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INOCULATION WITH RHIZOBIUM STRAINS IMPROVE COMMON BEAN *PHASEOLUS VULGARIS* TOLERANCE OF HYDROUS CONSTRAINT

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Abstract

In many African countries, drought is a major environmental stress affecting agricultural productivity. The potential solution to this problem for the common bean is to enhance its yield by the effective use of rhizobia. To improve the osmotic stress tolerance of a drought-sensitive common bean cultivar (Coco Blanc) consumed in Tunisia, plants were inoculated either with the reference strain *Rhizobium tropici* CIAT899 or with the local soil *Rhizobium* strain Ar02. Plants were grown under well-watered or medium to severe water-deficit conditions (100%, 75%, 50%, and 25% of the pot's useful reserves). Nodulation parameters measured included nodule biomass; growth (length and biomass of shoots and roots), leaf area, leaf perimeter, nitrogen content, chlorophyll and carotenoid content, soluble sugar content, and proline content were measured at the flowering stage. A significant reduction in parameters was observed due to water shortfall. However, plants inoculated with Ar02 and CIAT899 showed some tolerance to water stress; the number of nodules increased from 1 nodule/plant at 50% Useful Reserve (RU) to 28 nodules/plant at 50% RU + CIAT899. At 50% RU, the reduction in nodule biomass was 92.57% in plants inoculated with Ar02 and CIAT899 and 99.85% in control plants. Inoculation with CIAT899 increased dry biomass at 75% RU, 50% RU, and 25% RU and root length at 75% RU and 50% RU compared to the control treatment. Inoculation with CIAT899 also increased leaf area and perimeter. The effect of drought stress was lower in plants inoculated with rhizobia as demonstrated by chlorophyll a, chlorophyll b, chlorophyll a + b and carotenoid content. Rhizobia inoculation, especially with Ar02 at 50% RU and CIAT899 at 75% RU, increased nitrogen content. Inoculation also increased the soluble sugar and proline content. This study suggests that rhizobial inoculation of the common bean improves its performance and stress tolerance under adverse conditions.

Key words: Rhizobia, Common bean, Nodulation, Nitrogen, Water deficit.

Introduction

The common bean, *Phaseolus vulgaris* L. is one of the chief legumes consumed by humans. It is highly enriched in elements such as calcium, potassium, phosphorus, and iron. A bean seed contains on average about 18–32% protein (Numan & Nuri, 2005). The yield of common bean is relatively low, particularly under drought conditions, drought being the significant abiotic factor limiting plant growth, thereby badly affecting crop production. Such stresses can cause abnormal physiological processes that highly affect one or a combination of environmental and biological factors. Stress resulting from abnormal metabolism can reduce plant growth or worse, result in plant death. Various estimates suggest that only 10% of the world's arable land is free of stress. Therefore, environmental stress is one of the significant factors causing deviation between potential performance and actual plant yield. Stress and drought are considered the most common environmental stresses that affect around 25% of agricultural land and farm production.

In Tunisia, symbiotic N_2 fixation by the common bean and its yield are constrained by several factors, which include salinity, paucity of favourable soil rhizobia, low concentrations of soil nutrients (e.g. phosphorus), and water stress (Tajini *et al*., 2012). The impact of drought on N_2 fixation is relatively noticeable due to the responsiveness of nodule initiation, activity, and growth to water stress compared to the responses of shoot and root metabolism (Kaymakanova *et al*., 2008). Water stress is more detrimental to nitrogen fixation and nodulation during vegetative growth than at the reproductive stage (Naylor & Coleman-Derr, 2018). Using the acetylene-reduction assay, Tulumello *et al*., (2021) found that nitrogen derived from N_2 fixation was significantly reduced by common bean about 26% due to water stress.

Moreover, significant changes in N_2 fixation due to drought conditions were observed in genotypes of *Vigna radiata* (Ranawake *et al*., 2011) and *Trifolium repens* (Wang, 2011). Under water stress, leaf area is reduced, which decreases evapotranspiration and intercepted radiation levels, and leads to reduced photosynthesis (Levitt 1982; Lawlor & Cornic, 2002; Chaves *et al.* 2003; Shah & Paulsen, 2003). This causes inhibition of ATP synthesis and accumulation of some secondary metabolites, such as amino acids, particularly proline (Tahri *et al*., 1998; Simon-Sarkadi *et al*., 2005), and hormones, such as abscisic acid (Zhang *et al*., 2001), in stressed plants.

In many parts of the world, due to its high cost, irrigation is not suitable for cultivating the common bean. Thus, there is a pressing need to develop cost-effective means to lessen drought stress to enhance yield, mainly in semi-arid environments. Therefore, it is crucial that highly drought-tolerant crops are developed for improving food security in these harsh agricultural regions. Inoculation with drought-tolerant rhizobial strains can bring significant improvements to the symbiotic relationship under water stress (Athar & Johnson, 1996; Hussain *et al*., 2018), provided that the strain used is selected for its efficiency (Mnasri *et al*., 2007; Jahansooz *et al*., 2007; Ben Romdhane *et al*., 2009; Mhadhbi *et al.*, 2008) and tolerance to osmotic stress (Athar & Johnson, 1996). Several findings suggest a substantial role for N_2-fixing *Rhizobium*–legume symbioses in improving soil fertility in arid as well as semi-arid environments (Yanni *et al.*, 2016). We hypothesis that inoculated common bean with rhizobial strains can improve the plant performance at morpho-physiological level at flowering stage under different level of drought stress positively. Our study aims to assess the impact of water stress on common bean–rhizobia symbiosis using parameters related to nodulation, growth, chlorophyll content, N percentage, proline accumulation, and soluble sugars with the purpose of examining whether it is appropriate to inoculate the legume with introduced rhizobia.

Material and Methods

Biological material and experimental treatments: This study was conducted with the common bean variety Coco Blanc frequently cultivated in Tunisia. Common bean seeds were treated with 6.7% calcium hypochlorite ($Ca(ClO)_2$) for at least 15 minutes. They were then carefully washed in sterile distilled water. Seeds were germinated for a maximum of three days in Petri dishes comprising sterile moistened blotting paper. The reference *Rhizobium tropici* strain CIAT899 (S2), local *Rhizobium* strain Ar02 (S1) and common bean seeds were obtained from the Agronomic Sciences and Technology laboratory of the National Institute of Agronomic Research (INRAT), Tunisia. Liquid culture of rhizobial inoculants prepared in Yeast Extract Mannitol (YEM) medium was applied by soaking seedlings for approximately 30 minutes in the culture before transplanting them in plastic pots (785g Ariana soil/pot; Table 1).

The trial was conducted in a greenhouse with different water treatments (100%, 75%, 50%, and 25% RU). The test comprised twelve treatments; each treatment was repeated 4 times, and water stress was applied from the lifting stage (Table 2). Nodulation parameters (the number and biomass of nodules), plant growth (shoot and root length and shoot and root dry biomass), leaf area, leaf perimeter, nitrogen content, chlorophyll content, proline accumulation and soluble sugars were determined at the flowering stage.

Harvest and data analysis: Four plants in the early stage of flowering were harvested for each treatment. Nodules were then removed from the roots and the plant was separated into roots and shoots. Nodulation parameters (dry nodule weight and nodule number) and fresh weight and length of aerial plant parts and roots were determined. Cumulative leaf area and perimeter of all leaves on a single plant were determined using a planimeter. Plants were then dried in an oven at 70°C for 72 h. After measuring their dry weight, each plant's shoots were ground separately, and the nitrogen content was measured using the Kjeldahl procedure.

Table 1. Chemical and Physical characterization of soil.

Site	PH	CE	Nt	K2O	P2O5	LA	LT	Clay+Silt	Sand
Ariana	7.72	0.30	0.11	230	48	14	26	80	20

Nt: Nitrogen total; CE: Conductivity electrical; LA: Active lime; LT: Total lime; P_2O_5: Available phosphorus; K_2O: Ex-changeable potassium

Table 2. The treatments of the experience.

Treatments	100% RU	75% RU	50% RU	25% RU
Inculated	T1	T4	T7	T10
Inoculated by Ar02	T2	T5	T8	T11
Inoculated by CIAT899	T3	T6	T9	T12

RU=1/2 RT (Ducrop, 1990) or **RU**: Useful reserve; **RT**: Total reserve= Wet weight at field capacity (100%) – Dry weight after drying

Table 3. Effect of drought stress (100% RU, 75% RU, 50% RU, 25% RU) on chlorophyll content of common bean variety (Coco Blanc) grown in pots and inoculated by rhizobia strains under greenhouse conditions. Plants were harvested 45 DAS (the day after sowing). Data are the means ±SD of four replicates.

Treatments	Chlorophyll a (mg/g MF)	Chlorophyll b (mg/g MF)	Chlorophyll a+b (mg/g MF)	Carotenoid (mg/g MF)
100% Ru	29.9 ± 0.55	61.7 ± 0.5	91.7 ± 0.1	10.0 ± 0.3
75% Ru	28.5 ± 1.2	35.2 ± 0.3	63.7 ± 0.5	6.3 ± 0.5
50% Ru	24.6 ± 0.2	25.3 ± 0.5	49.9 ± 0.8	4.9 ± 1.1
25% Ru	20.5 ± 1.4	15.8 ± 0.3	36.3 ± 1.3	4.9 ± 1.5
100% Ru + Ar02	30.4 ± 0.7	68.4 ± 1.1	98.8 ± 1.1	9.9 ± 1.7
75% Ru + Ar02	31.7 ± 0.5	35.9 ± 0.5	67.6 ± 1.5	9.1 ± 1.2
50% Ru + Ar02	28.9 ± 0.3	26.9 ± 1.1	55.8 ± 0.5	7.8 ± 0.5
25% Ru + Ar02	26.8 ± 0.55	16.4 ± 1.2	43.2 ± 0.9	6.7 ± 0.2
100% Ru + CIAT889	31.3 ± 0.3	63.9 ± 1.5	95.2 ± 1.2	8.7 ± 1.1
75% Ru + CIAT889	29.9 ± 1.1	35.0 ± 0.55	64.9 ± 0.3	7.2 ± 0.5
50% Ru + CIAT889	28.8 ± 1.5	29.9 ± 0.2	58.7 ± 1.3	5.2 ± 0.55
25% Ru + CIAT889	22.6 ± 1.7	16.3 ± 0.1	38.9 ± 1.5	1.7 ± 0.7

Soluble sugar content: The phenol method proposed by Dubois *et al.*, (1956) was used to determine soluble sugars. Sugar was extracted by adding 3 ml of 80% ethanol to 100 mg of fresh material placed in test tubes. The solution was left at room temperature for 48 hours. At the time of dosing, the tubes were heated at 80°C to evaporate the alcohol; 20 ml of distilled water was added to the test tube, and the solution thus obtained was analysed. Next, 2 ml of the test solution was placed in a clean test tube, and 1 ml of 5% phenol solution was added. The phenol added was also diluted with distilled water. After adding 5 ml of sulphuric acid at 95% concentration, an orange–yellow solution was obtained. After rapid vortexing, the tubes were placed in a water bath for about 20 minutes at 30°C. Optical density was reported according to the standard curve for soluble sugars by measuring absorbance at a wavelength of 640 nm.

Proline content: The Troll & Lindsley (1955) method, further simplified and developed by Dreier & Goring (1974), was used to determine the proline content. The method consisted of placing 100 mg of lyophilised dry matter in a test tube containing 5 ml of methanol at 40% concentration. The test tubes were heated at 85°C for half an hour. Once the solution cooled down, 2 ml of acetic acid and 80 ml of absolute orthophosphoric acid at 1.7 density was added to 1 ml of the solution. The mixture was then boiled at 100°C for 30 minutes. When the solution turned red, it was further cooled, and 5 ml of toluene was added. Two phases emerged after stirring several times. The upper phase contained proline, while the lower was without it. The upper phase, which was red in colour, was recovered. Next, a pinch of sodium sulphate (Na_2SO_4) was added to remove the water content. Readings were taken with a spectrophotometer at 528 nm wavelength. Values ranging from 0.01 to 0.2 mg were plotted on a standard curve. A blank was prepared by replacing the extracts with 1 ml of distilled water under the same conditions.

Chlorophyll and carotenoid content: The Arnon (1949) method was used to determine the chlorophyll content. The process involved crushing 2 g of green leaves with a pinch of calcium carbonate, 80% acetone and 25 g of sand in a mortar. Optical density (OD) was measured using a spectrophotometer at 663 and 645 nm after filtration. The chlorophyll content and carotenoid concentrations were determined with the following formulae:

Chl a = 12 (DO 663) - 2, 67 (DO 645),
Chl b = 22, 5 (DO 645) - 4, 68 (DO 663),
Total Chl = Chl a + Chl b, and
C = 1000 DO (470) - 1.90 Chl (a) - 63.14 Chl (b) / 214.

Statistical analysis

SPSS 20 was used for statistical analysis. ANOVA was performed and means were compared with Fisher's LSD test at $p<0.05$.

Results

Nodule number and biomass: Nodule number was highest in plants inoculated with the Ar02 strain (164 nodules/plant) at 100% RU. In plants inoculated with CIAT899, nodule number was highest at 75% RU compared to other water stress treatments, including 100% RU. Nodule number was very low in the 50% RU treatment, with plants inoculated with the CIAT899 strain showing a higher nodule number (28 nodules/plant) than plants inoculated with Ar02 (24 nodules/plant) and non-inoculated plants (1 nodule/plant). The 25% RU treatment showed a complete absence of nodulation (Fig. 1A).

Plants inoculated with Ar02 and CIAT899 strains had a higher nodulation biomass at 100% RU. At 75%, nodulation biomass was reduced by 28.169% and 17.142% in control plants and those inoculated with Ar02, respectively, but in plants inoculated with CIAT899, the reduction in biomass was only 5.714%. At 50% RU, the reduction in nodule biomass was 92.57% in plants inoculated with the Ar02 and CIAT899 strains, and 99.85% in the control plants (Fig. 1B).

Biomass production: Dry biomass was higher in non-inoculated plants at 100% RU (1.64 g/plant). In contrast, inoculation with strain CIAT899 improved dry biomass at 75% RU (1.522 g/plant), 50% RU (1.40 g/plant) and 25% RU (0.542 g/plant) compared to the control and plants inoculated with the Ar02 strain (Fig. 1C). We noted that at 100%, 75% and 50% RU, inoculation did not increase dry root mass relative to 25% RU; however, in the severe water-stress treatment, root biomass was higher in the inoculated plants (Fig. 1D).

Growth parameters: The 100% and 75% RU levels were more suitable for the growth of common bean plants in all treatments. At these levels of stress, inoculation did not improve aerial growth compared to the control. In contrast, at 50% RU, inoculation with CIAT899 improved shoot length (42.25 cm; Fig. 2A). Inoculation with CIAT899 improved root length at 75% RU (35.25 cm) and 50% RU (27.5 cm); however, Ar02 improved root length at 25% RU (40.25 cm; Fig. 2B).

Leaf area and perimeter: Figure 2C shows that plants inoculated with CIAT899 at 75% and 50% RU had a higher leaf area, with an increase in leaf surface of 44.82% and 53.83%, respectively. Plants inoculated with CIAT899 had a larger leaf perimeter at 50% RU (53.893 cm/plant; Fig. 2D). Inoculation with CIAT899 improved leaf perimeter by 27.60% and 27.52% at 25% RU and 50% RU, respectively.

Chlorophyll and carotenoid content: Water stress significantly reduced the content of chlorophyll a, chlorophyll b, total chlorophyll a + b, and carotenoids (Table 3). On the other hand, the effect of drought stress was lower in plants inoculated with rhizobia; for example, at 50% RU without inoculation, chlorophyll a content was 24.6 mg/g MF, while plants inoculated with Ar02 and CIAT889 had a chlorophyll a content of 8.9 mg/g MF and 28.8 mg/g MF, respectively. At 50% RU without inoculation, the chlorophyll a + b content was 49.9 mg/g MF, while plants inoculated with Ar02 and CIAT889 had a chlorophyll a + b content of 55.8 mg/g MF and 58.7 mg/g MF, respectively. At 50% RU without inoculation, the carotenoid content was 4.9 mg/g MF, while plants inoculated with Ar02 and CIAT889 had a carotenoid content of 7.8 mg/g MF and 5.2 mg/g MF, respectively.

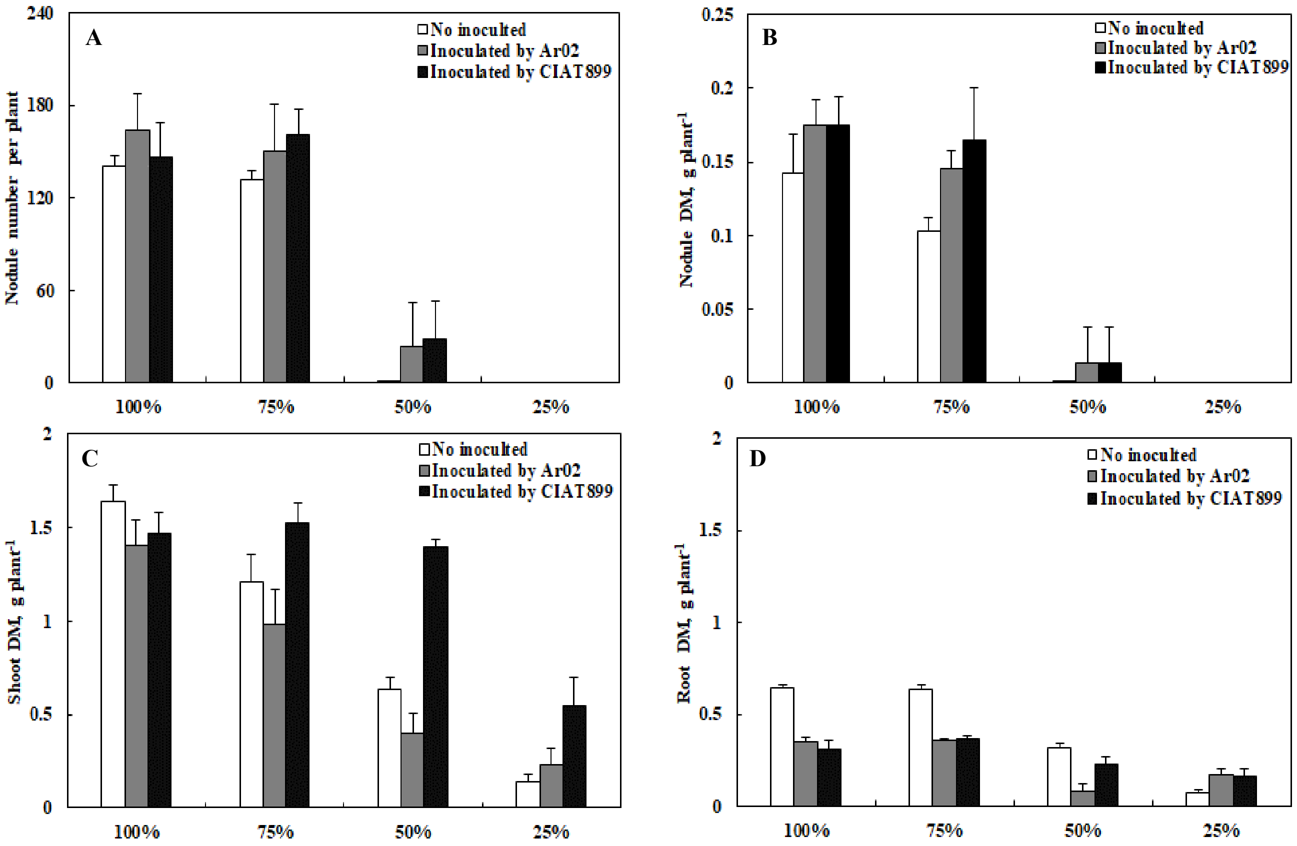

Fig. 1. Effect of drought stress (100%, 75%, 50%, 25%) on nodule number (A), nodule biomass (B) shoot biomass (C) and root biomass (D) of common beans variety (Coco Blanc) grown in pots and inoculated by rhizobia strains under greenhouse conditions. Plants were harvested 45 DAS (the day after sowing). Data are the means ±SD of four replicates.

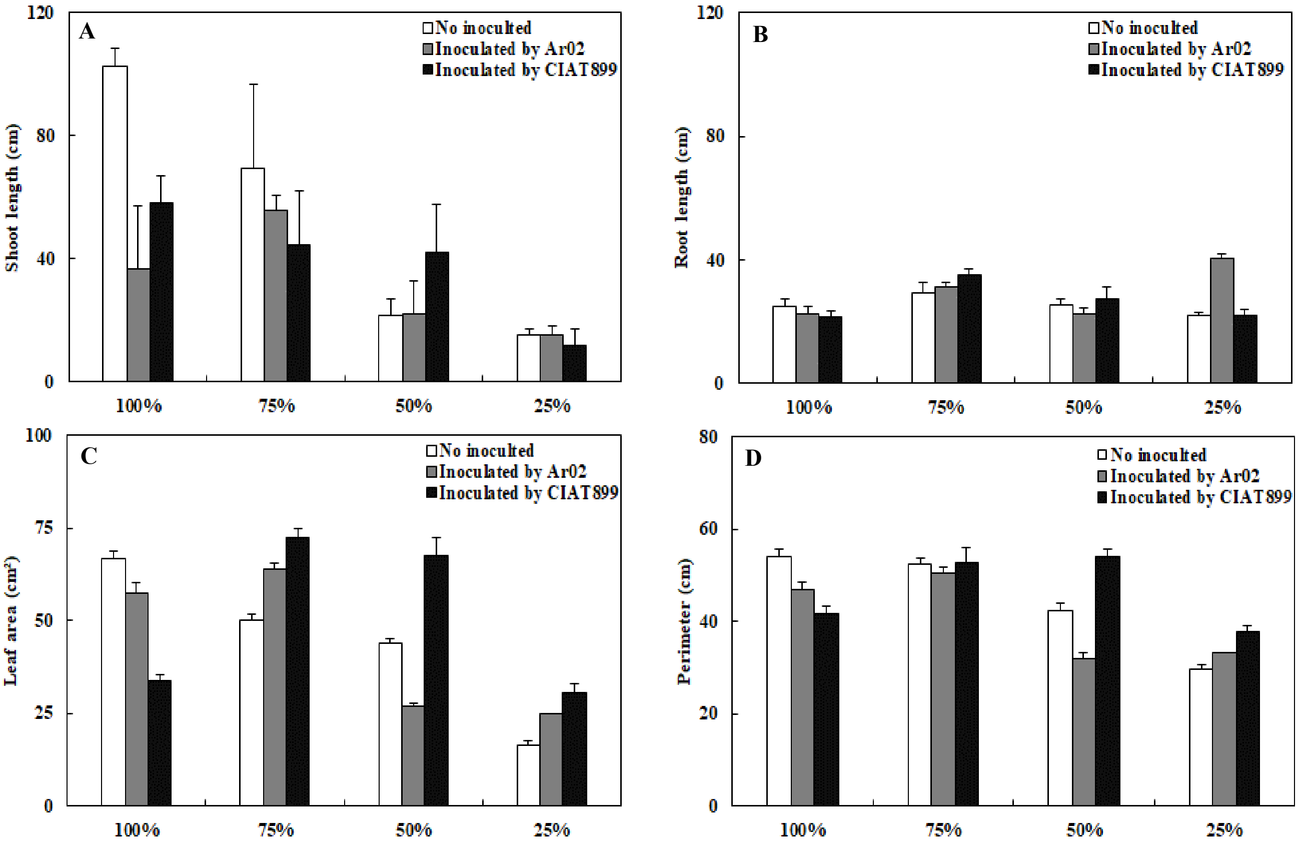

Fig. 2. Effect of drought stress (100%, 75%, 50%, 25%) on shoots length (A), roots length (B) leaf area (C) and leaf perimeter (D) of common beans variety (Coco Blanc) grown in pots and inoculated by rhizobia strains under greenhouse conditions. Plants were harvested 45 DAS (the day after sowing). Data are the means ±SD of four replicates.

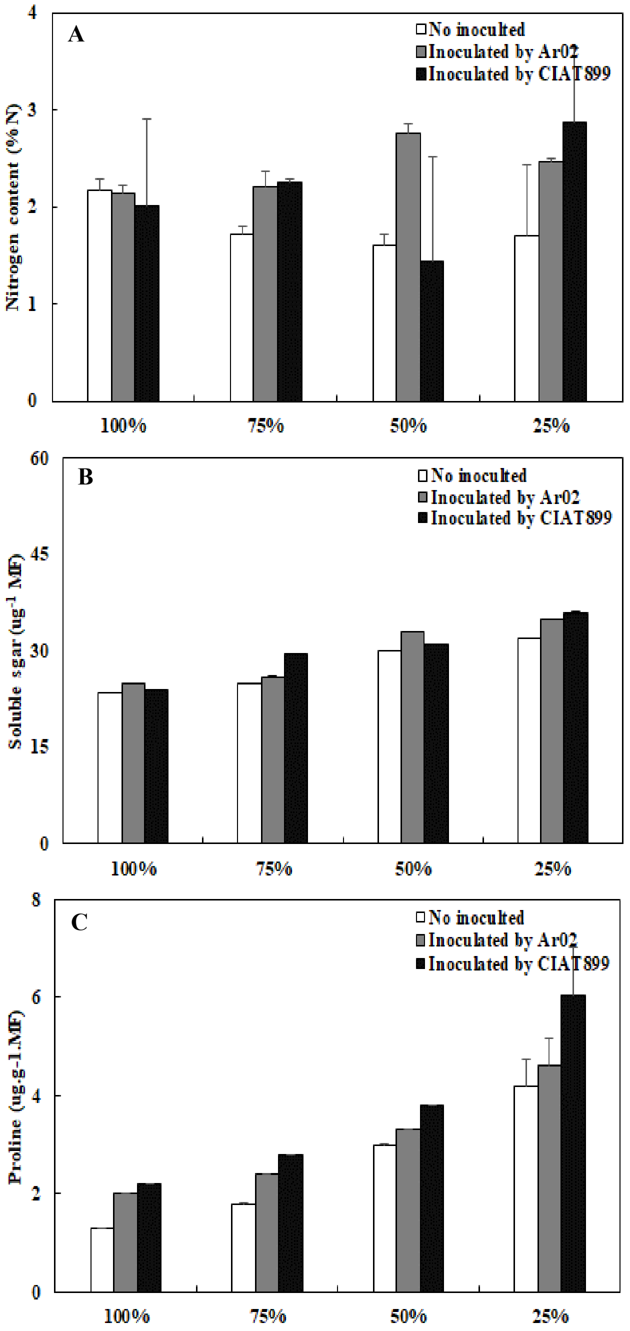

Fig. 3. Effect of drought stress (100%, 75%, 50%, 25%) on nitrogen content (A), proline content (B), soluble sugar content (C) of common beans variety (Coco Blanc) grown in pots and inoculated by rhizobia strains under greenhouse conditions. Plants were harvested 45 DAS (the day after sowing). Data are the means ±SD of four replicates.

Nitrogen content: Water stress reduced the total nitrogen content in non-inoculated common bean plants; however, despite water stress, inoculation increased the nitrogen content, especially with the Ar02 strain at 50% RU and CIAT899 at 75% RU (Fig. 3A).

Soluble sugar content: Water deficit (75% RU, 50% RU, and 25% RU) amplified the accumulation of soluble sugars in common bean. Under these conditions of water deficit, the soluble sugar content in non-inoculated plants was 25 µg.g^{-1} MF, 30 µg.g^{-1} MF, and 32 µg.g^{-1} MF, respectively. Inoculation with CIAT899 and Ar02 improved the soluble sugar content compared to control treatments to reach 36 µg.g^{-1} MF with CIAT899 and 35 µg.g^{-1} MF with Ar02 at 25% RU (Fig. 3B).

Proline content: Water deficit (75% RU, 50% RU, and 25% RU) also increased the accumulation of proline in common bean plants. Under these conditions of water deficit, the proline content in non-inoculated plants was 1.8µg. g^{-1} MF, 3 µg.g^{-1} MF, and 4.2 µg.g^{-1} MF, respectively. Inoculation with strains CIAT899 and Ar02 improved proline levels compared to control treatments to reach 6.05 µg.g^{-1} MF with CIAT899 and 4.6 µg.g-1 MF with Ar02 at 25% RU (Fig. 3C).

Discussion

Our study shows that the variation in nodulation depends strongly on the rhizobial strain used. For instance, the Ar02 strain gave the highest number of nodules at 100% RU. However, the highest number of nodules occurred with the CIAT899 strain when water stress increased to 75% RU (Fig. 1A). Krasova-Wade *et al.*, (2006) also reported variation in common bean nodulation under different water stress conditions. Under favourable humid conditions, the Ar02 strain no longer nodulated, whereas CIAT899 nodulated under limiting water conditions. Williams & De Mallorca (1984) found that under low water stress, a fewer number of nodules formed in common bean roots. In contrast, severe or moderate water stress reduced both biomass and the number of nodules. In our study, inoculation with CIAT899 reduced biomass by 5.714% only. In plants inoculated with Ar02 and CIAT899 strains, the reduction in nodule biomass was 92.57%, and in control plants, it was 99.85% (Fig. 1B).

We found that at 100%, 75%, and 50% RU, inoculation did not improve dry root mass; however, at 25% RU, an improvement was seen under inoculation (Fig. 1D). The increased dry root weight was offset by the plant's dry shoot weight; this resulted in an improvement in the PR: PA ratio. According to Kafkai (1991), the increase in the PR:PA ratio under water stress is due to the elongation of roots in search of water, whereas under severe water-stress conditions, resource allocation to aerial parts is reduced due to transportation of photoassimilates towards roots, especially towards the affected parts (Figueiredo *et al.*, 2008; Bayuelo-Jimenez *et al.*, 2002). However, all growth parameters deteriorated in plants in symbioses with both strains, but the reductions were higher in the control treatments. This indicates that the symbiosis under study was effective in maintaining higher growth potential under low water availability. This may be explained partially by the maintenance of a higher root:shoot ratio and lower leaf area. It is possible that under osmotic stress, plants spend most of the photosynthetic energy on root production to facilitate the search for water and to reduce water stress (Kafkafi, 1991), enabling the common bean to avoid the detrimental effects of osmotic stress (Sassi *et al.*, 2008a).

Nitrogen content increased under inoculation, especially with the Ar02 strain at 50% RU and the CIAT899 strain at 75% RU (Fig. 3A). The ability of the Ar02 and CIAT899 strains to improve nitrogen fixation under severe water stress may be related to the efficiency of the strains and their tolerance of water stress (Zahran, 1999). One probable reason behind the improvement is the capacity of the CIAT899 strain to release phytohormones directly or indirectly, which may be responsible for moderating the effects of water stress on the plant (Boiero *et al*., 2007; Figueiredo *et al*., 2008; Belimov *et al*., 2009). Common bean plants inoculated with rhizobia showed improved growth compared to non-inoculated control plants. However, the microorganisms' direct action – optimisation of symbiotic fixation of atmospheric nitrogen due to the phytohormones produced by the bacteria (Juszczuk *et al*., 2004) or the indirect action of the rhizosphere following the release of exopolysaccharides (EPS) may explain the results. Kaci *et al*., (2005) reported that inoculation of the soil with a *Rhizobium sullae* strain (KYGT207) from the arid region of southern Algeria improved the physical properties of the rhizosphere of cereals. The introduction of an efficient strain of *Rhizobium* promotes nodulation and the biological fixation of nitrogen (Sultan *et al*., 2002). However, the underlying condition for this to occur is the competitiveness of the introduced strain (Graham *et al*., 1994), which depends on several factors, including energy sources available in the soil (Bromfield *et al*., 1985; Murphy *et al*., 1987; Streit *et al*., 1996), the interaction between *Rhizobium* strains (Robleto *et al*., 1998; Orsenik *et al*., 1999), and the indigenous rhizospheric bacteria which can either be positive or negative (Dashti *et al*., 1998; Mrabet *et al*., 2005).

Non-inoculated common bean plants in the control treatments showed a significant increase in proline and sugars under stress conditions and in the full flowering stage (Fig. 3B and 3C). On the other hand, inoculation with Ar02 and CIAT899 strains improved the soluble sugar content, reaching 36 µg.g^{-1} MF with strain CIAT899 and 35 µg.g^{-1} MF with strain Ar02 at 25% RU (Fig. 3B) compared to control treatments. In addition, inoculation with CIAT899 and Ar02 improved proline levels, reaching 6.05 µg.g^{-1} MF with CIAT899 and 4.6 µg.g-1 MF with Ar02 at 25% RU (Fig. 3C). Kameli & Losel (1995) and Sanchez *et al.* (1998) consider sugars to be good osmoregulatory compounds that can play a significant role in osmotic adjustment and maintenance of turgor for plant adaptation to drought (Morgan, 1984; Zhang *et al*., 1999). According to Bohnert *et al*., (1995) and Ingram & Bartels (1996), soluble sugars serve as osmoprotectors under water stress conditions. Their presence allows the maintenance of phosphorylation reactions by stabilising proteins and membranes, especially mitochondrial membranes (Strauss & Hauser, 1986). The process of concentration of soluble sugars or proline in leaf tissue under stress is highlighted as an adaptive characteristic to ensure osmotic adjustment (Kameli & Losel, 1995). The causes of accumulation of soluble sugars in the common bean include reduced transpiration, which increases the diffusion of carbon dioxide and water vapour in the vegetative stage. Similar results have been obtained in broad common bean (Bousba, 2001), barley (Kara, 2001), and *Quercus coccifera* (Losch *et al*., 1982). The increased concentration of soluble sugars can also be attributed to poor translocation, slower consumption due to decreased growth, and other changes, including leaf starch hydrolysis (Kameli & Losel, 1996).

Proline can play the role of an osmoticum by intervening in the regulation of cytoplasmic pH (Pesci & Beffegna, 1984) or by accumulating nitrogen reserves after a period of stress (Tal & Rosenthal, 1979). The evolution effect of water stress on proline accumulation in wheat, both in drought-tolerant and drought-sensitive cultivars, signifies that the rate of proline accumulation may be significant in drought-tolerant cultivars (Nayyar & Walia, 2003).

In our study, the chlorophyll content of leaves decreased with increasing proline content. The decrease was seen in chlorophyll a, chlorophyll b, chlorophyll a + b and carotenoids. Furthermore, the effect of drought stress was reduced in plants inoculated with rhizobia. The decrease in chlorophyll content is caused by a reduction in stomata opening (Brown & Tanner, 1983; Bashir *et al*., 2020) to limit water loss by evaporation and increase resistance to the entry of atmospheric CO_2 essential for photosynthesis (Slatyer, 1974). Relative turgor is less affected by stress due to water saving, leading to the dilution of chlorophyl

l. Our results suggest a possible connection between proline and the biosynthetic pathways of chlorophyll pigments. At the origin of these evolutions is the competition between the two compounds for their common precursor glutamate. In our study, inoculated plants showed an increase in osmoregulatory (soluble sugars and proline) and a decrease in chlorophyll content compared to control plants. Inoculation with rhizobia helped common bean plants to grow and develop under drought.

Conclusion

Presented study demonstrates a positive association between osmotic stress tolerance in plants and inoculation with rhizobial strains, which is attributable to effective nodulation and increased shoot and root dry weight, chlorophyll content, nitrogen, soluble sugars, and proline content. We recommend more effective use of rhizobial strains Ar02 and CIAT899 for inoculation of the common bean to increase NF potential under stressful conditions. Nevertheless, additional research is required to elucidate the osmotic stress tolerance in the common bean in symbiosis with rhizobial strains to better understand the effect of osmotic stress on limiting rhizobial growth, nodulation, and root hair colonisation and infection.

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