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Alleviation of water stress effects in cowpea by *Bradyrhizobium* spp. inoculation

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Abstract

Experiments were carried out to investigate the effects of different degrees of water stress on cowpea in the presence and absence of *Bradyrhizobium* spp. inoculation and to evaluate physiological responses to stress. The soil used was Yellow Latosol, pH 6.3 and the crop used was cowpea (*Vigna unguiculata* (L.) Walp.) cv. 'IPA 204'. Stress was applied continuously by the control of matric potential (ψ_m) through a porous cup. The lowered soil ψ_m had a direct effect on the N_2 fixation, but the strains *Bradyrhizobium* introduced by inoculation in the cowpea plants were superior to the indigenous strain demonstrating the importance of inoculation in the stressed plants. At the more negative ψ_m plants inoculated with the strains EI 6 formed associations of greater symbiotic efficiency which helped the cowpea plants to withstand drought stress better than the strain BR 2001 and the uninoculated control. The leghaemoglobin concentration was not inhibited in the drought-stressed plants at ψ_m -70 kPa when inoculated with the strain EI 6, which confered a differential degree of drought resistance in plants. The ψ_w declined in the stressed plants reaching values of -1.0 MPa which was sufficient to cause disturbance in nodulation and biomass production.

Introduction

The effects of water stress on a plant's physiology vary depending on the species and degree of tolerance, as well as on the magnitude of the water deficit and how fast the plants experience this water deficit. Generally, drought quickly affects the processes related to cell turgidity and particularly the growth of the meristem. If the drought persists other physiological processes will be affected. Drought is one of a range of environmental stresses which can cause considerable reductions in N_2 fixation (Sinclair et al., 1987). However, it is not obvious which particular function of the stressed plant is actually affecting the nodule (Streeter, 1993). The relationship between the water status in the plant, photosynthesis and N_2 fixation, under water stress and the changes in nodule morphology have been studied for some temperate legumes (Sprent, 1981). However, tropical legumes growing in arid regions, have not received adequate attention. Even where information is available, the degree of water stress in the plants was not clearly defined which makes it difficult to make comparisons. The structural basis for the difference in sensivity of N_2 fixation in tropical legumes, under water stress, is not clearly understood (Venkateswarlu et al., 1990).

Nitrogen fixation has been shown to be sensitive to reductions in soil water availability for numerous crop species (González et al., 1995; Guerin et al., 1991; Lecoeur and Sinclair, 1996; Sinclair et al., 1987). Cowpea, although tolerant to prolonged drought, is quite susceptible to a lack of moisture during the phase approaching flowering (Stamford et al, 1990 and Venkateswarlu et al., 1990). The possibility of selecting rhizobial strains of cowpea according to their

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capacity to recover from water stress has been investigated (Stamford et al 1990; Walker and Miller Jr. 1986). However, the non specificity of the strains and the occurrence of ineffective indigeneous strains in soils, limits the introduction of selected strains, thus limiting the potential contribution of N_2 fixation in cowpea.

The hypothesis that drought causes a decline in nodulation and N_2 fixation and that the inoculation with strains *Bradyrhizobium* can confer a differential degree of drought resistance in plants, is tested and examined here. The concentration of ureide-N and leghaemoglobin, nitrogenase activity, nitrogen content, nodule and shoot biomass in the presence and absence of *Bradyrhizobium* spp. inoculation in cowpea plants subjected to different degrees of drought stress was measured.

Materials and methods

Soil preparation

The experiment was conducted in a greenhouse at a temperature of 27–35 °C (minimum-maximum) and a relative humidity of 50–80% (minimum-maximum). Pots were filled with soil samples (0–20 cm) of a sandy loam Yellow Latosol (Jacomine et al., 1973), from the Araripina Experimental Station, in a semi-arid region of Pernambuco State, Brazil. The soil was air dried, sieved (<5.0 mm), and adjusted to pH 6.3 by the addition of calcium and magnesium oxides in the ratio 3:1. Fourteen kilograms of soil were used in each 15 L pot. Chemical and physical analyses of the soil were conducted at the Pernambuco Enterprise of Agricultural and Livestock Research (Empresa Pernambucana de Pesquisa Agropecuária-IPA) in accordance with the EMBRAPA (1979) methods, and presented the following results: pH (water) 4.8; Ca^{2+} 7.0 $mmol_c$ kg^{-1}; Mg^{+} 4.0 $mmol_c$ kg^{-1}; K^{+} 0.7 $mmol_c$ kg^{-1}; Na^{+} 0.4 $mmol_c$ kg^{-1}; Al^{3+} 3.0 $mmol_c$ kg^{-1}; P 6.1 mg kg^{-1}; N 0.6 g kg^{-1}; clay 190 g kg^{-1}; silt 50 g kg^{-1}; fine sand 90 g kg^{-1}; coarse sand 670 g kg^{-1}; porosity 493 m^3 m^{-3}; particle density 2650 kg m^{-3}, and bulk density 1420 kg m^{-3}.

Inoculation and planting

The cultivar used was cowpea (*Vigna unguiculata* (L.) Walp.) cv. 'IPA 204' (L. 1429). The seeds were inoculated with a strain of *Bradyrhizobium* spp. BR-2001 host legume *Crotalaria juncea* L. origin EMBRAPA/CNPAB (National Agrobiology Research Centre), Rio de Janeiro, Brazil, and EI-6 host legume *Vigna unguiculata* (L.) Walp origin IPA-Pernambuco Enterprise of Agricultural and Livestock Research-Araripina Experimental Station-PE, Brazil. In the preparation of the inoculants, the strains were purified (Vincent, 1970) and then replated into agar manitol yeast extract medium with bromothymol blue as an indicator. In a following step they were transferred in duplicate into 125 mL Erlenmeyer flasks containing 25 mL of liquid mannitol yeast extract medium and incubated in a rotary agitator at a controlled temperature of 28 °C for 144 h BR-2001 and the EI-6 for 72 h. After this period of time, the inoculum contained 10^9bacterial cells cm^{-3}, evaluated by direct count in a Petroff-Hauser chamber, as well as by count of colony-forming units, performed by dilution and counting in Petri dishes. Five seeds of cowpea were surface sterilized (Vincent, 1970) and sown in each pot, and then inoculated with 5 mL pot^{-1} of liquid culture of *Bradyrhizobium* spp.. After emergence three plants were left per pot. Hoagland and Arnon solution without N was applied weekly at a rate of 2 mL Kg^{-1} of soil.

Water stress application and analysis

Water stress treatments were applied through a porous cup arrangement similar to that one described by Bataglia (1989). The auto irrigation system consisted of a porous ceramic filter cup (3.5 cm diameter and 14 cm height) placed in the center of the pot. The porous cup was connected to a constant level water reservoir through a flexible transparent tube (6 mm outside diameter and 3 mm inside diameter). The porous cup and tubing were filled with distilled water. The different soil water stress degrees were obtained by setting the vertical distances between the middle of the cups and the reservoir with a constant level of matric potential (ψ_m) at 15, 20, 40, 60, 80, and 100 cm equivalents to S1 = −1.5 kPa, S2 = −2.0 kPa, S3 = −4.0 kPa, S4 = −6.0 kPa, S5 = −8.0 kPa, and S6 = −10.0 kPa, representing the ψ_m values at the porous cup walls and consequently of the soil water when in equilibrium. As the plant roots absorb water there is a potential gradient development between the bulk soil and the surface of the cup, inducing water flow from cup to soil. Water stress was applied beginning on the fifteen day after germination and sampling were performed on seven days interval basis, for evaluation of the following parameters: Leaf transpiration rate and stomatal diffusive resistance using the 'Steady porometer' LI COR, Mod. LI 1600 with auxiliar quantic sensor LI COR, inc.sr.n° Q (12231) coupled to the porometer (readings were taken from 0900 to 1000 h, at the abaxial side of the more recently expanded leaf from each plant); leaf water potential (ψ_w) (Scholander, 1964), in order to obtain standardized measures, the following precautions were taken: (a) the determination was made on the same leaf for which the rate of transpiration and diffusive resistance of the leaf was determined; (b) the readings were taken from 0900 to 1000 h; (c) leaves of the same age, located on the apical part of the plant, were enveloped in transparent PVC film with an oblique razor-cut 2 cm from the petiole; (d) the rate of increase of the inlet of gas into the cylinder was maintained constant for all the measuring; (e) the apparatus was installed as close as possible to the plants to agility the measuring; soil water ψ_m using the tensiometer, Soil Moisture, mod. 2725 was installed at 11 cm depth just about half of the pot hight(readings were taken daily at 1000 h, throughout the entire drought period); and air relative humidity and temperature using the thermohygrograph.

The plants were harvested 45 days after germination and the following parameters concerning symbiosis were evaluated: the sap in shoot was exuded by pressurization using the Scholander chamber, collected in calibrated microcapillaries, and stored at −20 °C until assayed; ureide-N concentration was colorimetrically analysed according to Vogels and van der Drift (1970); leghaemoglobin concentration in nodules was assayed spectrophotometrically (540 nm) using Drabkin solution as 'blank' according to Wilson and Reisenauer (1963); and N_2ase activity in nodulated roots was determined by GC 30.S chromatography analysis, using a flame ionization detector and a Poropak N column, measuring ethylene production after nodulated roots incubation in a sealed flask under an atmosphere containing C_2 H_2 (10% v.v.) (Hardy et al., 1968). The following were also determined: leaf area using the portable area meter, model L1 3000, LI COR, Lincoln, Nebraska, USA; shoot, nodule, and root dry weights (65 °C for 72 h); shoot/root ratio; number of nodules; nodules fresh weights; nodules water content, nodules size and total N using the autoanalyser Tecator 1030 by Kjeldahl method (Bremner, 1965).

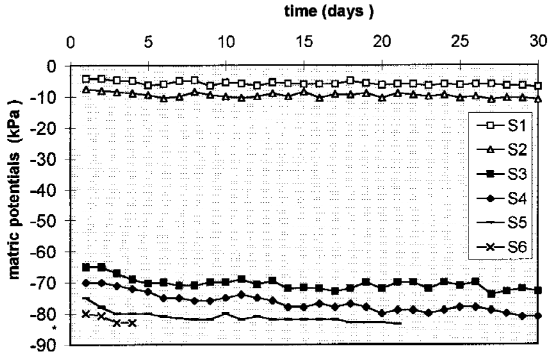

Figure 1. Matric potentials of the soil during 30 days, in respect to the different degrees of water stress *matric potentials exceeded −85 kPa).

Statistical design and analysis

The experimental design adopted was in randomised complete blocks with subdivided plots, each plot containing different stress degrees S1 (well-watered control), S2 (relativily-watered control), S3, S4, S5, and S6 divided in subplots containing the strains of *Bradyrhizobium* spp. BR-2001 and EI-6 and C (control-without inoculation) with three blocks. The variance analysis were studied according to the mathematical model of the experimental layout adopted by Steel and Torrie (1960). Differences between treatments were analysed through an ANOVA test with an F test for a $p<0.01$. The mean separation results were calculated by Tukey test ($p<0.05$). A contrast study was also performed in the treatments separated in two groups: the watered control, represented by the degrees Sl and S2, and the stressed, represented by the degrees S3, S4, S5, and S6, combined in a medium stress treatment.

Results and discussion

The effects of water stress on physiological processes

The cowpea plants were subjected to different degrees of water stress varying the water matric potentials (ψ_m) between −1.5 kPa (S1), −2.0 kPa (S2), −4.0 kPa (S3), −6.0 kPa (S4), −8.0 kPa (S5) and −10.0 kPa (S6), at the porous cup walls. As the plant roots absorb water there is a development of a potential gradient, inducing water flow from cup to soil. For this reason the ψ_m at the treatment S1 represents a soil ψ_m of −6.0 kPa; at the treatment S2 a soil ψ_m of −9.5 kPa, at the treatment S3 a soil ψ_m of −70 kPa, at the treatment

S4 a soil ψ_m of -80 kPa, and at the treatments S5 and S6 the soil ψ_m exceeded -85 kPa (the exact value has not being measured due to the limited range of the tensiometer) (Figure 1).

The ψ_m ranges to which plants were subjected with the increase of the head of water can be explained by analysing the unsaturated water flow through the soil as described by the Darcy law: q = $-$K $(\psi_m).\Delta\psi/_L$, where: q = water flow through soil; K(ψ_m) = unsaturated hydraulic conductivity, a function of matric potential; and $\Delta\psi/_L$ = hydraulic gradient. Unsaturated hydraulic conductivity decreases exponentially with soil moisture and consequently with ψ_m. Due to evapotranspiration, an hydraulic gradient is therefore necessary in order to maintain water flow, and consequently an ever greater difference in ψ_m to compensate the decrease in hydraulic conductivity. This explains the rising bands of ψ_m as the head of water increases. Since the ψ_m of the porous cup is fixed and equal to the head of water, it is necessary to reduce the soil ψ_m in order to increase the hydraulic gradient.

Kramer (1963) emphasizes that measurements of soil water content or soil water potential are not sufficient to determine the effects of water supply on plant processes and yields. Plant water stress can be characterized directly by measuring leaf water potential (ψ_w), but the levels of leaf water potential limiting plant growth are not generally known and must be determined for each species or crop. The ψ_w can be explained in terms of transpiration rate and leaf diffusion resistance, in order to provide principles and means to obtain more effective irrigation.

From Table 1 it can be seen that there was a significant difference between the degrees of water stress in the cowpea, however it presented no significance at the *F* test for the strains (subplot). The leaf water potential (ψ_w) declined in water stressed plants reaching values of -1.0 MPa, respectively. This response was in great contrast to the well-watered control plants (S1), where transpiration increased with increase of the $\psi_{w.}$ The negative effects on the metabolism of N_2 assimilation occurred every time that ψ_w fell below -0.75 MPa as happened in the stressed treatments in accordance with the results of Boyer et al. (1980) and Stamford et al. (1990).

In this work there was a correlation among ψ_w vs. leaf transpiration rate (r = 0.78** by comparing the stressed plants with the control (Sl), an increase in stomatal diffusive resistance can be observed in those subjected to water stress, but in contrast, the leaf transpiration rate of the plants fell down considerably with the increase of the stress application.

Water stress response on dinitrogen fixation and plant growth

Since an important objective of the experimental design was to ascertain responses to drought stress, the water potential that plants were allowed to reach was not extremely low. Nevertheless they were suficient to cause disturbance in nodulation, N_2 fixation and biomass production (Tables 2–6).The *Bradyrhizobium* strains introduced by inoculation in the cowpea plants were superior to the indigenous strain demonstrating the importance of inoculation in the cowpea stressed plants. At the more negative ψ_m plants inoculated with the strain EI-6 had the highest nitrogenase activity, nodule size, nitrogen content, shoot/root ratio, and concentration of ureide-N and leghaemoglobin (LHb), indicating that the strain was effective, helping the cowpea plants to withstand the water stress better than the strain BR-2001 and the uninoculated control (C).

Walker and Miller Jr. (1986) found a reduction in the N_2ase activity, number and weight of nodules, concluding that ψ_w is related to N_2 fixation by the plants. Similar results were found in the present work with cowpea, showing a correlation between N_2ase activity vs. ψ_w (r = 0.77**).

The analysis of nitrogen compounds in the xylem sap has been suggested as an efficient method for selection of the best symbiotic systems (Silva et al. 1996). In Table 2 the highest ureide-N concentration in the cowpea plants was presented by strain EI-6 and it can also be observed that drought affects the xylem water potential. The ureide-N concentration in the shoot demonstrated a positive correlation with N_2ase activity (r = 0.86**) and N total (r = 0.80**), which has been suggested to quantify plant dependence concerning N_2 fixation (Herridge et al., 1996).

The stress applied by porous cup strongly influenced N_2 fixation. However drought-stressed plants inoculated with strain EI 6 maintained the LHb concentration until ψ_m -70 kPa (S3), presented no significance ($p < 0.05$) in relation to the control ψ_m -6.0 kPa (S1), as well as, confered a differential degree of drought resistance in plants. Guerin et al. (1991) suggested that the decline in nodule N_2 fixation brought on by water stress be due to significant declines in LHb. The correlation between LHb concentration and the N_2ase activity was low (r = 0.59**). In this case

Table 1. Leaf water potential (ψ_w), leaf transpiration rate (T), and leaf diffusive resistance (Rf) in cowpea with (BR-2001 and EI-6) and without (C) *Bradyrhizobium* spp. inoculation at different degrees of water stress

Matric potentials[(1)] (kPa)	ψ_w (MPa)			T (mmol m^{-2} s^{-1})			(Rf) (s cm^{-1})		
	Strains								
	BR-2001	EI-6	C	BR-2001	EI-6	C	BR-2001	EI-6	C
S1	−0.50a	−0.47a	−0.51a	6.16a	6.05a	6.53a	2.77d	2.41d	2.74e
S2	−0.57a	−0.55a	−0.61ab	5.97a	5.55a	6.47a	3.36d	2.87d	3.70e
S3	−0.75ab	−0.70ab	−0.78b	1.80b	1.96b	2.17b	7.59c	6.90c	7.45d
S4	−0.82bc	−0.78ab	−0.84bc	1.79b	1.77b	1.84bc	10.83b	10.14b	10.70c
S5	−1.02c	−1.00b	−1.05cd	1.52b	1.35b	1.75bc	19.13a	18.16a	18.23b
S6	−1.10c	−1.07b	−1.08d	1.04b	1.08b	0.94c	20.00a	20.20 a	21.56a
F (plot)		32.98**			465.30**			476.49**	
F (supbplot)		2.85^{ns}			2.67^{ns}			2.19^{ns}	
% CV (plot)		15.57			10.60			9.97	
% CV (subplot)		11.95			13.97			8.99	

[(1)] SI −6.0 (well watered control), S2 −9.5 (relatively watered control), S3 −70.0, S4 −80.0, S5 and S6 exceeded −85.0. ^{ns}Not significant. *, **Significant at the 0.05 and 0.01 probability level. In each column the means followed by the same letter do not differ statistically ($p < 0.05$) from each other, according to Tukey's test.

Table 2. Leghaemoglobin concenration (LHb), ureide N concentration (UN), and nitrogenase activity (N_2ase) in cowpea with (BR-2001 and EI-6) and without (C) *Bradyrhizobium* app. inoculation at different degrees of water stress

Matric potentials[(1)] (kPa)	LHb (mg g^{-1} nod.DM)			UN (nmol ml^{-1})			N_2ase (nmol C_2 H_4 pl^{-1} h^{-1})		
	Strains								
	BR-2001	EI-6	C	BR-2001	EI-6	C	BR-2001	EI-6	C
S1	26.16ab^{A}	28.06ab^{A}	23.00ab^{B}	5179a^{A}	5351ab^{A}	4000a^{B}	10697a^{A}	11274b^{A}	9288a^{B}
S2	28.56a^{A}	29.70a^{A}	24.23a^{B}	5840a^{A}	6099a^{A}	4402a^{B}	11670a^{A}	12410a^{A}	9571a^{B}
S3	25.40b^{A}	27.23ab^{A}	20.40bc^{B}	3740b^{B}	4488bc^{A}	2906b^{C}	2563b^{B}	3400c^{A}	2093b^{B}
S4	25.40b^{A}	26.50b^{A}	20.40bc^{B}	3366bc^{B}	4114cd^{A}	2733b^{B}	2403b^{AB}	2633cd^{A}	1630bc^{B}
S5	24.03b^{A}	25.90b^{A}	20.30bc^{B}	2819bc^{B}	3567cd^{A}	2388b^{B}	1130c^{B}	2277d^{A}	1000c^{B}
S6	23.36b^{B}	25.90b^{A}	19.26c^{C}	2589c^{B}	3337d^{A}	2071ab^{B}	1121c^{AB}	1756d^{A}	837c^{B}
F (plot)		13.90**			132.87**			142.15**	
F (subplot)		132.36**			67.67**			67.48**	
% CV (plot)		5.54			7.44			23.94	
% CV (subplot)		4.57			9.54			20.25	

[(1)] For S1 to S6 see Table 1. *, **Significant at the 0.05 and 0.01 probability level. In each column (lower letters) and in each line (capital letters), the means followed by the same letter do not differ statistically ($p < 0.05$) from each other, according to Tukey's test.

changes in N_2ase function can not be attributed to declining LHb.

Even at the more negative ψ_m N_2ase activity was maintained (though reduced) in the different strains studied, resulting from the strong association of the water in the nodule**.** It can also be observed that the N_2ase activity was highly correlated with the nodule water content ($r = 0.92^{**}$), being this result consistent with Djekoun and Planchon (1991) with soybean. Water deficit response in cowpea appears to be directly related to a reduction in nodule mass (Table 3), which may (after a severe stress, S6) have affected nodule structural constituents. However, in moderate stress

Table 3. Nodule dry matter (NDM) and nodule water content (NWC) in cowpea with (BR-2001 and EI-6) and without (C) *Bradyrhizobium* spp. inoculation at different degrees of water stress

Matric potential[1] (kPa)	NDM mg pot^{-1}			NWC (%)		
	Strains					
	BR-2001	EI-6	C	BR-2001	EI-6	C
S1	402.66a^{A}	385.00a^{A}	333.33a^{A}	474.91a	452.03a	415.03a
S2	495.00a^{A}	400.00a^{B}	380.00a^{B}	381.37a	393.97a	387.11a
S3	230.00b^{A}	200.00b^{AB}	150.00b^{B}	127.15b	148.75b	130.14b
S4	180.00bc^{A}	130.00bc^{A}	108.00bc^{A}	107.94b	148.51b	119.82b
S5	90.00cd^{A}	75.33c^{A}	80.00bc^{A}	99.16b	125.79b	117.95b
S6	80.00d^{A}	75.00c^{A}	50.00c^{A}	96.50b	117.43b	102.77b
F (plot)		167.55**			72.29**	
F (subplot)		11.77**			0.71^{ns}	
% CV (plot)		16.43			24.43	
% CV (subplot)		18.20V			23.66	

For symbols see Table 1, 2.

Table 4. Shoot accumulation nitrogen (SAN), nodule size (NS), and nodule number (NN) in cowpea with (BR-2001 and EI-6) and without (C) *Bradyrhizobium* spp. inoculation at different degrees of water stress

Matric potential[1] (kPa)	SAN mg pot^{-1}			NS (mg nod.$^{-1}$)			NN[2] (nr. pot^{-1})		
	Strains								
	BR-2001	EI-6	C	BR-2001	EI-6	C	BR-2001	EI-6	C
S1	492.06a^{AB}	559.03a^{A}	412.93a^{B}	2.65a^{B}	5.45ab^{A}	3.53a^{B}	12.58a^{A}	8.35a^{B}	9.88a^{B}
S2	432.23a^{AB}	495.65a^{A}	350.74a^{B}	2.64a^{B}	6.57a^{A}	4.01a^{B}	13.93a^{A}	8.08a^{B}	9.71a^{C}
S3	142.71b^{A}	185.94b^{A}	107.15b^{A}	2.56a^{AB}	4.54ab^{A}	3.12a^{B}	9.73b^{A}	6.70ab^{B}	7.09b^{B}
S4	123.02b^{A}	150.42b^{A}	107.05b^{A}	2.46a^{A}	3.96b^{A}	3.01a^{A}	8.60b^{A}	5.82bc^{B}	6.14bc^{B}
S5	124.69b^{A}	149.04b^{A}	107.15b^{A}	2.39a^{A}	3.81b^{A}	2.95a^{A}	6.32c^{A}	4.44c^{AB}	5.42bc^{B}
S6	119.70b^{A}	150.50b^{A}	94.67b^{A}	2.00a^{AB}	3.83b^{A}	2.26a^{B}	6.29c^{A}	4.43c^{B}	4.68c^{B}
F (plot)		810.45**			1.52*			28.27**	
F (subplot		7.13**			33.33**			84.58**	
% CV (plot)		7.50			24.64			17.21	
% CV (subplot)		28.33			24.59			10.20	

For symbols see Table 1, 2. [2] Values changed $\sqrt{x}$.

(S3) the impact on nodule water content was higher than on the changes in nodule mass.

As shown in Table 4 the strain EI-6 inoculated in cowpea presented a smaller number of nodules, but the size of the nodule was larger than in the other treatments. This is an evidence that the strains introduced by inoculation in cowpea, as well as the indigenous strain presented a good nodulation capacity and that the host plant benefited from nitrogen fixation.

The N_2ase activity, ureide-N and LHb concentration were superior in the S2 treatment in relation to the S1 treatment, but the shoot dry matter and leaf area (Table 5) were superior in the S1 treatment in relation to the S2 treatment, which indicates that the energy requirements for the N_2 fixation in the cowpea can demand the dry matter reduction, which presented a decline of about 15%. The nitrogen fixation process demands a continuous supply of

Table 5. Shoot dry matter (SDM) and leaf area (LA) in cowpea with (BR-2001 and EI-6) and without (C) *Bradyrhizobium* spp. inoculation at different degrees of water stress

Matric potential[(1)] (kPa)	SDM g pot^{-1}			LA (cm^2)		
	Strains					
	BR-2001	EI-6	C	BR-2001	EI-6	C
S1	20.10a^{AB}	21.83a^{A}	18.63a^{B}	2903a^{A}	3205a^{A}	2488a^{B}
S2	17.43b^{A}	18.73b^{A}	15.20b^{B}	2260b^{B}	2677b^{A}	2177a^{B}
S3	5.29c^{AB}	6.45c^{A}	4.46c^{B}	990c^{A}	1023c^{A}	886b^{A}
S4	4.68c^{A}	5.46c^{A}	4.27c^{A}	800c^{A}	887c^{A}	765b^{A}
S5	4.66c^{A}	5.30c^{A}	4.10c^{A}	684c^{A}	767c^{A}	640b^{A}
S6	4.34c^{A}	5.29c^{A}	3.76c^{B}	6.50c^{A}	747c^{A}	543b^{B}
F (plot)		611.89**			41.07**	
F (subplot)		23.13**			9.89**	
% CV (plot)		9.25			18.70	
% CV (subplot)		9.84			14.35	

For symbols see Table 1, 2.

carbohydrates (Neves, 1981). However, the carbon consumption in the nodules is shared between the compounds of growth processes and nodule maintenance, N_2 reduction through N_2ase, and assimilation and transport of fixed N from the nodule (Mahon, 1983).

The shoot dry matter and leaf area (Table 5) and accumulated nitrogen (Table 4) of the cowpea plants showed significant differences for the strains, indicating higher efficiency of nitrogen biological fixation in the inoculated plants compared to those nodulated by indigenous strain (that is, the uninoculated control) demonstrating the importance of inoculation in the cowpea plants. It was verified that even with a low dry matter production by the stressed plants, the nutrient concentration at the shoot was greater, where the nitrogen content in the shoots (Table 6) did not present significant difference to the stress. However, it was significant in relation to the strains showing that inoculated treatment was superior in relation to the uninoculated control.

In Tables 7 and 8 the contrast study in the treatments watered × stressed (contrast 1) showed significant difference in the LHb and ureide-N concentration, leaf area, accumulation nitrogen, shoot and nodule dry matter, number of nodule, nodule water content, ψ_w, leaf diffusive resistance, transpiration rate ($p < 0.01$) and nodule size ($p < 0.05$).

Table 6. Nitrogen content (NC) in cowpea with (BR-2001 and EI-6) and without (C) *Bradyrhizobium* spp. inoculation

Strains	NC (mg N g^{-1} DM)
BR-2001	26.28ab
EI-6	27.33a
C	24.07b
F (plot)	1.65^{ns}
F (subplot)	4.40*
% CV (plot)	11.21
% CV (subplot)	13.00

For symbols see Table 1, 2.

In relation to the watered treatments (contrast 2) did not show significant difference to ψ_w, leaf diffusive resistance, transpiration rate, number and size nodule and nitrogenase activity, but presented significant difference in LHb and ureide-N concentration, leaf area, nodule dry matter ($p < 0.05$), accumulation nitrogen and shoot dry matter ($p < 0.01$).

Conclusion

The stress applied strongly influenced N_2 fixation, but the *Bradyrhizobium* strains introduced by inoculation

Table 7. F-values from analysis of variance by contrast study, between watered χ stressed treatment (contrast 1) and among the watered treatment (contrast 2), of leghaemoglobin (LHb) and ureide-N (UN) concentration, nitrogenase activity (N_2ase), shoot nitrogen accumulation (SNA), leaf area (LA), and nodule (NDM) and shoot (SDM) dry matter, in cowpea plants

Variation source	LHb (mg g^{-1} nod. DM)	UN (nmol ml^{-1})	N_2ase (nmol C_2 H_4 pl^{-1} h^{-1})	SNA (mg pot^{-1})	LA (cm^2)	NDM (mg pot^{-1})	SDM (g pot^{-1})
Contrast 1	55.71**	571.02**	699.82**	3990.06**	197.41**	756.02**	2998.43**
Contrast 2	7.41*	20.17*	2.10^{ns}	53.43**	5.37*	9.62*	55.42**

For symbols see Table 1, 2.

Table 8. F-values from analysis of variance by contrast study, between watered χ stressed treatment (contrast 1) and among the watered treatment (contrast 2), of leaf water potential (ψ_w), leaf transpiration rate (T), leaf diffusive resistance (Rf), nodule number (NN), nodule size (NS), and nodule water content (NWC), in cowpea plants

Variation source	ψ_w (MPa)	T (mmol $m^{-2}s^{-1}$)	Rf (s cm^{-1})	NN (nr. pot^{-1})	NS (mg nod $^{-1}$)	NWC (%)
Contrast 1	105.10**	2280.26**	1392.64**	102.43**	6.11*	369.42**
Contrast 2	2.13^{ns}	2.46^{ns}	1.82^{ns}	0.62^{ns}	0.53^{ns}	5.61*

For symbols see Table 1, 2.

in the cowpea plants were superior to the indigenous strain demonstrating the importance of inoculation in the cowpea stressed plants. At the more negative ψ_m,plants inoculated with the strain EI 6formed associations of greater symbiotic efficiency helping the cowpea plants to withstand water stress better than the strain BR 2001 and the uninoculated control. The LHb concentration was not inhibited in the drought-stressed plants at ψ_m -70 kPa when inoculated with the strain EI 6, which confered a differential degree of drought resistance in plants, compared to strain BR 2001. The ψ_w declined in the droughted plants reaching values of -1.0 MPa and was sufficient to cause disturbance in nodulation and biomass production.

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