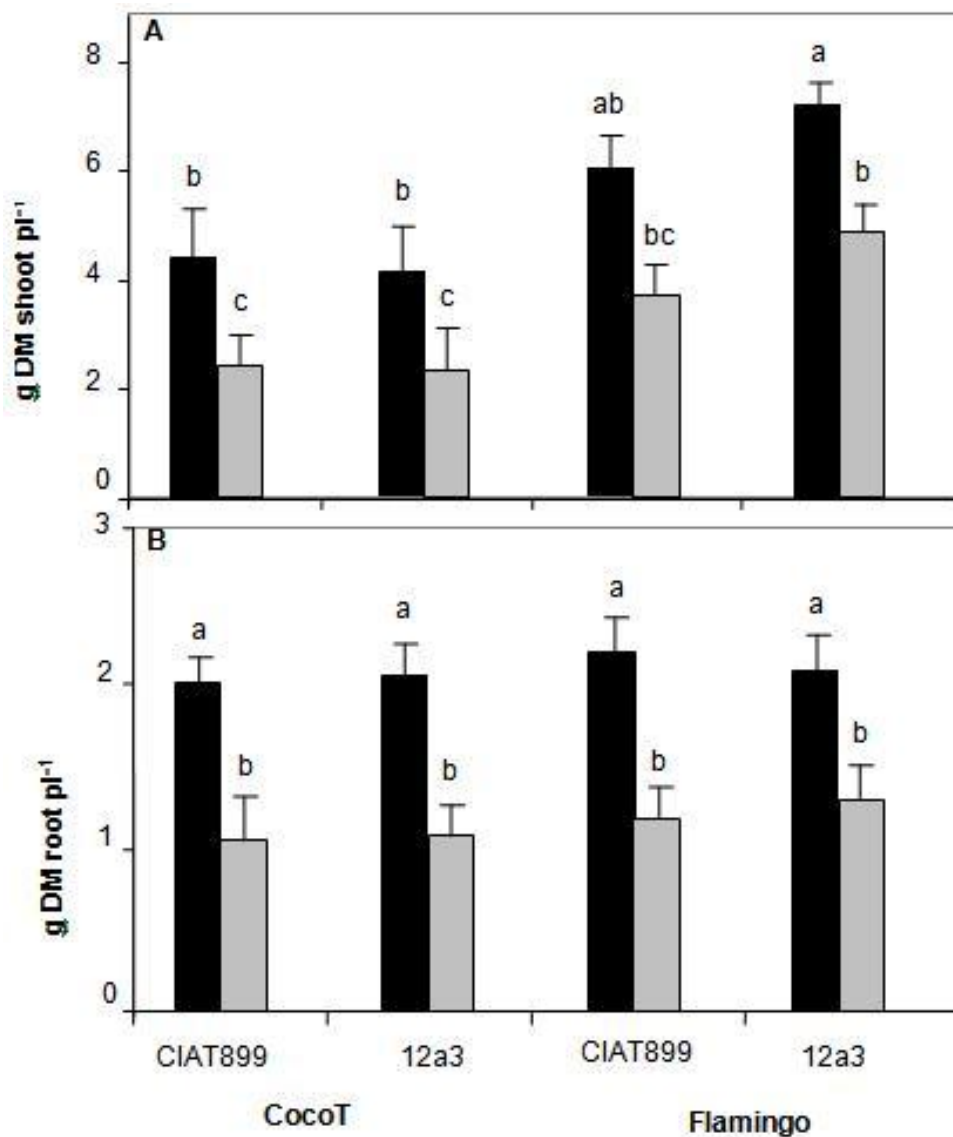
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Figure 4. Effect of water stress on shoot (A) and root dry mass (B) of common bean genotypes CocoT and Flamingo inoculated with *R. tropici* CIAT899 and *R. etli* 12a3: control plants (black bars) and plants with stress (grey bars). Data are means ± SD of 15 replicates harvested at 60 days after sowing; different letters indicate significant differences between treatment means according to Duncan's multiple range test (P < 0.05).

rhizobia, does not support the conclusion of Thies et al. (1992) that the native rhizobia are generally more competitive than introduced ones.

On the other hand, the present work shows the evaluation of the interaction between rhizobia strains (CIAT899 and 12a3) and common bean genotypes on nitrogen fixation efficiency under water stress. Many parameters were conducted which includes; nodulation, nitrogen percentage, plant growth, chlorophyll formation and proline accumulation. The results showed that water stress decreased the mean number of nodules. Thus, nodule formation was sensitive to water stress under this

experiment conditions which agreed with Daniel et al. (2007) who found that the deficit of water inhibits nodule formation by the inhibition of initial steps of rhizobia-legume symbioses. However, the combination of Flamingo and 12a3 was more tolerant to water stress than the other symbioses (Figures 1 and 2), not only for the nodulation process but also for the nodule growth and the nodule function like previously described for other symbioses (Drevon et al., 2001). Parallely, nitrogen percentage decreased with water stress for all symbioses. In symbiosis with CIAT899, no significant difference between both genotypes. But, in symbiosis
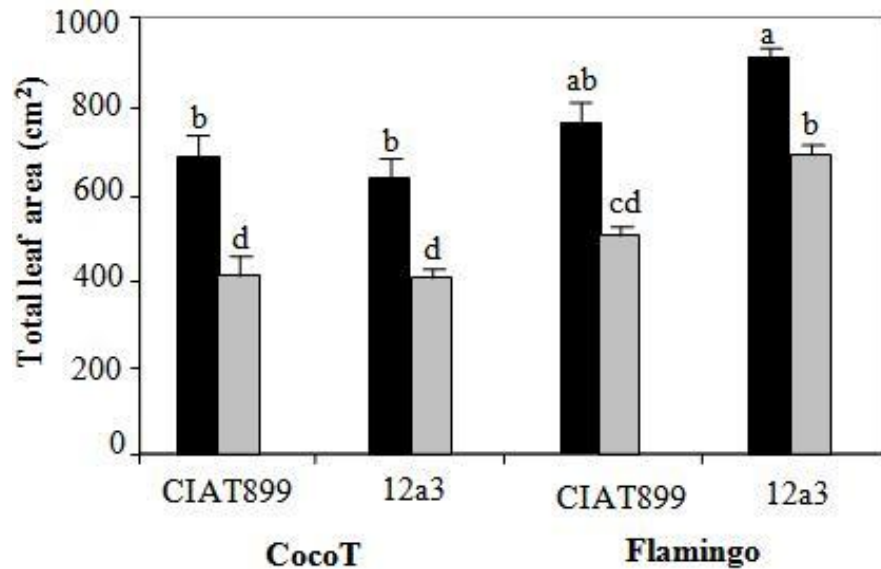
Figure 5. Effect of water stress on leaf area of common bean genotypes CocoT and Flamingo inoculated with *R. tropici* CIAT899 and *R. etli* 12a3: control plants (black bars) and plants with stress (grey bars). Data are means ± SD of 15 replicates harvested at 60 days after sowing; different letters indicate significant differences between treatment means according to Duncan's multiple range test (P < 0.05).

with 12a3, CocoT was more sensitive than Flamingo. However, differences in this tolerance may also be due to other determinants such as the interaction with the plant genotype, as illustrated by the lower efficiency of 12a3 with CocoT than with Flamingo (Tajini et al., 2008). Gordon et al. (1997) reported that the reduction of N_2-fixing activity by water stress is attributed to a reduction in respiration of the nodules. Also, the water stress induced distortions in nodule structure could be the reason for the decline in the N_2 fixation rate by legumes subjected to water stress (Daniel et al., 2007). The depressive effect of water stress on N_2 fixation by legumes is directly related to the water deficiency induced decline in dry weight and N content in the shoot (Sellstedt et al., 1993), who found that N derived from N_2 fixation in soybean was decreased by about 26% as a result of water deficiency when measured by the acetylene reduction assay. In the other hand, Zahran (1999) found that the plant growth was less affected by the water stress than N_2 fixation because nodule initiation, growth, and activity are all more sensitive to water stress than are general root and shoot metabolism.

The correlation of nitrogen content as a function of nodules biomass (Figure 3), establishes that the water stress is a major threat of nitrogen fixation and bean growth and suggests a major factor that limit the nodulation and prevented the symbiosis contributing to plant-growth.

The results presented in this study show that water stress reduce plant growth in both genotypes (Razmjoo et al., 2008; Ramirez et al., 2008), and Flamingo with

both rhizobia was more tolerant than CocoT. Also, Mufioz-Perea et al. (2007) found that the major effect of drought stress is the reduction in dry mass accumulation in common bean and results in significant yield reductions. This effect was more related to a reduction of total leaf area, agrees with the previous observation of Santos et al. (2009) and Jaleel et al. (2008) who reported that the drought stress is characterized by reduction of water content, diminished leaf water potential and turgor loss, closure of stomata and decrease in cell enlargement and growth. Also, other studies show that water deficit stress mostly reduced leaf growth and in turn the leaf areas in many species of plant like *Populus* (Wullschleger et al., 2005), soybean (Zhang et al., 2004) and many other species (Farooq et al., 2008 and 2009).

For Chlorophyll concentration, plants inoculated with both rhizobia showed reduction under water deficit and the decrease was pronounced in CocoT than Flamingo genotype with both rhizobia. This result agreed with Kiani et al. (2008) and Farooq et al. (2009) who noticed that water stress has the ability to reduce the tissue concentrations of chlorophylls and carotenoids. Also, Santos et al. (2006) found that in even moderate drought stress can reduce the net photosynthetic rate in water stress sensitive plants, such as common beans. The decline in photosynthesis observed under water stress could be attributed to stomatal factors. In this condition, the concentration of CO_2 in chloroplasts decreases because of a reduction in stomatal conductance (Daniel et al., 2007; Gama et al., 2007).
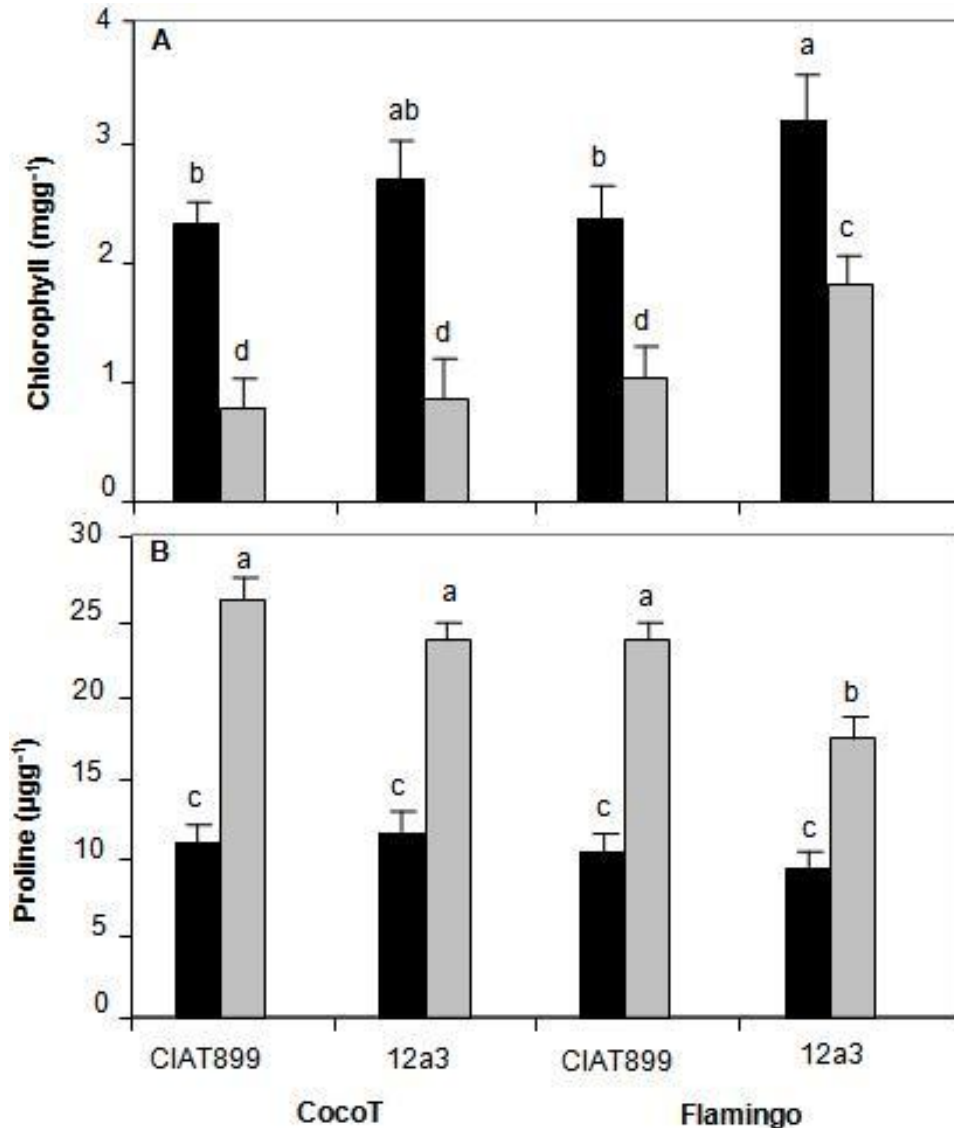
Proline content in plants inoculated with both rhizobia

Figure 6. Effect of water stress on chlorophyll (A) and proline concentration (B) of common bean genotypes CocoT and Flamingo inoculated with *R. tropici* CIAT899 and *R. etli* 12a3: control plants (black bars) and plants with stress (grey bars). Data are means ± SD of 15 replicates harvested at 60 days after sowing; different letters indicate significant differences between treatment means according to Duncan's multiple range test (P < 0.05).

showed increment under water stress in all symbioses, while Flamingo inoculated with 12a3 showed the least proline levels. The concentrations of proline in the plant tissues were generally very low but the levels were significantly affected by water stress (Kapuya et al., 1995; Djibril et al., 2005). Proline has been suggested to play multiple roles in plant stress tolerance. It acts as a mediator of osmotic adjustment, protects macromolecules during dehydration and serves as a hydroxyl radical scavenger.

In conclusion, this work showed the existence of variability among symbioses in the response to water stress: Flamingo inoculated with 12a3 was the most tolerant symbiosis, expressed by higher values in number of nodules, nitrogen content, chlorophyll content, plant dry mass and total leaf area, but there were the least in proline accumulation. The compatibility of Flamingo and 12a3 might be considered as drought tolerant under our experimental conditions which might have potential for application in field under water stress in arid and semi-arid regions.

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