Water deficit stress effects on N_2 fixation in cowpea inoculated with different *Bradyrhizobium* strains

M. V. B. Figueiredo[1,4], H. A. Burity[2], and F. P. de França[3]

[1]*EMBRAPA-EPEAL Doutorado, IM/UFRJ, CP 699, CEP 57025.050 Maceio-AL. Brazil;* [2]*EMBRAPA-IPA, CP 1022, CEP 50761.000 Recife-PE. Brazil; and* [3]*EQ/CT-UFRJ, CP 6841, CEP 21949.900 Rio de Janeiro-RJ. Brazil.* *Received 3 October 1997, accepted 29 June 1998.*

Figueiredo, M. V. B., Burity, H. A. and de França, F. P. 1998. **Water deficit stress effects on N_2 fixation in cowpea inoculated with different *Bradyrhizobium* strains**. Can. J. Plant Sci. **78**: 577–582. The objectives of this experiment were to select strains of *Bradyrhizobium* sp. resistant to water stress, envisaging an increase in N_2 fixation in cowpea (*Vigna unguiculata* [L.] Walp.), and to verify the plant's adaptive physiological responses to water stress. The experiment was carried out in greenhouse conditions using random complete blocks subdivided into plots adjusted to soil water potential levels of –6.0, –75.0, and –85.0 kPa, and subplots containing strains of *Bradyrhizobium* sp. (SEMIA 6145, 6086, 6002 and NFB 700), with four blocks. The soil was a Yellow Latosol with pH 6.3. The crop used was cowpea cv. IPA 204. Stress was applied continuously beginning 15 d after planting, by the control of water potential through a porous cup. Various parameters were evaluated every 7 days, until final harvest at 45 d. There was significant interaction between *Bradyrhizobium* strains and water stress. At the more negative ψ_m, plants inoculated with the SEMIA 6145 had higher LHb concentration, ureide-N, ψ_w and root dry matter, forming associations of greater symbiotic efficiency, while plants inoculated with SEMIA 6086 were not resistant to stress. LHb concentration apparently was not inhibited at ψ_w –1.0 MPa in cowpea. The *Bradyrhizobium* strains may have affected the metabolism of N assimilation and/or transport.

Key words: *Bradyrhizobium,* N_2 fixation, porous cup, *Vigna unguiculata,* water stress, Yellow Latosol

Figueiredo, M. V. P., Burity, H. A. et de França, F. P. 1998. **Effets du stress de déficit hydrique sur la fixation de N_2 chez le dolique inoculé par diverses souches de *Bradyrhizobium***. Can. J. Plant Sci. **78**: 577–582. L'objet de nos recherches était de trouver des souches de *Bradyrhizobium* sp. résistantes au stress hydrique dans la perspective d'un accroissement de la fixation de N_2 chez le dolique (*Vigna unguiculata* [L.] Walp.) et de vérifier les réactions d'adaptation physiologique de la culture au stress hydrique. L'expérience, réalisée en serre selon un dispositif en blocs aléatoires complets à quatre répétitions, comprenait quatre niveaux de potentiel hydrique du sol : –6,0, –75,0 et –85,0 kPa comme parcelles principales et quatre souches de rizobium SEMIA 6145, 6086, 6002 et NFB 700 comme sous-parcelles. Le sol utilisé était un latosol jaune de pH 6,3 et la culture le cultivar de dolique IPA 204. Le stress hydrique était appliqué en permanence dès le 15^{e} jour après la germination par la régulation du potentiel hydrique au moyen d'une coupe poreuse. Divers paramètres étaient évalués tous les 7 jours jusqu'à la récolte à 45 jours. On observait une interaction significative entre les souches et le stress hydrique. Aux valeurs négatives les plus fortes du ψ_m les plantes ensemencées avec l'inoculum SEMIA 6145 fournissaient une concentration de leghémoglobine (LHb), une teneur en N sous forme uréide, un potentiel hydrique foliaire (ψ_w) plus élevé et une matière sèche racinaire plus abondante, formant des associations symbiotiques plus efficientes. Par comparaison, les plantes mises en présence de SEMIA 6086 ne résistaient pas au stress hydrique. La concentration de LHb n'était semble-t-il pas inhibé au ψ_w de –1,0 Mpa chez le dolique. Les diverses souches de *Bradyrhizobium* peuvent avoir un effet sur le métabolisme de l'assimilation et ou du transport de l'azote.

Mots clés: *Bradyrhizobium,* fixation de N_2, porous cup, *Vigna unguiculata,* stress hydrique, latosol jaune

Water shortage is one of the greatest selective pressures in the evolution of plants, and their ability to survive during drought is a major determining factor in the distribution and productivity of cultivated plants (Boyer 1980). Drought can either weaken vital functions or stimulate adaptive reactions that enable plants to survive prolonged water deficits (Pimentel et al. 1990). While the effects of the environment on growth, development and production are evident, it is essential to evaluate the effects of these factors on the growth and physiology of species that are economically important (Perez 1995). Water deficiency affected N accumulation, suggesting that cowpea, although tolerant to prolonged drought, is quite susceptible to a lack of water during the phase approaching flowering (Venkateswarlu et al. 1989; Stamford et al. 1990). The possibility of selecting rhizobial strains applicable to cowpea for their ability to recover from water stress has been investigated (Walker and Miller 1986). However, the unspecificity of the strains and the occurrence of ineffective native strains in soils, limit the introduction of selected strains, thus reducing potential N_2 fixation by the cowpea. Nevertheless, the effect of water stress on the growth of the plant and fixation of N_2 must be evaluated in order to determine the extent to which it is possible to increase N_2 fixation through resistance to drought (Walker and Miller 1986).

[4]*To whom correspondence should be addressed: Rua Sá e Souza, 1020 / 1401, CEP 51030-060 Recife, PE, Brazil. E-mail: mbarreto@elogica.com.br.*

Abbreviations: $\boldsymbol{\psi_m}$, soil water potential; $\boldsymbol{\psi_w}$, leaf water potential; $\mathbf{N_2}$**ase**, nitrogenase; **LHb**, leghemoglobin

The relation among water status of the plant, photosynthesis and N_2 fixation under water stress, and the changes in nodule morphology have been studied in some temperate legumes (Sprent 1981). However, tropical legumes grown in dry regions, have not received adequate attention in these studies. Even where information is available, the levels of stress are not clearly defined in terms of plant water status, which makes comparisons difficult among rhizobia cultures. According to Sprent (1981) and González et al. (1995), N_2 fixation in legumes requires that the soil be capable of supplying water and certain gases to the nodules and symbiont plant. Intermittent water supply may bring about temporary conditions of water excess or deficiency. Both situations can directly hinder the development of the crop and affect biological fixation of nitrogen, through influencing the physiological activities of microorganisms, as well as, their survival. When there is excess water, N_2 fixation can be totally inhibited, which appears to be related to the inability of rhizobia cells to withstand physical-chemical and biological alteration caused by water variations (Osa-Afiana and Alexander 1982).

Reduction in nitrogenase activity in stressed plants has already been reported in different legumes by several writers (Huang et al. 1975; Finn and Brun 1980; Guerin et al. 1990). The leghemoglobin in nodules in stressed plants may have unusual influence and instability on nitrogenase activity, assisting the respiration of bacteroides (Sprent 1981). However, the mechanism by which water stress causes loss in N_2 fixation is still controversial.

The objectives of this study were to select strains of *Bradyrhizobium* sp. resistant to water stress envisaging an increase in N_2 fixation in cowpea, and to verify the plant's adaptive physiological responses to water stress.

MATERIALS AND METHODS

The experiment was carried out in pots using soil (0–20 cm) of a sandy loam Yellow Latosol (Jacomine et al. 1973) taken from the Araripina Experimental Station in the semiarid region of Pernambuco State, Brazil. The soil was air dried, sieved (5.0 mm), corrected to pH 6.3 by the addition of calcium and magnesium oxides in the ratio 3:1, and autoclaved for 30 min at a temperature of 121°C and pressure of 101 kPa at 24-h intervals for 3 consecutive days. Fourteen kilograms of soil were used in each 15-L pot. Chemical and physical analysis of the soil was carried out at the Pernambuco enterprise of Agricultural and Livestock Research (Empresa Pernambucana de Pesquisa Agropecuária-IPA) in accordance with EMBRAPA (1979) methods (Table 1).

Table 1. Chemical and physical characteristics of Yellow Latosol soil

ph (water)	4.8	Clay	(g kg^{-1})	190
Ca^{2+} (mmol $_c$$kg^{-1}$)	7.0	Silt	(g kg^{-1})	50
Mg^{+} (mmol $_c$$kg^{-1}$)	4.0	Fine sand	(g kg^{-1})	90
K^{+} (mmol $_c$$kg^{-1}$)	0.7	Coarse sand	(g kg^{-1})	670
Na^{+} (mmol $_c$$kg^{-1}$)	0.4	Porosity	(m^3 m^{-3})	493
Al^{3+} (mmol $_c$$kg^{-1}$)	3.0	Particle density	(kg m^{-3})	2650
P (mg kg^{-1})	6.1	Bulk density	(kg m^{-3})	1420
N (g kg^{-1})	0.6			

The cultivar used was cowpea (*Vigna unguiculata* [L.] Walp.) cv. IPA 204 (L. 1429) and the seeds were inoculated with four strains of *Bradyrhizobia* culture (Table 2). The strains of *Bradyrhizobium* sp. were purified (Vincent 1970) and replated in duplicate into 125-mL Erlenmeyer flasks containing 25-mL of liquid mannitol yeast extract medium. All strains were incubated in a rotary agitator at 28°C for 72 h (SEMIA 6002 and NFB 700) or 144 h (SEMIA 6086 and 6145). After this period, the inoculum contained 10^9 bacterial cells cm^{-3}, evaluated by direct count in a Petroff-Hanser chamber, as well as by the count of colony-forming units by dilution and counting in Petri dishes.

Seeds of cowpea were sterilized (Vincent 1970) and sown five seeds per pot prior to inoculation with 5 mL pot^{-1} of liquid culture of *Bradyrhizobium* sp. 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 treatments were applied by means of a porous cup arrangement similar to that described by Bataglia (1989). The auto-irrigation system (Fig. 1) consisted of a porous ceramic filter cup (diameter 3.5 cm; height 14 cm) 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 deaerated water, avoiding the presence of air bubbles. The different soil water stress levels were accomplished by setting the vertical distances between the middle of the cups and the constant level of the water ψ_m reservoir at 15 cm (L1), 40 cm (L2), and 100 cm (L3) equivalent to –1.5 kPa (S1), –4.0 kPa (S2), and –10.0 kPa. (S3), representing the ψ_m values at the porous cup walls, and consequently, the soil water ψ_m when in equilibrium. As the plant roots absorbed water, a potential gradient developed between the bulk soil and the surface of the cup, inducing water flow from cup to soil.

Water stress was applied beginning at 15 d after germination, and sampling was carried out every 7 d to evaluate the following parameters: leaf water potential (ψ_w) (PMS Instrument Company, Carvalho, OR, USA, according to Scholander et al. [1964] readings were taken from 09:00 to 10:00 h); soil water potential (ψ_m) using a tensiometer (Soil Moisture, mod. 2725) (the readings were taken daily at 10:00 h, throughout the entire drought period); and air relative humidity and temperature using a thermohygrograph.

The plants were harvested 45 d after germination. Shoot xylem sap was exudated by pressurization using the Scholander chamber, collected in calibrated microcapillaries, and stored at –20°C until assayed; ureide-N concentration of the sap was colorimetrically analyzed according to Vogels and van der Drift (1970); LHb 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 chromotography analysis, using a flame ionization detector and a Poropak N column, measuring ethylene production after incubation of nodulated

Table 2. Original hosts of the *Bradyrhizobium* sp.

Strains	Host legumes	Origin
SEMIA 6145[z]	*Crotalaria juncea* L.	EMBRAPA/CNPAB (National Agrobiology Research Centre) – Brazil
SEMIA 6086[z]	*Vigna unguiculata* (L.) Walp.	IITA (International Institute of Tropical Agriculture) – Nigeria
SEMIA 6002[z]	*Vigna unguiculata* (L.) Walp.	CSIRO (Division of Plant Industry) – Australia
NFB 700	*Cajanus cajan* (L.) Mills	UFRPE (Federal Rural University of Pernambuco) – Brazil

[z]Supplied by IPAGRO (Soil Microbiology Section – MIRCEN) – Porto Alegre, RS, Brazil.

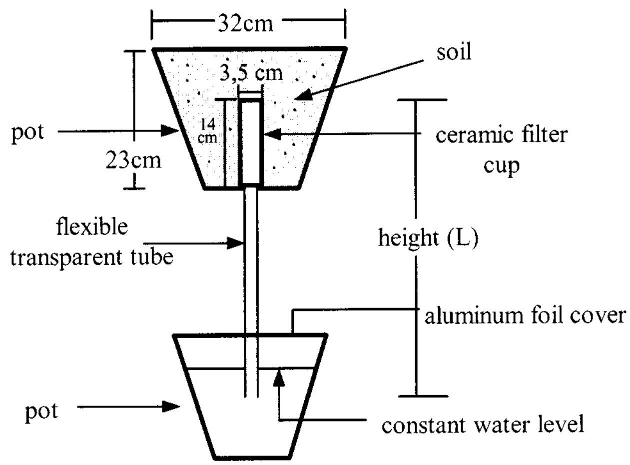

Fig. 1. Irrigation system using a porous cup technique.

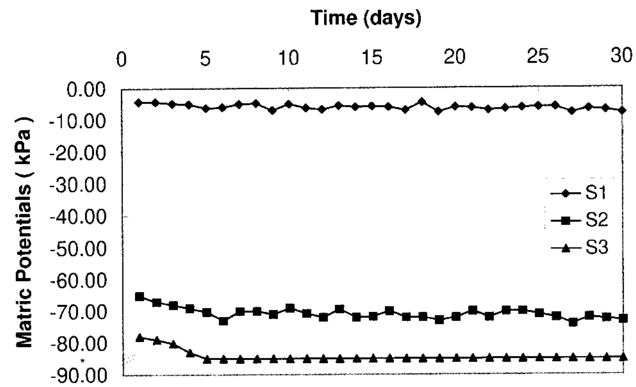

Fig. 2. Matric potentials of the soil during 30 d under different levels of water stress (* matric potentials exceeded –85 kPa).

roots in a sealed flask under an atmosphere containing C_2H_2 (10% vol/vol) (Hardy et al. 1968). Leaf area was determined using a portable area meter (model L1 3000, LI COR, Lincoln, NE, USA). Other measurements included: specific leaf area, shoot, nodule, and root dry weights (65°C for 72 h) and shoot/root ratio. Total N was determined using a Tecator 1030 auto-analyser by the Kjeldahl method (Bremner 1965).

The experimental design was a random complete block with subdivided plots, each main plot containing different water stress levels, one without stress (control S1) and two with stress (S2 and S3), divided in subplots containing the various *Bradyrhizobium* strains (SEMIA 6145, 6086, 6002, and NFB 700) with four blocks. All data were subjected to analysis of variance ($P < 0.01$) in accordance with the experimental layout adopted (Steel and Torrie 1960). Differences among treatment means were determined by Tukey's test ($P < 0.05$).

RESULTS AND DISCUSSION

The Impact of Water Stress on Physiological Processes, Nodulation and N_2 Fixation

Soils varied in water potential (ψ_m) from –6.0 kPa (S1), –70.0 kPa (S2) and in excess of –85.0 kPa (S3, the exact value could not be measured due to the limited range of the tensiometer) (Fig. 2). There was significant interaction between *Bradyrhizobium* strains and water stress level in regard to our measured parameters (leaf water potential, nodule numbers, nodule dry matter, nitrogenase activity, nitrogenase specific activity, N accumulation, leghemoglobin, ureide-N, shoot and root dry matter, leaf area, specific leaf area and shoot/root ratio), except for N content.

At the lowest ψ_m (S3), the plants inoculated with strain SEMIA 6145 showed higher leaf water potential (ψ_w) during stress, than two of the three other strains. Studies carried out by Turk et al. (1980) on cowpea and González et al. (1995) on soybean showed ψ_w of –1.2 MPa with values similar to results shown in Table 3.

Lowering soil ψ_m reduced ($P < 0.05$) the efficiency of the N_2 fixation process (Table 3). Water stress reduced ($P < 0.05$) N_2ase activity and presented a correlation between N_2ase activity and ψ_w ($r = 0.63$**), as well as, a reduction in the number and weight of nodules, suggesting that ψ_w is related to N_2 fixation by the plant.

The S2 treatment did not show a significant difference ($P < 0.05$) in nodule dry matter (Table 3) among the strains studied. However, in the S3 treatment, plants inoculated with SEMIA 6086 were not resistant to water stress.

N_2ase activity per gram of nodule (specific activity) is an important indicator of the N assimilatory efficiency. Extremely low values of specific N_2ase activity were observed (Table 3). At the most negative ψ_m, plants inoculated with SEMIA 6086 did not nodulate. With the application of stress, total N accumulated in the shoot, which represents a measure of the N fixed, was highly affected by water deficiency (Table 4). The negative effects on the metabolism of N_2 assimilation occurred every time ψ_w fell below –0.8 MPa (Tables 3 and 4), in agreement with the work of Huang et al. (1975), Stamford et al. (1990), and

Table 3. Leaf water potential (ψ_w), nodule number, nodule dry matter, N_2ase activity, and N_2ase specific activity in cowpea inoculated with four strains of *Bradyrhizobium* sp. at different levels of water stress

	ψ_w (MPa)			Nodule numbers[z] (no. nod.)			Nodule dry matter (mg pot^{-1})			N_2ase activity (µmol C_2H_4 h^{-1} plant^{-1})			N_2ase specific activity (µmol C_2H_4 h^{-1} p^{-1} g dry wt nod^{-1})		
	Water stress level[y]														
Strains	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
SEMIA 6145	–0.50*a*	–0.82*c*	–1.01*a*	14.01*a*	3.40*a*	2.80*a*	544.2*a*	80.5*a*	25.9*a*	11.93*a*	0.13*a*	0.12*a*	66.0*ab*	4.0*a*	14.0*a*
SEMIA 6086	–0.46*a*	–0.78*bc*	–1.08*b*	13.68*a*	4.13*a*	0.70*b*	443.8*c*	63.6*a*	0.0*b*	5.21*c*	0.12*a*	0.00*b*	35.0c	6.0*a*	0.0*b*
SEMIA 6002	–0.50*a*	–0.74*ab*	–1.08*b*	2.86*b*	1.31*a*	2.00*b*	486.7*b*	67.8*a*	14.9*a*	10.24*b*	0.12*a*	0.08*a*	63.0*b*	5.0*a*	12.0*a*
NFB 700	–0.48*a*	–0.83*c*	–1.04*ab*	13.51*a*	4.46*a*	3.35*a*	493.8*b*	71.0*a*	23.8*a*	11.80*a*	0.13*a*	0.12*a*	72.0*a*	5.0*a*	15.0*a*
F(plot))		2336.91**			867.95**			1810.44**			257.54**			212.50**	
F (subplot)		6.20**			178.87**			248.54**			119.86**			103.22**	
F (interaction)		2.33*			66.85**			148.11**			112.52**			45.32**	
% CV (plot)		3.31			13.15			14.18			46.71			35.98	
% CV (subplot)		5.48			17.40			12.52			20.83			22.21	

*,**Significant at $P < 0.05$ and $P < 0.01$ respectively.
[z]Values changes. $\sqrt{x + 0.5}$
[y]S1 –6.0 kPa; S2 –70.0 kPa; S3 excess – 85.0 kPa.
a–c In each column, means (4 replicates) followed by the same letter do not differ statistically from each other at $P < 0.05$ according to Tukey's test..

Table 4. Nitrogen accumulation, leghemoglobin (LHb), ureide-N and N content in cowpea inoculated with four strains of Bradyrhizobium sp. at different levels of water stress

	Nitrogen accumulation (mg pot^{-1})			Leghemoglobin (mg g^{-1} DM nod.)			Ureide-N (µmol mL^{-1})			N content (mg N g^{-1} DM)
	Water stress level(1)									
Strains	S1	S2	S3	S1	S2	S3	S1	S2	S3	X(2)
SEMIA 6145	551*a*	100*a*	76*a*	27.40*a*	23.00*a*	22.00*a*	5.6*a*	4.5*a*	3.6*a*	26.5*a*
SEMIA 6086	367*c*	61*c*	36*c*	16.82*b*	16.10*b*	0.00*c*	3.8*b*	2.8*b*	2.6*bc*	23.1*b*
SEMIA 6002	454*b*	74*b*	52*b*	26.43*a*	21.62*ab*	15.87*b*	4.6*bc*	4.0*a*	3.0*ab*	25.7*a*
NFB 700	539*a*	99*a*	71*a*	25.23*a*	22.59*ab*	19.94*ab*	4.9*ab*	4.3*a*	3.2*ab*	26.2*a*
F(plot))		9515.85**			34.67**			70.65**		5.93*
F (subplot)		2221.93**			116.45**			58.91**		41.51**
F (interaction)		1138.11**			5.58**			2.33**		1.41NS
% CV (plot)		5.08			8.36			9.39		9.75
% CV (subplot)		3.15			15.87			11.30		6.84

*,**Significant at $P < 0.05$ and $P < 0.01$ respectively. NS, not significant.
[z]S1 –6.0 kPa; S2 –70.0 kPa; S3 excess – 85.0 kPa.
[y]*x* = average.
a–c In each column, means (4 replicates) followed by the same letter do not differ statistically from each other at $P < 0.05$ according to Tukey's test.

Nilsen (1995). Among the *Bradyrhizobium* strains studied, SEMIA 6145 and NFB 700 were superior to the others ($P < 0.05$) in their resistance to water stress.

There was significant interaction between *Bradyrhizobium* strains and water stress in the LHb concentration (Table 4), and the greatest concentration in nodules was produced by SEMIA 6145. This increase was proportional to the greater N_2 fixation by this strain. LHb concentration decreased with increasing stress, although the reduction was small in relation to the decreases on the N_2ase activity at the most negative ψ_m (S3).

In the present study, the correlation between LHb and N_2ase activity was low ($r = 0.60$**). It is, therefore, unlikely that changes in N_2ase can be attributed to declining LHb. One theory supported by the data of Guerin et al. (1991) and Irigoyen et al. (1992), is that LHb concentration may decline significantly during water stress. Results of the present study suggest that when water stress was applied gradually, the LHb concentration apparently was not inhibited at $\psi_w = -1.0$ MPa in cowpea (Table 3), at the most negative ψ_m (S3). Similar results were found by González et al. (1995) studying the role of *Sucrose synthase* in the response of soybean (*Glycine max*) nodules to drought.

An alternative measure of N_2 fixation is based on the concentration of ureide-N where it can be clearly observed that water stress reduced ureide concentration. Plants inoculated with SEMIA 6145 had higher ureide-N concentration than those inoculated with *Bradyrhizobium* strains, this strain again showed superiority to other strains at the lowest ψ_m (S3) (Table 4). This strain was also shown to induce higher ureide concentration in soybean (Neves et al. 1985). This suggests that strain SEMIA 6145 may affect the metabolism of N assimilation and/or transport. The ureide concentration of xylem was positively correlated with N_2ase activity ($r = 0.82$**), agreeing with data obtained by Khadri et al. (1996) who studied N_2 fixation and the metabolism of ureide in

Table 5. Shoot and root dry matter, leaf area, specific leaf area and shoot/root ratio in cowpea inoculated with four strains of *Bradyrhizobium* sp. at different levels of water stress

	Shoot dry matter ($g\ pot^{-1}$)			Root dry matter ($g\ pot^{-1}$)			Leaf area (cm^2)			Specific leaf area ($cm^2\ g^{-1}$)			Shoot/root ratio ($g\ pot^{-1}$)		
	Water stress level[z]														
Strains	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
SEMIA 6145	22.34*a*	3.84*a*	2.70*a*	9.26*a*	3.15*a*	0.88*a*	3028*a*	435*a*	283*a*	342.85*b*	200.06*bc*	322.69*b*	2.41*a*	1.22*c*	3.06*a*
SEMIA 6086	15.84*d*	2.57*b*	1.59*b*	8.92*b*	1.68*c*	0.69*bc*	2422*c*	356*b*	240*a*	418.26*a*	318.85*a*	387.28*a*	1.77*b*	1.53*abc*	2.30*bc*
SEMIA 6002	19.31*c*	2.81*b*	1.88*b*	9.13*a*	1.61*c*	0.75*abc*	2527*b*	369*ab*	266*a*	326.09*b*	244.40*ab*	422.04*a*	2.11*ab*	1.73*ab*	2.51*b*
NFB 700	21.84*b*	3.68*a*	2.52*a*	9.22*a*	2.75*b*	0.82*ab*	3016*a*	356*b*	276*a*	396.29*b*	193.87*bc*	404.41*a*	2.36*a*	1.34*bc*	3.07*a*
F(plot))		8959.88**			29539.82**			14145.11**			105.14**			31.77**	
F (subplot)		2110.06**			2450.85**			1462.45**			15.56**			5.84**	
F (interaction)		1289.74**			1355.88**			1001.88**			10.64**			12.60**	
% CV (plot)		5.39			2.77			4.52			9.65			9.38	
% CV (subplot)		2.75			2.24			3.78			9.79			9.86	

**Significant at $P < 0.01$.
[z]S1 –6.0 kPa; S2 –70.0 kPa; S3 excess – 85.0 kPa.
a–d In each column, means (4 replicates) followed by the same letter do not differ statistically from each other, at $P < 0.05$, according to Tukey's test.

beans (*Phaseolus vulgaris*). There was also a correlation between ureide and total N ($r = 0.80$**). A similar observation led Herridge et al. (1996) to suggest that ureide concentration be used to quantify plant dependence on N_2 fixation.

Influence of Water Stress on Vegetative Development

Leaf area, shoot and root dry matter of the cowpea were reduced by water stress ($P < 0.05$) (Table 5). Further, water stress influenced the rankings of shoot biomass, though differences in the presence of stress were small. SEMIA 6145 consistently induced the highest shoot biomass across all water treatments. In stressed plants, SEMIA 6145 and NFB 700 strains showed no differences, although in normal water supply conditions, plants inoculated with SEMIA 6145 showed a greater accumulation of dry matter than the other strains. Plants inoculated with SEMIA 6002 showed an accumulation of root dry matter, which was comparable with the most efficient strains in the control treatment (S1). However, in stressed plants, SEMIA 6145 was superior ($P < 0.05$) to the other strains forming associations of greater symbiotic efficiency with the cowpea.

Leaf area of cowpea subjected to S2 treatment was greatest in plants inoculated with SEMIA 6145. However, specific leaf area was greatest ($P < 0.05$) for those plants inoculated with SEMIA 6086. SEMIA 6145, at the most negative ψ_m (S3), produced the lowest specific leaf area ($P < 0.05$), and the highest yield of leaf dry matter, suggesting again that this strain is superior to the other strains studied.

The shoot/root ratios (Table 5) produced by SEMIA 6145 and NFB 700 in treatments S1 and S3 were higher than those produced by other strains. Also, it was observed that the shoot/root ratio was highest at the highest water stress level. This is in keeping with the work of Costa et al. (1997) who also found increased shoot/root ratio in water stressed cowpea. However, work by González et al. (1995) has shown that the shoot/root ratio of drought and control plants diverged throughout the study period, increasing in the control and decreasing in drought stressed plants.

The N content in the shoots of stressed plants (25.3 mg N g^{-1} DM), which was greater in control plants (22.8 mg N g^{-1} DM), was represented by the relationship $Y = 22.82 - 0.02x$ ($R^2 = 0.81$). This suggests that although the dry matter production of stressed plants was low (Table 5), the nutrient concentration in the shoot was high. These data agree with most of the related research by Viets (1972) on the effects of water deficiency on the availability of nutrients.

Strains SEMIA 6145 and NFB 700 in cowpea cv. IPA 204 displayed similar behavior, but at the more negative ψ_m SEMIA 6145 was superior in LHb concentration, ureide-N, ψ_w and root dry matter, forming associations of greater symbiotic efficiency. Strain SEMIA 6086 was not resistant to water stress.

CONCLUSIONS

There was significant interaction between *Bradyrhizobium* strains and water stress. At the more negative ψ_m, the inoculation with SEMIA 6145 formed associations of greater symbiotic efficiency, helping the cowpea plants to better withstand water stress. *Bradyrhizobium* strains may have affected the metabolism of N assimilation and/or transport. Water stress applied by a porous cup method strongly influenced N_2 fixation, but the LHb concentration apparently was not inhibited at ψ_w – 1.0 MPa in cowpea.

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