Soybean tolerance to drought depends on the associated *Bradyrhizobium* strain

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Abstract

We evaluated the effect of three different *Bradyrhizobium* strains inoculated in two soybean genotypes (R01-581F, drought-tolerant, and NA5858RR, drought-sensitive) submitted to drought in two trials conducted simultaneously under greenhouse. The strains (SEMIA 587, SEMIA 5019 (both *B. elkanii*), and SEMIA 5080 (*B. diazoefficiens*)) were inoculated individually in each genotype and then submitted to water restriction (or kept well-watered, control) between 45 and 62 days after emergence. No deep changes in plant physiological variables were observed under the moderate water restriction imposed during the first 10 days. Nevertheless, photosynthesis and transpiration decreased after the severe water restriction imposed for further 7 days. Water restriction reduced growth (-30%) and the number of nodules (-47% and -58% for R01-581F and NA5858RR, respectively) of both genotypes, with a negative effect on N-metabolism. The genotype R01-581F inoculated with SEMIA 5019 strain had higher photosynthetic rates compared with NA5858RR, regardless of the *Bradyrhizobium* strain. On average, R01-581F showed better performance under drought than NA5858RR, with higher number of nodules (51 vs. 38 nodules per plant, respectively) and less accumulation of ureides in petioles (15 μmol g^{-1} vs. 34 μmol g^{-1}, respectively). Moreover, plants inoculated with SEMIA 5080 had higher glutamine synthetase activity under severe water restriction, especially in the drought-tolerant R01-518F, suggesting maintenance of N metabolism under drought. The *Bradyrhizobium* strain affects the host plant responses to drought in which the strain SEMIA 5080 improves the drought tolerance of R01-518F genotype.

Keywords Biological nitrogen fixation · Inoculation · Nodulation · Ureides · Water stress

Introduction

Climatic changes have been observed worldwide, with predictions of increase by 0.2 °C per decade in Earth's temperature and occurrence of extreme events such as torrential rains, heat or cold waves, tropical cyclones, and drought [1, 2], leading to negative effects on agricultural activities. The rainfall patterns are expected to decrease up to 20% by the end of century [1], with intensification of drought in normally rainy areas.

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Water restriction is one the most limiting abiotic factors to soybean (*Glycine max* L. Merrill) yield, particularly because the biological nitrogen fixation (BNF) is highly sensitive to drought, even more than transpiration and photosynthesis rates [3, 4]. Drought impairment of the BNF activity limits the plant N-supply and reduces grain yield.

Many studies have made efforts to characterize and select soybean genotypes tolerant to drought [3–5]. The genotypes R01-581F (PI 647961) and R01-416F (PI 647960) are considered tolerant [5] and show higher BNF activity and more N accumulation in their shoots under drought, in addition to higher grain yields compared with the parental "KS4895" [3]. This trait is also named nitrogen fixation drought tolerance (NFDT) [3–5]. The advantage of NFDT genotypes is attributed to less accumulation of ureides in the whole plant that could cause a feedback inhibition of the BNF [6].

In addition to the host genotype, differences among *Bradyrhizobium* strains for efficiency of BNF under adverse conditions have been evidenced [7, 8]. The diversity of

Bradyrhizobium has been studied [9, 10], and the selection of efficient and competitive strains adapted to local conditions is essential for an effective BNF [8, 9]. Different symbiotic traits have been observed among *Bradyrhizobium* species, in which *B. elkanii* is more competitive and forms more nodules than *B. japonicum* strains [7]. Within species, the strain SEMIA 5019 (= 29 W) of *B. elkanii* was more competitive in the Brazilian Cerrado soils than SEMIA 587 (also *B. elkanii*), which showed better performance in the southern Brazil [11]. Batista et al. [10] also reported intraspecific variations between *Bradyrhizobium* strains, where *B. japonicum* strain SEMIA 5079 (=CPAC 15) showed higher saprophytic capacity and competitiveness than SEMIA 5080 (=CPAC 7) under regular water supply in the Brazilian Cerrados.

In South Africa, under drought, the inoculation with *Sinorhizobium fredii* strain SMH12 promoted more nodulation of soybean than *B. diazoefficiens* strain WB74-1 [8]. *B. japonicum* strain CPAC 390 stimulated higher photosynthetic rates than CPAC 7 (=SEMIA 5080) in soybean due to sink stimulation [12]. However, little is known about the behavior of BNF traits promoted by *Bradyrhizobium* spp. in host plants exposed to drought. This work is pioneer in characterizing the performance of *Bradyrhizobium* strains employed in the production of inoculants for soybean in Brazil, under water restriction.

The aim of this study was to evaluate the effect of inoculation of three different *Bradyrhizobium* strains in two soybean genotypes contrasting in NFDT on traits related to plant physiology and BNF under water restriction. We hypothesized that the performance of the soybean genotype with NFDT under water restriction depends on the associated *Bradyrhizobium* strain.

Material and methods

Experimental design and installation

Two simultaneous experiments were performed under greenhouse with the soybean genotypes R01-581F, known as drought-tolerant by having NFDT [4, 5, 13], and NA5858RR, a drought-sensitive, non-NFDT genotype. The experimental designs were completely randomized, in 3×2 factorial arrangement, with six replications. The first factor was the inoculation with one of the three *Bradyrhizobium* strains: SEMIA 587 (*B. elkanii*), SEMIA 5019 (= 29 W) (*B. elkanii*), or SEMIA 5080 (=CPAC7) (*B. diazoefficiens*); the second factor consisted of exposure to water restriction between 45 and 62 days after emergence (DAE), or normal water supply during all growth period. Soybean genotypes were not considered a factor in the analysis.

Physiological attributes related to gas exchanges were assessed at the end of two periods of water restriction with different intensities: moderate (between 45 and 55 DAE), for acclimation, followed by severe (between 55 and 62 DAE), as will be further detailed. The intensity of water restriction was also not considered a factor in the analysis. Destructive assessments of plant growth and attributes related to BNF were made only once, by the end of severe water restriction at 63 DAE.

A soil sample from an agricultural site cropped with soybean, obtained at 0–20 cm depth (Typic Acrudox, USDA soil taxonomy), was used as a substrate, with the following characteristics: pH (CaCl₂) = 4.7; organic matter = 33 g kg⁻¹; P (Mehlich I) = 2.14 mg dm⁻³; K = 0.31 cmol꜀ dm⁻³; Ca = 4.02 cmol꜀ dm⁻³; Mg = 0.64 cmol꜀ dm⁻³; H + Al = 5.6 cmol꜀ dm⁻³; CEC = 10.5 cmol꜀ dm⁻³; particle sizes: sand = 732 g kg⁻¹, silt = 30 g kg⁻¹, clay = 238 g kg⁻¹. Dolomitic limestone was applied to raise pH to 6.5, and 2-kg aliquots were placed into plastic pots, watered, and incubated for 30 days. Before sowing, each pot received 27 mg of P, 158 mg of K (both as K_2HPO_4), 115 mg of Mg, 155 mg of S (both as $MgSO_4.7H_2O$), and 50 mL of micronutrient solution containing 0.0014 mg of $CoSO_4$, 0.0054 mg of Na_2MoO_4, and 0.5 g of H_3BO_3 in 5 L of water. N was provided by inoculating one of each strain previously grown in the YM broth (K_2HPO_4 0.5 g L⁻¹, $MgSO_4.7H_2O$ 0.2 g L⁻¹, NaCl 0.1 g L⁻¹, manitol 5.0 g L⁻¹, yeast extract 0.4 g L⁻¹), containing 1×10^9 viable cells mL⁻¹, 1 mL per seed. Each pot received three seeds that were thinned to one plantlet at 7 DAE.

During the trial, average night/day temperatures ranged between 18.4 and 32.9 °C, respectively, and the average day/night relative humidity ranged from 28 to 94%, respectively. The photosynthetically active radiation inside the greenhouse reached up to 1200 µmol m⁻² s⁻¹ photon flux density at noon in clear days.

Drought induction

For adjustment of soil moisture, the water-holding capacity was determined on a tension table and Richards's extractor device resulting in a water-retention curve correlating the water content and the soil water potential (ψ_w). During the first 45 DAE, all plants received water to maintain the ψ_w at − 13 kPa (300 mL of water per L of soil), which represent a fraction of available water (FAW) in the soil of 0.9. After 45 DAE, during the flowering stage (R1-R2), the plants to be submitted to water restriction were initially subjected to moderate water restriction for 10 days for acclimation, keeping the ψ_w at − 200 kPa (90 mL of water per L of soil), with a FAW of 0.27. The control plants under normal water supply continued to receive water to keep the ψ_w at − 13 kPa. After 10 days under moderate condition, the water restriction was intensified to severe water restriction for further 7 days, receiving water only to reach ψ_w at − 500 kPa (60 mL of water per L of soil),

corresponding to a FAW of 0.18. Soil moisture was monitored daily by weighing each pot on an electronic scale, with correction of moisture in the morning (between 8 and 10 a.m.). We considered the fresh mass of plants at well-watered condition at 45 DAE from extra pots to correct the effect of the plant weight on water reposition in the pots containing plants subjected to water restriction.

Physiological analysis

On the 10th and 17th days under water restriction (moderate and severe, respectively), physiological parameters were measured in both stressed and non-stressed plants with a portable gas exchange meter, model LI-6400 (Li-Cor, Biosciences Inc., Nebraska, USA). Determinations included net photosynthetic (A) and transpiration (E) rates, stomatal conductance (g_s), intercellular CO_2 concentration (Ci), and temperature of leaves. Gas exchanges were assessed in the central foliolate of the third recently expanded trifoliolate in the morning (9–11 a.m.).

N-metabolism traits

The experiments were harvested at 63 DAE; root samples containing nodules were immediately frozen in liquid N_2 and stored at − 80 °C for assessment of glutamine synthetase (GS) activity [14] in extracts prepared using a Sephadex G-25 column [15]. Shoots and the remaining nodulated roots were dried at 60 °C for 48 h for determination of the shoot dry weight, number, and dry weight of nodules; the shoot N concentration was determined in sulfuric extracts by the green salicylate colorimetric method [16] and then converted into shoot total N content based on the shoot dry biomass. The concentrations of ureides were determined, in dried petioles and nodules, after extraction [15] based on the colorimetric method of Vogels and van der Drift [17].

Statistical analysis

The datasets were submitted to tests of normality and homogeneity of variances for each experiment, followed by ANOVA with application of F test at $p \leq 0.05$. Once the effects of treatments or interactions between factors were detected, means were compared by Tukey's test at $p \leq 0.05$.

Results

Physiological parameters

Water restriction and inoculation affected net photosynthesis, gas exchanges, and leaf temperature in the NFDT genotype R01-581F (Table 1). Inoculation with SEMIA 5019 increased the net photosynthesis under well-watered conditions compared with the other two strains. However, no differences were found among strains on photosynthesis under dry conditions. Severe water restriction reduced the photosynthetic rate, regardless of the strain. Transpiration rate did not change under moderate but dropped sharply under severe water restriction to less than 1% of the wet control plants. Inoculation with SEMIA 5019 increased the transpiration rate, in the average of water status. Water restriction decreased stomatal conductance, especially under severe restriction. Inoculation with SEMIA 5019 promoted higher stomatal conductance under wet conditions compared with plants inoculated with SEMIA 587 or SEMIA 5080. Intercellular CO_2 decreased by 10% under moderate stress in the average of strains. Under severe stress, the CO_2 concentration was twice as high in plants inoculated with SEMIA 5019. The leaf temperature increased with water restriction in both drought severity levels, but inoculation with SEMIA 5019 decreased leaf temperature compared with plants inoculated with SEMIA 587 or SEMIA 5080. There was no effect of strains on leaf temperature of the well-watered control plants.

Considering the drought-sensitive genotype N5858RR (Table 2), the water status did not affect the net photosynthetic rate under moderate stress, but SEMIA 5080 increased this trait in the average of water conditions compared with SEMIA 5019. Severe stress decreased net photosynthesis, regardless of the strain, but inoculation with SEMIA 5080 under wet conditions increased the photosynthetic rate compared with SEMIA 5019. Transpiration rate was not affected under moderate water restriction, but severe restriction reduced it sharply, regardless of the *Bradyrhizobium* strains, which had no effect on this trait. Stomatal conductance reduced by 33% under moderate and almost 100% under severe water stress, with no effect of *Bradyrhizobium* strains. Intercellular CO_2 concentration was reduced by 15% under moderate stress regardless of the strain. However, under severe water restriction, SEMIA 5019 led to the highest Ci while SEMIA 5080 to the lowest. There was no effect of strains under wet conditions on Ci. There was an increase in leaf temperature by 1.3 and 4.5 °C under moderate and severe water restriction, respectively, regardless of the *Bradyrhizobium* strain.

Plant biomass, nodulation, and N in shoots

Water restriction negatively affected plant biomass and nodulation in both genotypes (Fig. 1). In R01-581F, stressed plants accumulated 35% less shoot biomass compared with well-watered plants, in the average of strains (Fig. 1a). Drought stress limited the number of nodules by 47% less, in the average of the strains, as compared with well-watered plants, in which plants inoculated with SEMIA 587 nodulated almost 15% more than plants inoculated with the other two strains. However, there was no effect of strains on the number of

Table 1 Net photosynthesis, gas exchanges, and leaf temperature of R01-581F drought-tolerant genotype inoculated with different *Bradyrhizobium* strains, submitted to moderate water restriction (during 10 days) and severe (during 7 days), between 45 and 62 days after emergence

Net photosynthetic rate, A (μmol CO_2 m^{-2} s^{-1})						
	Moderate water restriction			Severe water restriction		
Strain	Dry	Wet	Average	Dry	Wet	Average
SEMIA 587	17.8 aA	18.5 bA	18.1	0.58 aB	12.1 cA	6.31
SEMIA 5019	18.3 aB	20.9 aA	19.6	0.54 aB	18.3 aA	9.42
SEMIA 5080	18.4 aA	17.7 bA	18.0	0.12 aB	14.6 bA	7.35
Average	18.2	19.1		0.41	15.04	
Transpiration rate, E (mmol H_2O m^{-2} s^{-1})						
	Moderate water restriction			Severe water restriction		
Strain	Dry	Wet	Average	Dry	Wet	Average
SEMIA 587	2.82	2.96	2.89 a	0.04	3.19	1.62 ab
SEMIA 5019	2.69	3.44	3.07 a	0.09	3.98	2.03 a
SEMIA 5080	2.96	3.00	2.99 a	0.04	3.18	1.57 b
Average	2.83 A	3.13 A		0.03 B	3.45 A	
Stomatal conductance, g_s (mol H_2O m^{-2} s^{-1})						
	Moderate water restriction			Severe water restriction		
Strain	Dry	Wet	Average	Dry	Wet	Average
SEMIA 587	0.301 aA	0.341 bA	0.321	0.015 aB	0.261 bA	0.137
SEMIA 5019	0.261 aB	0.452 aA	0.362	0.005 aB	0.386 aA	0.195
SEMIA 5080	0.272 aA	0.313 bA	0.292	0.000 aB	0.278 bA	0.139
Average	0.278	0.369		0.007	0.308	
Intercellular CO_2 concentration, Ci (μmol CO_2 mol^{-1})						
	Moderate water restriction			Severe water restriction		
Strain	Dry	Wet	Average	Dry	Wet	Average
SEMIA 587	265	289	277 a	268 bA	194 aA	281
SEMIA 5019	265	308	286 a	548 aA	289 aB	419
SEMIA 5080	265	291	278 a	277 bA	280 aA	279
Average	265 B	296 A		364	288	
Temperature of leaves (°C)						
	Moderate water restriction			Severe water restriction		
Strain	Dry	Wet	Average	Dry	Wet	Average
SEMIA 587	30.7 aA	29.1 aB	29.9	34.1 aA	30.3 aB	32.2
SEMIA 5019	28.1 bB	29.3 aA	28.7	31.7 bA	29.7 aB	30.7
SEMIA 5080	30.6 aA	29.5 aB	30.1	33.8 aA	30.1 aB	31.9
Average	29.8	29.3		33.2	30.1	

Means followed by the same letter, capital in lines and small in columns, do not differ from one another (Tukey, $p < 0.05$) ($n = 6$). Comparison within each factor level denotes significant interaction; comparison in the average of one factor denotes isolated effect

nodules under water restriction (Fig. 1c). The nodule dry weight followed the same trend as the number of nodules, with 54% less in the average of strains as a consequence of water restriction (data not shown).

NA5858RR genotype accumulated by 30% less biomass under water restriction (Fig. 1b) compared with normal water supply, in the average of the strains. Inoculation with SEMIA 5019 increased the shoot dry weight in well-watered condition compared with SEMIA 587; under water restriction, there was no significant effect of strains on plant biomass. The number of nodules also decreased with drought (Fig. 2d). Under water

restriction, plants inoculated with SEMIA 587 had more nodules than plants inoculated with SEMIA 5019. Water restriction limited nodulation by 44% in plants inoculated with SEMIA 587, whereas plants inoculated with SEMIA 5019 and SEMIA 5080 had 71% and 59% less nodulation, respectively, than the well-watered counterparts. Water restriction also limited the nodule dry weight by 47% less compared with well-watered plants, in the average of strains (data not shown).

There was no interaction between water condition and strains for total shoot nitrogen content in the shoots of R01-581F and NA5858RR genotypes. There was only a

Table 2 Net photosynthesis, gas exchanges, and leaf temperature of NA5858RR drought-sensitive genotype inoculated with different *Bradyrhizobium* strains, submitted to moderate water restriction (during 10 days) and severe (during 7 days), between 45 and 62 days after emergence

Net photosynthetic rates, A (μmol CO_2 m^{-2} s^{-1})

	Moderate water restriction			Severe water restriction		
Strain	Dry	Wet	Average	Dry	Wet	Average
SEMIA 587	17.1	19.4	18.2 ab	0.22 aB	16.1 abA	8.13
SEMIA 5019	17.0	17.7	17.2 b	0.07 aB	15.3 bA	7.68
SEMIA 5080	19.3	20.3	19.8 a	− 0.23 aB	16.8 aA	8.30
Average	17.8 A	19.1 A		0.02	16.1	

Transpiration rates, E (mmol H_2O m^{-2} s^{-1})

	Moderate water restriction			Severe water restriction		
Strain	Dry	Wet	Average	Dry	Wet	Average
SEMIA 587	2.99	3.36	3.18 a	0.02	3.82	1.92 a
SEMIA 5019	3.08	3.32	3.20 a	0.09	3.46	1.78 a
SEMIA 5080	3.47	3.57	3.52 a	0.15	4.00	2.10 a
Average	3.18 A	3.42 A		0.09 B	3.76 A	

Stomatal conductance, g_s (mol H_2O m^{-2} s^{-1})

	Moderate water restriction			Severe water restriction		
Strain	Dry	Wet	Average	Dry	Wet	Average
SEMIA 587	0.281	0.443	0.362 a	0.000	0.378	0.19 a
SEMIA 5019	0.289	0.382	0.335 a	0.003	0.399	0.20 a
SEMIA 5080	0.290	0.455	0.372 a	0.003	0.390	0.20 a
Average	0.287 B	0.427 A		0.002 B	0.389 A	

Intercellular CO_2 concentration, Ci (μmol CO_2 mol^{-1})

	Moderate water restriction			Severe water restriction		
Strain	Dry	Wet	Average	Dry	Wet	Average
SEMIA 587	273	330	301 a	283 bA	302 aA	293
SEMIA 5019	271	315	293 a	387 aA	279 aB	334
SEMIA 5080	283	309	296 a	231 cB	279 aA	255
Average	276 B	318 A		300	287	

Temperature of leaves (°C)

	Moderate water restriction			Severe water restriction		
Strain	Dry	Wet	Average	Dry	Wet	Average
SEMIA 587	29.7	28.6	29.2 a	34.2	29.8	32.0 a
SEMIA 5019	30.8	28.9	29.8 a	34.4	29.9	32.2 a
SEMIA 5080	29.8	28.8	29.3 a	34.5	29.7	32.1 a
Average	30.1 A	28.8 B		34.3 A	29.8 B	

Means followed by the same letter, capital in lines and small in columns, do not differ from one another (Tukey, $p < 0.05$) ($n = 6$). Comparison within each factor level denotes significant interaction; comparison in the average of one factor denotes isolated effect

significant effect of water condition, with less accumulated N under drought by 32% and 28% for R01-581F and NA5858RR, respectively, compared with the well-watered plants (Fig. 1e, f).

Ureides concentration and GS activity

Ureides concentration in petioles of R01-581F varied with *Bradyrhizobium* strains only under normal water supply (Fig. 2a). In this case, plants inoculated with SEMIA 5080 had 41 and 27% higher concentration of ureides than plants inoculated with SEMIA 587 and SEMIA 5019, respectively.

Under water restriction, the concentration of ureides in petioles remained steady among strains. Considering the water condition, plants inoculated with SEMIA 587 showed 27% higher concentrations under water restriction; plants inoculated with SEMIA 5019 had similar concentrations in both water conditions; and plants inoculated with SEMIA 5080 had 27% lower concentration of ureides under water restriction. Considering the NA5858RR genotype, the concentration of ureides in petioles of plants under water restriction increased to 89%, 42%, and 49% when inoculated with SEMIA 587, SEMIA 5019, and SEMIA 5080, respectively (Fig. 2b). Plants inoculated with SEMIA 5080, however, had higher

Fig. 1 Shoot dry weight (**a** and **b**), number of nodules (**c** and **d**), and total shoot N content (**e** and **f**) in drought-tolerant R01-581F and drought-sensitive NA5858RR soybean genotypes inoculated with different *Bradyrhizobium* strains (SEMIA 587, SEMIA 5019, or SEMIA 5080) submitted to moderate (during 10 days) and severe (during 7 days) water restriction between 45 and 62 days after emergence. Means followed by the same letters do not differ from one another (Tukey, $p < 0.05$); capital letters compare genotypes at wet condition; small letters compare genotypes at dry condition. *Significant effect between water condition in each genotype. Vertical bars represent the standard deviation ($n = 6$ for **a**, **b**, **e**, and **f**; $n = 4$ for **c** and **d**)
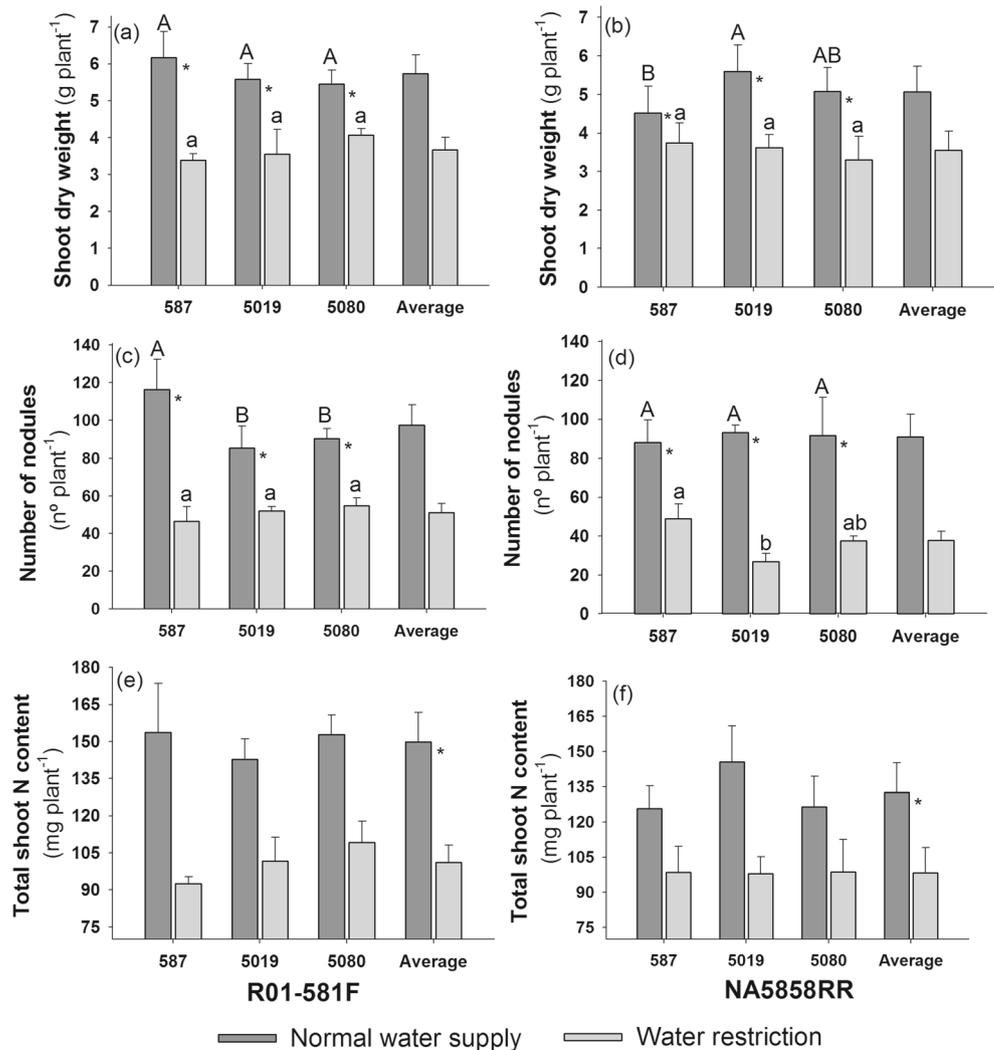
concentrations of ureides in petioles in both water conditions, over 50% the average between SEMIA 587 and SEMIA 5019.

The concentration of ureides in nodules of the R01-581F increased under water restriction, independently of the inoculated strain (Fig. 2c). At normal water supply, plants inoculated with SEMIA 5019 and SEMIA 5080 showed 66% more concentrations of ureides in nodules than plants inoculated with SEMIA 587. Under water restriction, concentrations were higher in plants inoculated with SEMIA 5080, i.e., 50% and 27% higher than in plants inoculated with SEMIA 587 and SEMIA 5019 strains, respectively. The relative increase in the concentration of ureides in nodules under water restriction compared with normal water supply was 276%, 104%, and 129% for inoculation with SEMIA 587, SEMIA 5019, and SEMIA 5080, respectively. For the NA5858RR, the drought-sensitive genotype, the concentration of ureides in nodules also increased under water restriction, but at a lesser extent than the NFDT genotype. Plants inoculated with SEMIA 587 and SEMIA 5019 had increases in ureides in nodules by 19 and 51%, respectively; however, plants

inoculated with SEMIA 5080 had no significant increase due to water restriction. There was no significant effect of *Bradyrhizobium* strains under normal water supply on the concentration of ureides in nodules (Fig. 2d).

The activity of GS in nodules differed according to the inoculated strain in both soybean genotypes (Fig. 2e and f); in R01-581F, at normal water supply, plants inoculated with SEMIA 587 and SEMIA 5019 (both *B. elkanii*) showed GS activity 37% higher than plants inoculated with SEMIA 5080 (Fig. 2e). Under water restriction, plants inoculated with SEMIA 587 and SEMIA 5080 showed GS activity 18% higher than plants inoculated with SEMIA 5019. Considering the water condition, plants inoculated with SEMIA 587 did not differ between dry and wet conditions; plants inoculated with SEMIA 5019 dropped the GS enzyme activity by 23%, whereas plants inoculated with SEMIA 5080 had the GS activity increased by 55% under drought.

For NA5858RR under normal water supply, the inoculation with SEMIA 5019 and SEMIA 5080 resulted in higher activity of GS by 21% over the plants inoculated with SEMIA

Fig. 2 Ureides in petioles (**a** and **b**) and nodules (**c** and **d**) and glutamine synthetase activity in nodules (**e** and **f**) in drought-tolerant R01-581F and drought-sensitive NA5858RR soybean genotypes inoculated with different *Bradyrhizobium* strains (SEMIA 587, SEMIA 5019, or SEMIA 5080) submitted to moderate (during 10 days) and severe (during 7 days) water restriction between 45 and 62 days after emergence. Means followed by the same letters do not differ from one another (Tukey, $p < 0.05$); capital letters compare genotypes at wet condition; small letters compare genotypes at dry condition. *Significant effect between water condition in each genotype. Vertical bars represent the standard deviation ($n = 6$ for **a** and **b**; $n = 4$ for **c**, **d**, **e**, and **f**)
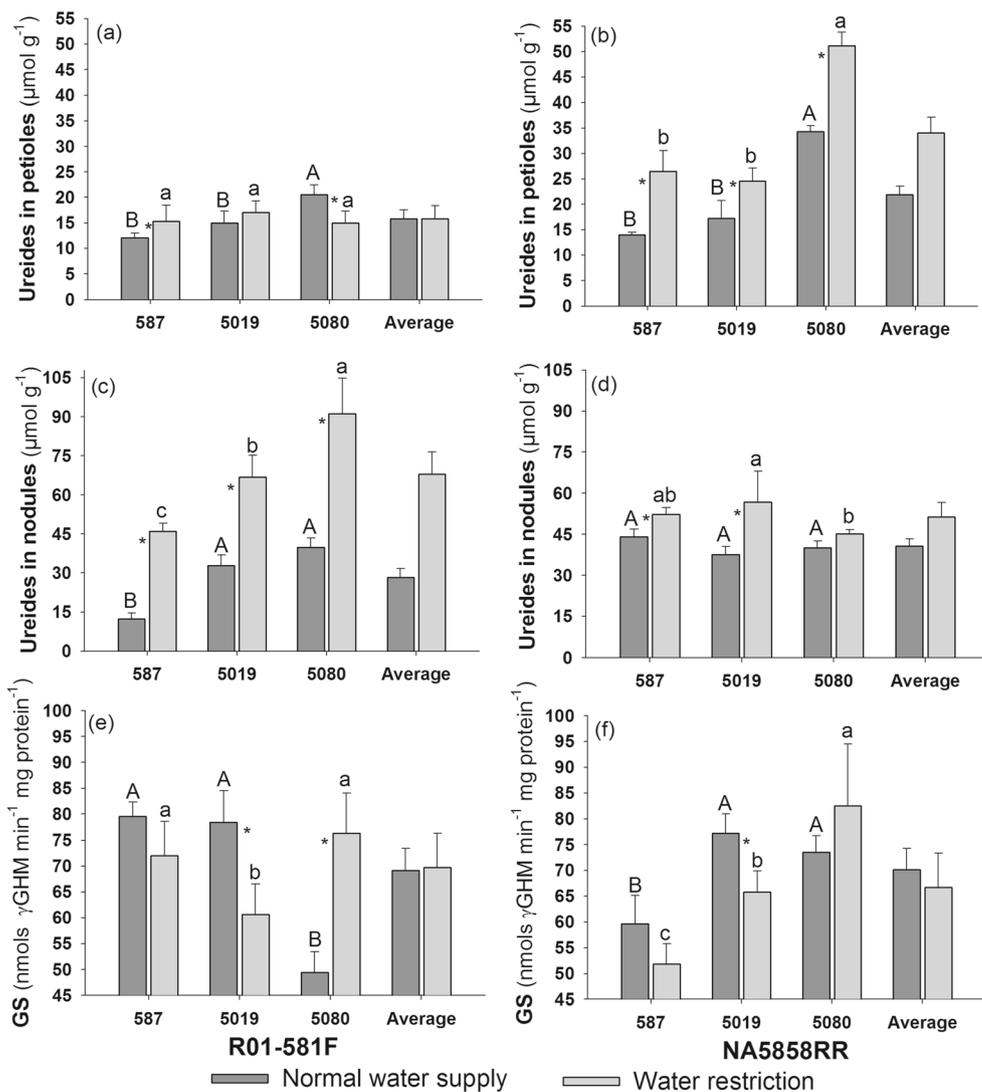
587 (Fig. 2f). Under water restriction, plants inoculated with SEMIA 5080 had GS activity 37% and 20% higher than plants inoculated with SEMIA 587 and SEMIA 5019, respectively. Considering the water condition, only plants inoculated with SEMIA 5019 had 15% reduction in GS activity under drought, compared with plants under normal water supply. Plants inoculated with SEMIA 587 and SEMIA 5080 did not show significant changes in the GS activity under drought.

Discussion

Efforts have been made to understand and select soybean genotypes with ability to keep their physiological processes and BNF under drought [3, 4, 6, 8]. Advantages among soybean lines regarding the ability to deal with drought by means of several mechanisms have been observed. Genotypes like R01-581F, R01-416F, and R02-1325 have NFDT; PI471937 presents limited transpiration under high-vapor pressure deficit; and PI471938 shows a slow-wilting phenotype. These genotypes have been tested in multi-disciplinary research programs aiming at increasing the stability of grain yield under drought [3, 4, 13].

The genotype R01-581F was evaluated during 7 years in the field in 28 environments, in six sites in Arkansas, Florida, and North Carolina, USA, and showed to be capable of maintaining high yield potential under non-irrigated conditions due to sustained nitrogen fixation under drought [3, 5]. Cerezini et al. [4, 13], in a study under moderate water restriction, observed that R01-581F can slow down the drought effects in the whole plant, keeping photosynthetic rates and N metabolism, despite negative effect of water restriction on nodulation. However, the symbiosis between soybean and different *Bradyrhizobium* strains may respond differently to environmental conditions [7–11, 18]. Depending on the host plant genotype, as observed in this trial, inoculation of *B. elkanii*

SEMIA 5019 resulted in better gas exchanges in the drought-tolerant R01-581F under severe water restriction than in the drought-sensitive NA5858RR host.

Previous studies reported different competitiveness and BNF effectiveness among *Bradyrhizobium* strains [10] and more effective nodulation of soybean by *S. fredii* strain SMH12 than by *B. diazoefficiens* strain WB74-1 under drought [8]. The most effective strain of *B. japonicum* in fixing N_2 increased the soybean photosynthetic rate to compensate the higher C sink [12]. However, the response to the strain may change with the soybean genotype as observed under wet conditions, when SEMIA 5019 stimulated the photosynthetic rates over plants inoculated with the other two strains in the drought-tolerant genotype R01-518F, but not in the drought-sensitive NA5858RR.

Sinclair and Nogueira [19] highlight that the role of the host plant in regulating the BNF activity has usually been neglected, and breeding programs have disregarded plant traits that might be related to increased N_2 fixation capacity, regardless of the water condition. Recent research on legume N_2 fixation showed that the host plant has a dominant role in regulating N_2 fixation [4, 12, 19] that is in agreement with our study wherein the N_2 fixation activity varies with the host, highlighting the importance of selecting plant genotypes that have more affinity with the microsymbiont and that promote greater protection of FBN, especially under drought.

Considering the gas exchanges, physiological plant responses to the strains varied with the water condition (wet or dry) and with the intensity of water restriction (moderate or severe). Under normal water supply, stomatal conductance increased in the drought-tolerant R01-581F genotype inoculated with SEMIA 5019. Moderate water restriction had no effects on gas exchanges, but severe restriction resulted in higher intercellular CO_2 in both genotypes inoculated with SEMIA 5019, which also promoted general increase of transpiration rate in the drought-tolerant R01-581F.

On the other hand, no effect of strains was observed in the drought-sensitive genotype NA5858RR under severe water restriction. Higher transpiration rate may have decreased leaf temperature in the R01-581F inoculated with SEMIA 5019, indicating that plants are transpiring and releasing heat with the transpiration flow, helping to maintain adequate leaf temperature to keep physiological processes [20].

Transpiration rate and transport of solutes across the plant are physiological processes strongly related. Thus, the increase in physiological capacity in R01-581F plants promoted by SEMIA 5019 may reflect in better performance and yield. Although SEMIA 5019 and SEMIA 587 belong to same species, gas exchanges in the host plant varied with the different strains. Kaschuk et al. [12] reported different effectiveness of BNF by two *B. japonicum* strains, where nitrate-fertilized plants had the lowest rates of photosynthesis and the highest

concentration of starch. Plants inoculated with the more efficient N-fixing strain CPAC 390 had higher photosynthesis rates and lower starch concentration than plants inoculated with the less effective strain CPAC 7.

R01-581F genotype kept photosynthetic and transpiration rates and stomatal conductance under moderate stress, but the intercellular CO_2 dropped in this condition. Consumption of CO_2 in the stomatal chamber via photosynthesis and limited influx of CO_2 through stomatal pore [21] due to beginning of stomatal closure may have been enough to start to affect the CO_2 assimilation. Reduction of stomatal conductance in soybean is associated to increasing levels of abscisic acid (ABA) in leaves as a strategy to cope with drought. As stomatal closure acts to save water at the beginning of water restriction, the crops may take a longer period before running severe dehydration [20, 22]. However, stomatal conductance reduction by 33% decreased the photosynthesis by 14% under high atmospheric vapor pressure deficit [20].

In our study, despite a reduction by 42% in stomatal conductance under moderate water stress, the photosynthesis dropped only 12% in R01-581F inoculated with SEMIA 5019, showing that this genotype-strain combination kept the photosynthetic activity at higher level. On the other hand, moderate water restriction reduced stomatal conductance by 33% in the drought-sensitive genotype NA5858RR, regardless of the inoculated strain, but the effect in photosynthesis was only 7%, not significant.

A general overview indicates a more negative effect of severe water restriction on the drought-sensitive genotype NA5858RR. The significant reduction of stomatal conductance, intercellular CO_2, and increase of leaf temperature under moderate water restriction did not affect the photosynthetic and transpiration rates. Under these conditions, the increase of photorespiration may act as a protective mechanism of the photosynthetic apparatus, acting in the dissipation of excessive energy, but at a high energetic cost to the plant [23]. Under severe water restriction, the negative net photosynthesis in NA5858RR inoculated with SEMIA 5080 indicates more production of CO_2 (respiration) than assimilation (photosynthesis).

Both NA5858RR, drought-sensitive, and R01-581F, tolerant, inoculated with SEMIA 5019 had increased intercellular concentrations of CO_2 under severe water restriction. The increase of intercellular concentration of CO_2 may result from damage to the photosynthetic apparatus, resulting from formation of reactive oxygen species, decrease of Rubisco activation, limited transport of electrons, and oxidation of photosynthetic pigments [21], causing accumulation of CO_2. In addition, as N is essential for synthesis of Rubisco and chlorophylls, the reduction of shoot N accumulation in both genotypes due to water restriction also contributed for impairment in C

metabolism, once C and N metabolisms are correlated [12].

Water restriction impaired not only physiological parameters and accumulation of biomass, but nodulation was also sensitive. In a previous study, the exposition to moderate drought for 10 days reduced the number and mass of nodules by 12% and 33%, respectively [4]. Despite reduction by 50% in number of nodules in our study, inoculation with SEMIA 587 promoted more nodulation in R01-581F under normal water supply and in NA5858RR under drought. Different nodulation rates among rhizobial strains have been previously observed [8]. Scholles et al. [18] reported that SEMIA 587 was more efficient in nodulation and nitrogen fixation and more tolerant to herbicides applied to soybean than SEMIA 5074 and SEMIA 5079 (both *B. japonicum*). However, higher nodulation was not translated into either root or shoot biomass, as also observed in our study. The exposure to drought was the strongest driver leading to reduction in nodulation, emphasizing the negative effect of drought on the BNF [3, 4, 6, 8, 13], regardless of the rhizobial strains.

The drought-tolerant R01-581F genotype showed slightly higher nodulation than NA5858RR, in both water conditions. Under drought, in the average of the strains, R01-581F had 51 nodules per plant, whereas NA5858RR only had 38. In particular, drought decreased the nodulation of the drought-sensitive NA5858RR inoculated with SEMIA 5019, but with no further consequences on shoot N accumulation. A general decrease in total shoot N content occurred in both genotypes exposed to drought, regardless of the inoculated strain. Many studies have confirmed the sensitivity of the BNF in soybean to drought, but variation may occur among genotypes [3, 4, 6, 13].

Both genotypes inoculated with SEMIA 5080 (*B. diazoefficiens*) showed higher concentration of ureides in petioles under wet condition, suggesting more effective symbiotic performance [4]. This strain is one of the most efficient in N-fixation and is largely used in commercial inoculants in Brazil since 1992 [24, 25]. Moreover, the NFDT genotype R01-581F inoculated with SEMIA 5080, and also SEMIA 5019, did not show increase of ureides in petioles under drought, which would be harmful to the BNF [6]. In addition, R01-581F showed only a slight increase of ureides in petioles due to exposure to drought compared with NA5858RR. This is an important feature in plants having NFDT phenotype, showing that ureides continue to be metabolized in the shoots even under drought [3, 4, 6]. In addition, the non-NFDT NA5858RR inoculated with SEMIA 5080 kept the concentration of ureides in nodules under water restriction, suggesting better performance of N-metabolism.

The more evident increase in the concentration of ureides in petioles of NA5858RR and in the nodules of R01-581F suggests failures, respectively, in the metabolism and in the transport of N-compounds caused by reduction of sap flow due to decrease in the transpiration rate under water restriction [26]. Accumulation of ureides impairs not only the N supply to the shoots but also the nodule development and function. Increase of ureides may also occur as consequence of remobilization by catabolism of purines [27, 28], a strategy for adaptation induced by stress, leading to suggest, based on only minor changes in ureides in petioles, that the drought-tolerant R01-581F better supported the stress caused by water restriction. Brychkova et al. [27] suggest that the accumulation of ureides may act in cell protection under oxidative stress. The higher concentration of ureides in nodules of R01-581F compared with NA5858RR under water restriction, especially when inoculated with SEMIA 5080, can also be attributed to more effective BNF and N metabolism.

GS-GOGAT is considered the major pathway for ammonia assimilation in soybean under normal growth conditions [15]. Drought and high temperatures generally decrease the GS activity [29], impairing the N assimilation in nodules, and leading to accumulation of ureides. Whereas the inoculation with SEMIA 5019 decreased GS activity in both genotypes under drought, plants inoculated with SEMIA 5080 showed higher activity, confirming that the N metabolism is differentially affected by the inoculated *Bradyrhizobium* strain. Despite no significant variations in terms of accumulated N and shoot biomass in both genotypes, these results provide evidences of interactions between host genotype and *Bradyrhizobium* strains in terms of N metabolism.

Despite the impairment of both genotypes due to water restriction, nodulation, ureides in petioles, shoot total-N, and GS activity suggest a more effective BNF process in R01-581F compared with NA5858RR under both water conditions. The strain SEMIA 5019 (*B. elkanii*) improved photosynthesis and stomatal conductance under normal water supply when inoculated in R01-581F. However, SEMIA 5080 (*B. diazoefficiens*) had higher a symbiotic performance based on higher GS activity under drought and concentration of ureides in petioles of well-watered plants of both genotypes. Thus, the best combination between soybean genotype and *Bradyrhizobium* strain is R01-581F and SEMIA 5080. The knowledge on the symbiotic performance of commercial *Bradyrhizobium* strains inoculated on soybean genotypes contrasting for NFDT can be useful to develop strategies to improve the BNF effectiveness to cope with the negative effect of drought on soybean.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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