The Effect of Water Stress on Soybean Plants in the Presence and Absence of *Bradyrhizobium* Strains

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ABSTRACT

Experiments were carried out to investigate the effect of water stress on soybean (*Glycine max* L.) Merr variety Giza 83 and to evaluate its physiological responses in the presence and absence of *Bradyrhizobium* spp. inoculation . The soil used was sandy loam and pH = 7.3 . Stress was applied continuously by the control of matric *potential* (Ψm) through a porous cup. The lowered soil Ψm had a direct effect on the N_2 fixation, but the strains *Bradyrhizobium* introduced by inoculation in the soybean plants were superior to the indigenous strain demonstrating the importance of inoculation in the stressed plants. At the more negative Ψm plants inoculated with the strain TAL 379 formed associations of greater symbiotic efficiency which helped the soybean plants to w and to evaluate physiological responses ithstand drought stress better than the strain ARC 517 and the uninoculated control. The leghaemoglobin concentration was not inhibited in the drought-stressed plants at ($\Psi m = -70$ KPa) when inoculated with the strain TAL 379, which conferred a differential degree of drought resistance in plants. The Ψw declined in the stressed plants reaching values of -1.0 MPa which was sufficient to cause disturbance in nodulation and biomass production.

Key words: *Bradyrhizobium* , N_2 fixation , symbiosis , water deficit , *Glycine max* L.

INTRODUCTION

The effects of water stress on a plant physiology vary depending on:(1) the species and degree of tolerance(2) the magnitude of the water deficit and(3) 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 (Streete, 1993). The relationship between 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 the 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 sensitivity 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 (Gonzaiez *et al.*, 1995; Guerin *et al.*, 1991; Lecoeur and Sinclair, 1996). Legumes 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 rhizobia strains of legumes according to their capacity to recover from water stress has been investigated (Stamford *et al.*, 1990; Walker and Miller, 1986). However, the non specificity of the strains and the occurrence of ineffective indigenous strains in soils, limits the introduction of selected strains, thus limiting the potential contribution of N_2 fixation in soybean.

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 soybean 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).Surface soil samples (0-20 cm) of a sandy loam, from Kafr El-Dawar city, Behera Governorate,Egypt were collected. The soil samples were air dried, sieved (< 5.0 mm), and adjusted to pH 7.3 by the addition of calcium and magnesium oxides in the ratio 3 : 1. Fourteen kilograms of soil samples were used (to fill) each of 15 L pot. Chemical and physical analysis of the soil were conducted at the Faculty of

Agric. Alex. Univ. El-Shatby in accordance with the Black (1965) methods, and presented in Table(1).

Table 1. Some physical and chemical properties of soil.

Characteristic	Value
pH (water)	7.8
Ca^{2+} mmol$_e$ kg^{-1}	7.0
Mg^{2+} mmol$_e$ kg^{-1}	4.0
K^+ mmol$_e$ kg^{-1}	0.7
Na^+ mmol$_e$ kg^{-1}	0.4
Al^{3+} mmol$_e$ kg^{-1}	3.0
P mg kg^{-1}	6.1
N g kg^{-1}	0.6
clay g kg^{-1}	190
silt g kg^{-1}	50
fine sand g kg^{-1}	90
coarse sand g kg^{-1}	670
porosity m^{3}m^{-3}	493
particle density kg m^{-3}	2650
bulk density kg m^{-3}	1420

Inoculation and planting

Bradyrhizobium bv. Soybean strains ARC 517 and TAL 379 were obtained from Microbiology Research Center, Cairo Mircen, Egypt (EMCC), Fac. of Agric., Ain Shams Univ., Cairo, Egypt.

The cultivar used was soybean (*Glycine max* L.) Merr variety Giza 83. The seeds were inoculated with a strain of *Bradyrhizobium* spp. ARC 517 and TAL 379. 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 ARC 517 and the TAL 379 for 72 h. After this period of time, the inoculum contained 10^9 bacterial cells cm^{-3}, as well as by count of colony-forming units, performed by dilution and counting in Petri dishes. Five seeds of soybean were 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.

Adjust the applied water stress at the porous cup walls

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 (3.5cm 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 cub 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 (Ψm) at 15, 20, 40, 60, 80, and 100 cm equivalents to S$_1$ = – 1.5 KPa, S$_2$ = – 2.0 KPa; S$_3$ = – 4.0 KPa, S$_4$ = – 6.0 KPa, S$_5$ = – 8.0 KPa, and S$_6$ = – 10.0 KPa, representing the Ψm values at the porous cup walls and consequently of the soil water when(T °C) 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 days after germination and sampling were performed on seven days interval basis, for evaluation . Leaf transpiration rate (T) , stomata diffusive resistance (Rf)and leaf water potential (Ψw) .

Measurements of (T),(Rf) and (Ψw)

The leaf transpiration rate and stomata diffusive resistance are measured using the "Steady porometer" LICOR, Mod. LI 1600 with auxiliary quantic sensor LICOR, 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 (Ψw) (Scholander *et al.*, 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.

Measurement of (Ψm), (θ), (RH) and (T °C)

Soil water (Ψm) using the tensiometer, soil Moisture, mod. 2725 was installed at 11 cm depth just about half of the pot hight (readings of (Ψm) and soil moisture (θ) were taken daily at 1000 h, throughout the entire

drought period); and air relative humidity (RH) and temperature (T °C) using the thermohygrograph.

Determination of Symbiosis Parameters

The plants were harvested 45 days after germination and the following parameters concerning symbiosis were evaluated:-(1) the sap in shoot was exuded by pressurization using the chamber, collected in calibrated microcapillaries, and stored at − 20°C until assayed;(2) ureide-N concentration was colorimetrically analysed according to Vogels and Drift (1970);(3) leghaemoglobin concentration in nodules was assayed spectrophotometrically (540 nm) using Drabkin solution as "blank" according to Wilson and Reisenauer (1963); and (4) N_2 ase 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_2H_2 (10% V.V.) (Hardy et al., 1968).

Determination of plant indicators nodules

Leaf area using the portable area meter, model LI 3000, LICOR, 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).

Statistical design and analysis

The experimental design adopted was in randomized complete blocks with subdivided plots, each plot containing different stress degrees S_1 (well-watered control), S_2 (relativily-watered control), S_3, S_4, S_5, and S_6 divided in subplot containing the strains of Bradyrhizobium spp. ARC 517 and TAL 379 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 S_1 and S_2, and the stressed, represented by the degrees S_3, S_4, S_5, and S_6, combined in a medium stress treatment.

RESULTS AND DISCUSSION

The effects of water stress on physiological processes

The soybean plants were subjected to different degrees of water stress varying the water matric potentials (Ψm) between − 1.5 KPa (S_1), − 2.0 KPa (S_2),

− 4.0 KPa (S_3), − 6.0 KPa (S_4), − 8.0 KPa (S_5) and − 10.0 KPa (S_6), at the porous cup walls. As the plant roots absorb water a potential gradient was created and water moved from cup to soil. For this reason the value of soil matric potential (Ψm) at the treatment S_1 became equal − 6.0 KPa; at the treatment S_2 a soil Ψm of − 9.5 KPa, at the treatment S_3 a soil Ψm of − 70 KPa, at the treatment S_4 a soil Ψm of − 80 KPa, at the treatment S_5 and S_6 the soil Ψm exceeded − 85 KPa (the exact value has not being measured due to the limited range of the tensiometer (Figure 1).

The Ψm ranges to which plants were subjected with the increase of the head of water can be explained by analyzing the unsaturated water flow through the soil as described by the Darcy low: q = − K (Ψm).Δ Ψ/L, Where: q = water flow through soil; K (Ψm) = insaturated hydraulic conductivity, a function of matric potential; and Δ Ψ/L = hydraulic gradient. Unsaturated hydraulic conductivity decreases expotentially with soil moisture and consequently with Ψm.
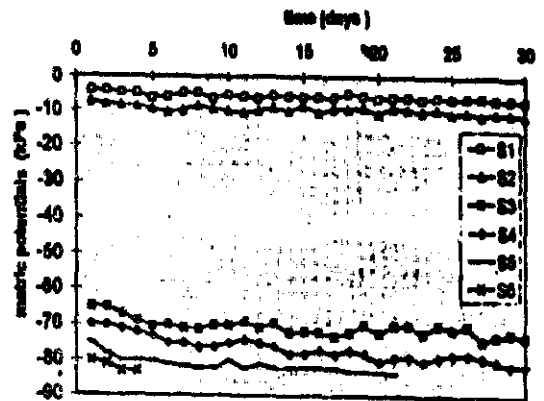

Figure 1. Matric potentials of the soil during 30 days, in respect to the different degrees of water stress *matric potentials exceeded −85 kPa).

Due to evapotranspiration, hydraulic gradient is therefore necessary in order to maintain water flow, and consequently an ever greater difference in Ψm to compensate the decrease in hydraulic conductivity. This explains the rising bands of Ψm as the head of water increases. Since the Ψm of the porous cup is fixed and equal to the head of water, it is necessary to reduce the soil Ψm in order to increase the hydraulic gradient.

Kramer (1963) and Knany et al. (2005) 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 (Ψw), but the levels of leaf water potential limiting plant growth are not generally known

Table 2. Leaf water potential (Ψw), leaf transpiration rate (T), and leaf diffusive resistance (Rf) in soybean withARC 517 and TAL 379 and without (C) *Bradyrhizobium* spp. inoculation at different degrees of water stress.

Matric potentials (1) (Kpa)	Ψw (Mpa)			T (m mol m^{-2} s^{-1})			Rf (s. cm^{-1})		
	Strains								
	ARC 517	TAL 379	C	ARC 517	TAL 379	C	ARC 517	TAL 379	C
S$_1$	-0.50 a	-0.47 a	-0.51 a	6.16 a	6.05 a	6.53 a	2.77 d	2.41 d	2.74 e
S$_2$	-0.57 a	-0.55 a	-0.61 ab	5.97 a	5.55 a	6.47 a	3.36 d	2.87 d	3.70 e
S$_3$	-0.75 ab	-0.70 ab	-0.78 b	1.80 b	1.96 b	2.17 b	7.59 c	6.90 c	7.45 d
S$_4$	-0.82 bc	-0.78 ab	-0.84 bc	1.79 b	1.77 b	1.84 bc	10.83 b	10.14 b	10.70 c
S$_5$	-1.02 c	-1.00 b	-1.05 cd	1.52 b	1.35 b	1.75 bc	19.13 a	18.16 a	18.23 b
S$_6$	-1.10 c	-1.07 b	-1.08 d	1.04 b	1.08 b	0.94 c	20.00 a	20.20 a	21.56 a
F (plot)	32.98**			465.30**			476.49**		
F (subplot)	2.85 n.s			2.67 n.s			2.19 n.s		
% CV (plot)	15.57			10.60			9.97		
% CV (subplot)	11.95			13.97			8.99		

(1) S$_1$ – 6.0 (well waterd control), S$_2$ – 9.5 (relatively watered control), S$_3$ – 70.0, S$_4$ – 80.0, S$_5$ and S$_6$ exceeded – 85.0 n.s

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.

and must be determined for each species or crop. The Ψw 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 2 it can be seen that there was a significant difference between the degrees of water stress in the soybean, however it presented no significance at the *F* test for the strains (subplot). The leaf water potential (Ψw) declined in water stressed plants reaching values of – 1.0 MPa, respectively

This response was in great contrast to the well-watered control plants (S$_1$), where transpiration increased with increase of the Ψw. The negative effects no the metabolism of N$_2$ assimilation occurred every time that Ψw fell below – 0.75 MPa as happened in the stressed treatments in accordance with the results of Boyer (1980); Stamford *et al.* (1990); Mokabel (2005) and Saad and Sinha (2005).

In this work there was a correlation among the leaf water potential (Ψw) vs. leaf transpiration rate(T) (*r* = 0.78**) by comparing the stressed plants with the control (S$_1$).Although, an increase in stomatal diffusive resistance (Rf) was observed in those plants subjected to water stress, but in contrast, the leaf transpiration rate

(T) felt 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 3- 7). The *Bradyrhizobium* strains introduced by inoculation in the soybean plants were superior to the indigenous strain demonstrating the importance of inoculation in the soybean stressed plants. At the more negative Ψm plants inoculated with the strain TAL 379 had the highest nitrogenase activity(12410 n mol C$_2$H$_4$ pl^{-1}h^{-1}) , nodule size(6.57mg nod.$^{-1}$), nitrogen content(27.33 mg N g^{-1} DM), shoot/root ratio(0.0498), and concentration of ureide-N(6099 n mol ml^{-1}) and leghaemoglobin (LHB)(29.70 mg g^{-1} nod. DM) , indicating that the strain was effective, helping the soybean plants to withstand the water stress better than the strain ARC 517 and the uninoculated control (C).

The results were found in the present work with soybean, showing a correlation between N$_2$ ase activity

vs. Ψw ($r = 0.77**$)were similar to findings of Walker and Miller (1986) and El-Sayed (2005)who found a reduction in the N_2 ase activity, number and weight of nodules, concluding that Ψw is related to N_2 fixation by the plants.

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; El-Sebsy, 2004 and Omara, 2004). In Table 3 the highest ureide-N concentration in the soybean plants was presented by strain TAL 379(6099 n mol ml^{-1})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_2 ase 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 and Abdel-Aziz et al.,2003).

The stress applied by porous cup strongly influenced N_2 fixation. However drought-stressed plants inoculated with strain TAL 379 maintained the LHb concentration until Ψm – 70 KPa (S_3), presented no significance ($P < 0.05$) in relation to the control Ψm – 6.0 KPa (S_1), as well as, conferred a differential degree of drought resistance in plants. Guerin et al. (1991) and El-Sayed and Ahmad (2003) suggested that the decline in nodule

N_2 fixation brought on by water stress may be due to significant declines in LHb. The correlation between LHb concentration and the N_2 ase activity was low ($r = 0.59**$). In this case changes in N_2 ase function can not be attributed to declining LHb.

Even at the more negative Ψm N_2 ase 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_2 ase 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 soybean appears to be directly related to a reduction in nodule mass (Table 4), (S_6) which may   have affected nodule structural constituents. However, in   stress (S_3) the impact on nodule water content was higher than on the changes in nodule mass.

As shown in Table 5 the strain TAL 379 inoculated in soybean presented a smaller number of nodules (4.43nr. Pot^{-1}), but the average size of the nodule was larger (4.69mg $nod.^{-1}$)than in the other treatments. This is an evidence that the strains introduced by inoculation in soybean, as well as the indigenous strain presented a good nodulation capacity and that the host plant benefited from nitrogen fixation.

Table3. Leghaemoglobin concentration (LHb), ureide N concentration (UN), and nitrogenase activity (N_2 ase) in soybean with ARC 517 and TAL 379 and without (C) *Bradyrhizobium* spp inoculation at different degrees of water stress.

Matric potentials [1] (KPa)	LHb (mg g^{-1} nod. DM)			UN (n mol ml^{-1})			N_2 ase (n mol C_2H_4 $pl^{-1}h^{-1}$)		
	Strains								
	ARC 517	TAL 379	C	ARC 517	TAL 379	C	ARC 517	TAL 379	C
S_1	26.16ab^A	28.06ab^A	23.00ab^B	5179a^A	5351ab^A	4000a^B	10697a^A	11274b^A	9288a^B
S_2	28.56a^A	29.70a^A	24.23a^B	5840a^A	6099a^A	4402a^B	11670a^A	12410a^A	9571a^B
S_3	25.40b^A	27.23ab^A	20.40bc^B	3740b^B	4488bc^A	2906b^C	2563b^B	3400c^A	2093b^B
S_4	25.40b^A	26.50b^A	20.40bc^B	3366bc^B	4114cd^A	2733b^B	2403b^{AB}	2633cd^A	1630bc^B
S_5	24.03b^A	25.90b^A	20.30bc^B	2819bc^B	3567cd^A	2388b^B	1130c^B	2277d^A	1000c^B
S_6	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 S_1 to S_6 see Table 2. *,** 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.

Table 4. Nodule dry matter (NDM) and nodule water content (NWC) in soybean with ARC 517 and TAL 379 and without (C) _Bradyrhizobium_ spp. inoculation at different degrees of water stress.

Matric potentials (1) (KPa)	NDM mg pot^{-1}			NWC (%)		
	Strains					
	ARC 517	TAL 379	C	ARC 517	TAL 379	C
S$_1$	402.66a^A	385.00a^A	333.33a^A	474.91a	452.03a	415.03a
S$_2$	495.00a^A	400.00a^B	380.00a^B	381.37a	393.97a	387.11a
S$_3$	230.00b^A	200.00b^{AB}	150.00b^B	127.15b	148.75b	130.14b
S$_4$	180.00bc^A	130.00bc^A	108.00bc^A	107.94b	148.51b	119.82b
S$_5$	90.00cd^A	75.33c^A	80.00bc^A	99.16b	125.79b	117.95b
S$_6$	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$^{n.s}$		
% CV (plot)	16.43			24.43		
%CV (subplot)	18.20			23.66		

For symbols see Tables 2 & 3.

Table 5. Shoot accumulation nitrogen (SAN), nodule size (NS), and nodule number (NN) in soybean with ARC 517 and TAL 379 and without (C) _Bradyrhizobium_ spp. inoculation at different degrees of wate stress.

Matric potentials (1) (KPa)	SAN (mg pot^{-1})			NS (mg nod.$^{-1}$)			NN (2) (nr. Pot^{-1})		
	Strains								
	ARC 517	TAL 379	C	ARC 517	TAL 379	C	ARC 517	TAL 379	C
S$_1$	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
S$_2$	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
S$_3$	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
S$_4$	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
S$_5$	124.69b^A	149.04b^A	107.15b^A	2.39ba^A	3.81b^A	2.95a^A	6.32c^A	4.44c^{AB}	5.42bc^B
S$_6$	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		

(1) For symbols see Tables2 &3.

(2) Values changed $\sqrt{x}$.

The N$_2$ ase activity, ureide-N and LHb concentration were superior in the S$_2$ treatment in relation to the S$_1$ treatment, but the shoot dry matter and leaf area (Table 6) were superior in the S$_1$ treatment in relation to the S$_2$ treatment, which indicates that the energy requirements for the N$_2$ fixation in the soybean can demand the dry matter reduction, which presented a decline of about 15%. The nitrogen fixation process demands a continuous supply of carbohydrates (Neves, 1981 and Abdel-Mawly and El-Sayed, 1999). However, the

Table 6. Shoot dry matter (SDM) and leaf area (LA) in soybean with ARC 517 and TAL 379 and without (C)
Bradyrhizobium **spp. inoculation at different degrees of water stress.**

Matric potentials (1) (KPa)	SDM (g pot^{-1})			LA (cm^2)		
	Strains					
	ARC 517	TAL 379	C	ARC 517	TAL 379	C
S$_1$	20.10a^{AB}	21.83a^A	18.63a^B	2903a^A	3205a^A	2488a^B
S$_2$	17.43b^A	18.73b^A	15.20b^B	2260b^B	2677b^A	2177a^B
S$_3$	5.29c^{AB}	6.45c^A	4.46c^B	990c^A	1023c^A	886b^A
S$_4$	4.68c^A	5.46c^A	4.27c^A	800c^A	887c^A	765b^A
S$_5$	4.66c^A	5.30c^A	4.10c^A	684c^A	767c^A	640b^A
S$_6$	4.34c^A	5.29c^A	3.76c^B	650c^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 Tables2 & 3.

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

The shoot dry matter and leaf area (Table 6) and accumulation nitrogen (Table 5) of the soybean 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 soybean 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 7) 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.

Table 7. Nitrogen content (NC) in soybean with ARC 517 and TAL 379 and without (C) *Bradyrhizobium* **spp. inoculation.**

Strains	NC (mg N g^{-1} DM)
ARC 517	26.28 ab
TAL 379	27.33 a
C	24.07 b
F (plot)	1.65 n.s
F (subplot)	4.40**
% CV (plot)	11.21
% CV (subplot)	13.00

For symbols see Tables 2 & 3.

In Tables 8 and 9 the contrast study in the treatments watered x 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, Ψw, leaf diffusive resistance, transpiration rate ($P < 0.01$) and nodule size ($P < 0.05$).

In relation to the watered treatments (contrast 2) did not show significant difference to Ψw, 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 in the soybean plants were superior to the indigenous strain demonstrating the importance of inoculation in the soybean stressed plants. At the more negative Ψm, plants inoculated with the strain TAL 379 formed associations of greater symbiotic efficiency helping the soybean plants to withstand water stress better than the strain ARC 517 and the uninoculated control. The LHb concentration was not inhibited in the drought-stressed plants at Ψm – 70 KPa when inoculated with the strain TAL 379, which conferred a differential degree of drought resistance in plants, compared to strain ARC 517. The Ψw declined in the droughted plants reaching values of – 1.0 MPa and was sufficient to cause disturbance in nodulation and biomass production.

Table 8. F-values from analysis of variance by contrast study, between watered x stressed treatment (contrast 1) and among the watered treatment (contrast 2), of leghaemoglobin (LHb) and ureide-N (UN) concentration, nitrogenase activity (N_2 ase), shoot nitrogen accumulation (SNA), leaf area (LA), and nodule (NDM) and shoot (SDM) dry matter, in soybean plants.

Variation source	LHb (mg g^{-1} nod.DM)	UN (n mol ml^{-1})	N_2 ase (n mol C_2H_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 n.s	53.43**	5.37*	9.62*	55.42**

For symbols see Tables 2 & 3.

Table 9. F-values from analysis of variance by contrast study, between watered x stressed treatment (contrast 1) and among the watered treatment (contrast 2), of leaf water potential (Ψw), leaf transpiration rate (T), leaf diffusive resistance (Rf), nodule number (NN), nodule size (NS), and nodule water content (NWC), in soybean plants.

Variation source	Ψw (MPa)	T (m mol $m^{-2}s^{-1}$)	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 n.s	2.46 n.s	1.82 n.s	0.62 n.s	0.53 n.s	5.61*

For symbols see Tables 2 & 3.

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الملخص العربي

تأثير إجهاد الماء لنباتات فول الصويا في وجود وغياب سلالات بريدي رايزوبيوم

سعيد عباس محمد السيد

نفذت تجارب لفحص تأثير درجات مختلفة من إجهاد الماء على نباتات فول الصويا صنف جيزة ٨٣ في تربة ذات قوام Sandy loam ، وذات pH = 7.3 في وجود وغياب سلالات بريدي رايزوبيوم وذلك لتقييم الإستجابات الفسيولوجية المختلفة لهذا الإجهاد عن طريق التحكم في :

matric potential (Ψm) بإستخدام a porous cup ثم تعريض النباتات لإجهاد مائي مستمر.

أوضحت النتائج ما يلي :

● أقل إجهاد لـ soil Ψm يؤثر تأثيرا مباشرا على النيتروجين المثبت حيويا.

● أوضحت سلالات بريدي رايزوبيوم للملقحة لنباتات فول الصويا أنها أكثر مقاومة لإجهاد الماء.

● عند زيادة سالبية Ψm للنباتات الملقحة بالسلالة TAL 379 أعطت كفاءة تكافلية مرتفعة ساعدت نباتات فول الصويا على مقاومة الجفاف عن السلالة ARC 517 مقارنة بالكنترول (بدون تلقيح).

● تركيز الهيموجلوبين كان غير مثبط عند إجهاد الجفاف للنباتات عند Ψm = −70 KPa عند التلقيح بالسلالة TAL 379 حيث تمنح النباتات درجات مختلفة من مقاومة إجهاد الجفاف.

● لإنخفاض قيمة Ψw إلى 1.0 MPa − سببت اضطرابا كافيا في قدرة النباتات على تكوين عقد بكتيرية وإنتاج خلايا حيوية.