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Physiological and N_2-fixation-related traits for tolerance to drought in soybean progenies

Paula Cerezini[1],
Biana Harumi Kuwano[1],
Walkyria Neiverth[1],
Anna Karolina Grunvald[2],
Antonio Eduardo Pípolo[3],
Mariangela Hungria[3] and
Marco Antonio Nogueira[3 ✉]

[1] Universidade Estadual de Londrina,
Departamento de Agronomia,
Rodovia Celso Garcia Cid, PR-445, Km 380,
Caixa Postal 10011,
CEP 86057-970 Londrina, PR, Brazil.
E-mail: paulacerezini@yahoo.com.br,
bianakuwano@hotmail.com,
walkybio@yahoo.com.br

[2] Conselho Nacional de Desenvolvimento
Científico e Tecnológico, SHIS QI 01,
Edifício Santos Dumont, Lago Sul,
CEP 71605-001 Brasília, DF, Brazil.
E-mail: annakgrunvald@gmail.com

[3] Embrapa Soja, Rodovia Carlos João Strass,
s/nº, Caixa Postal 231, CEP 86001-970
Londrina, PR, Brazil.
E-mail: antonio.pipolo@embrapa.br,
mariangela.hungria@embrapa.br,
marco.nogueira@embrapa.br

✉ Corresponding author

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Abstract – The objective of this work was to evaluate six soybean (*Glycine max*) genotypes for physiological traits and biological nitrogen fixation in drought conditions, and their capacity for recovery after rewetting, based on yield components. The genotypes evaluated in a greenhouse were the following: 'BRS 317', susceptible to drought; R01-581F and R02-1325, which show biological nitrogen fixation tolerant to drought; and the BRB14-207525, BRB14-207526, BRB14-207527 breed lines resulting from crossings between commercial genotypes and PI 471938, which shows a slow-wilting phenotype under drought. Drought conditions were applied to the genotypes at the full-flowering stage (R2) for ten days, whereas control plants were kept well-watered. Photosynthetic rate and gas exchanges under drought were more stable in the BRB14-207526 genotype. Root biomass increased 3.5 times in R02-1325 as an adaptive response to drought. In addition, leaf ureides – a trait related to biological nitrogen fixation in plants tolerant to drought – remained stable in genotypes R02-1325 and R01-581F exposed to drought, but decreased in BRB14-207527. Genotypes BRB14-207526, BRB14-207527, and R02-1325 show more favorable physiological performances and a lower accumulation of ureides under drought, besides a higher grain yield after rehydration. The BRB14-207526 and BRB14-207527 progenies show drought-tolerance traits from PI 471938, whereas R02-1325 is a promising source of tolerance to drought for soybean breeding.

Index terms: *Bradyrhizobium*, glutamine synthetase, photosynthesis, rewetting, ureides.

Características fisiológicas e relativas à fixação biológica de N_2 para tolerância à seca em progênies de soja

Resumo – O objetivo deste trabalho foi avaliar seis genótipos de soja (*Glycine max*) quanto aos parâmetros fisiológicos e à fixação biológica de nitrogênio, em condições de restrição hídrica, e sua capacidade de recuperação após reidratação, com base em componentes de produção. Os genótipos avaliados em casa de vegetação foram os seguintes: 'BRS 317', suscetível à seca; R01-581F e R02-1325, que apresentam fixação biológica de nitrogênio tolerante à seca; e os recombinantes BRB14-207525, BRB14-207526, BRB14-207527 resultantes de cruzamentos entre genótipos comerciais e PI 471938, que apresenta o fenótipo "slow wilting" em condições de seca. A restrição hídrica foi aplicada por dez dias aos genótipos em fase de florescimento pleno (R2), enquanto as plantas-controle foram mantidas bem irrigadas. A taxa fotossintética e as trocas gasosas sob restrição hídrica foram mais estáveis no genótipo BRB14-207526. A biomassa das raízes aumentou 3,5 vezes em R02-1325 como resposta adaptativa à seca. Além disso, os teores foliares de ureídos – uma característica relacionada à fixação biológica de nitrogênio em plantas tolerantes à seca – mantiveram-se estáveis nos genótipos R02-1325 e R01-581F, sob seca, e diminuíram em BRB14-207527. Os genótipos BRB14-207526, BRB14-207527 e R02-1325 apresentam melhores desempenhos fisiológicos e menor acúmulo de ureídos sob seca, além de maior produção de grãos após a sua reidratação. As progênies BRB14-207526 e BRB14-207527 apresentam características de tolerância à seca que provêm de PI 471938, enquanto R02-1325 é uma fonte promissora de tolerância à seca para o melhoramento da soja.

Termos para indexação: *Bradyrhizobium*, glutamina sintetase, fotossíntese, reidratação, ureídeos.

Introduction

Extreme climate events have affected the agricultural yield potentials, and several models have predicted the rainfall reduction and temperature increase by the second half of the 21st century (Shanker et al., 2014), which may result in severe agricultural losses of economically important crops. To face the global climate changes, researchers have strived to breed plant genotypes for more stable yield under drought (Cerezini et al., 2014; Devi et al., 2014; Shanker et al., 2014; Sinclair & Nogueira, 2018).

Important traits related to drought tolerance have been identified in soybean (*Glycine max* L. Merr.) such as, for instance, the limited transpiration under high-vapor pressure deficit (VPD) in PI 416937 (Devi et al., 2014), slow wilting in PI 471938 (Sadok et al., 2012; Devi & Sinclair, 2013), and nitrogen fixation tolerant to drought in 'Jackson' (Sall & Sinclair, 1991). Some progenies from 'Jackson' – R01-581F (PI 647961) and R01-416F (PI 647960) – also perform well under drought due to the maintenance of the biological nitrogen fixation (BNF) rates, contributing for the stability of yield (Chen et al., 2007; Cerezini et al., 2017).

BNF rates in soybean increase with the crop cycle and reach the maximum at the beginning of the reproductive stages, which is the most susceptible to failure for the BNF because of the high-demand for N (Mastrodomenico et al., 2013). The biologically fixed N in soybean is transported mainly as ureides (Baral et al., 2016), whose accumulation caused by failure in the transport and metabolism of N compounds in plants under drought results in a feedback inhibition on the BNF (Baral et al., 2016). Less accumulation of ureides in soybean shoots under drought is recognized as an important trait for BNF (Devi & Sinclair, 2013; Cerezini et al., 2014, 2017). Thus, the nitrogen fixation drought tolerance (NFDT) is a key trait for keeping the symbiotic process and helps the plant to cope with drought (Chen et al., 2007; Devi & Sinclair, 2013; Sinclair & Nogueira, 2018).

Besides the ability of sustaining the BNF and photosynthesis during soil drying, the recovery capacity after drought is important to ensure a superior plant performance (Mastrodomenico et al., 2013; Rosas-Anderson et al., 2014; Cerezini et al., 2016). Thus, the soybean capacity to recover the physiological activity after rewetting is a pivotal trait for stability of grain yield after moderate drought. Soybean breeders have been focusing an increased attention on drought tolerance in Brazil, as they have been facing crop losses caused by drought. However, among several traits that improve plant performance under drought, the BNF process has been neglected. Genotypes with less sensitive BNF to drought could result in plants with both more effective performance under drought and recovery after rewetting. The characterization of these genotypes could be useful for further use in breeding programs for drought tolerance.

The objective of this work was to evaluate the capacity of six soybean genotypes for physiological traits and BNF in drought conditions, and their capacity for recovery after rewetting, based on yield components.

Materials and Methods

The experiment was performed in a greenhouse at Embrapa Soja, in the municipality of Londrina (23°11'28.8"S, 51°11'01.6"W), in the state of Paraná, Brazil, during the 2014/2015 crop season. Six soybean genotypes, contrasting for their responses to drought, were evaluated. The commercial genotype 'BRS 317' is considered as drought susceptible (Cerezini et al., 2014, 2017). The genotypes R01-581F (Chen et al., 2007) and R02-1325 (Devi et al., 2014) were previously identified as NFDT. And the three recombinant genotypes BRB14-207525, BRB14-207526, BRB14-207527 – namely BRB-25, BRB-26 and BRB-27, respectively – were obtained from breeding between some commercial genotypes with PI 471938 that is known for showing the slow-wilting trait (Devi & Sinclair, 2013) (Table 1).

The experimental design was applied in randomized complete blocks, in 6×2 factorial arrangement, that is, six soybean genotypes and two water conditions – well-watered, or exposed to water restriction between 35 and 45 days after emergence, at the flowering stage R_1-R_2 –, with 10 replicates.

A soil sample taken at 0–20 cm soil depth, from an agricultural soil classified as Latossolo Vermelho-Amarelo distrófico (Santos et al., 2013), corresponding to Typic Acrudox (Soil Survey Staff, 2014) was used as substrate. The soil chemical properties and granulometric fractions were: pH ($CaCl_2$), 5.8; organic matter, 31.4 g kg^{-1}; P (Mehlich-I), 2.85 mg dm^{-3}; K, 0.10 cmol$_c$ dm^{-3}; Ca, 3.47 cmol$_c$ dm^{-3}; Mg, 1.10 cmol$_c$ dm^{-3}; H + Al, 4.12 cmol$_c$ dm^{-3}; CEC, 8.79 cmol$_c$ dm^{-3}; V, 53%; sand, 732 g kg^{-1}; silt, 30 g kg^{-1}; clay, 238 g kg^{-1}, corresponding to a sandy clay-loam texture.

Soil aliquots of 4 kg were placed into plastic pots, and received the following nutrients: 54 mg P and 320 mg K (both as K_2HPO_4); 230 mg Mg and 310 mg S (both as $MgSO_4.7H_2O$); and 150 mL of a micronutrient solution containing 2.85 mg $CoSO_4$, 10.8 mg Na_2MO_4, and 1.0 g H_3BO_3 in 15 L water. Nitrogen was provided biologically by inoculating the seed with a mixture of *Bradyrhizobium japonicum* (strain Semia 5079) and *B. diazoefficiens* (strain Semia 5080). The isolates were grown separately in YM broth (5 g L^{-1} mannitol, 0.4 g L^{-1} yeast extract, dibasic potassium phosphate -0.5 g L^{-1} K_2HPO_4, magnesium sulfate heptahydrate 0.2 g L^{-1} $MgSO_4.7H_2O$, sodium chloride 0.1 g L^{-1} NaCl, and pH adjusted to ~6.8), for six days at 28°C, and 180 rpm until reaching $1{\times}10^9$ viable cells mL^{-1}. Immediately before sowing, the two growth media were mixed, applied on seed, and mixed again, thoroughly, to provide $1.2{\times}10^6$ *Bradyrhizobium* cells per seed. Each pot received six seed; and one week later, seedlings were thinned to remain two of them per pot.

Soil water-holding capacity was determined as described by Cerezini et al. (2017). During the first 35 days after emergence, plants received water daily to maintain the soil-water potential (ψ_w) at -13 kPa (daily replacement to reach 300 mL of water L^{-1} of soil), corresponding to 0.9 available water fraction. At 35 days after emergence, during the flowering stage (R_1-R_2), plants were subjected to water restriction at -200 kPa (daily replacement to reach 90 mL of water L^{-1} of soil), corresponding to a fraction of available water of 0.27 for 10 days. Control plants continued to receive water supply at -13 kPa of ψ_w. Soil moisture was monitored daily by weighing the pots on an electronic scale; water replacement was performed in the morning (between 8:00–10:00 h). During the experiment, the averages of temperature and relative air humidity, measured with a thermohygrometer, were 33.5 and 21.7°C and 42 and 81.4%, in the day and at night, respectively.

Leaf gas exchange was measured simultaneously with two portable meter devices (LCpro-SD, ADC BioScientific Ltd., Hoddesdon, England) in 10 plants of each treatment (five plants with each device), in the third recently expanded trifoliolate, in the central leaflet, early in the morning (between 8:00-10:00 h), at the 5th and 10th day under water restriction. To minimize the effects of variations of temperature, irradiance, air moisture, and VPD along the measuring interval, one replicate of each treatment was assessed per round. Determinations included net photosynthetic (A) and transpiration rates (E), stomatal conductance (g_s), intercellular to atmospheric CO_2 concentration ratio (Ci/Ca), and leaf temperature. The greenhouse

Table 1. Characteristics and pedigree of soybean genotypes used in the experiment.

Soybean genotype	Type of growth	Pedigree	Characteristic
BRS 317	determinate	-	Drought sensitivity
R01-581F	determinate	'Jackson' × KS 4895	Nitrogen fixation drought tolerance (NFDT)
R02-1325	determinate	93705-35-1 × PI 227557	NFDT
BRB14-207525	indeterminate	PI 471938 × source BtRR2 – P	Slow-wilting
BRB14-207526	indeterminate	PI 471938 × source BtRR2 – P	Slow-wilting
BRB14-207527	indeterminate	PI 471938 × source BtRR2 – P	Slow-wilting

conditions during the measurements were: 700–1200 μmol m^{-2} s^{-1} photosynthetic photon flux density, 25–28°C air temperature, and 42–52% air moisture.

On the 10th day under water restriction, six out of 10 replicates per treatment were taken for destructive analysis. Fresh nodules (20 per pot) were weighed, then they were immediately frozen in liquid N_2 and stored at -80°C. Extracts were prepared using a Sephadex G-25 column (Hungria, 1994) for assessment of glutamine synthetase (GS) (EC 6.3.1.2) activity (Ferguson & Sims, 1971), a key enzyme involved in N assimilation. The remaining fresh nodules were counted for total number and weighed for the estimation of nodule dry matter, along with the estimated dry matter of the fresh nodules used for GS activity. Shoots, roots, and nodules were dried at 60°C, for 48 hours, for the determination of the respective dry matters; total N in the shoots was determined in sulfuric extracts by the green salicylate method (Searle, 1984). The concentration of ureides was determined (Vogels & Van Der Drift, 1970) in dry nodules, petioles, and leaves, after extraction according to Hungria (1994).

After the 10th day under water restriction, the remaining four replicates of each treatment previously subjected to water restriction and their respective well-watered controls were kept under -13 kPa of water supply, until their physiological maturity (R_8), for assessment of number of pods, and number and mass of grains (at 12% humidity).

The dataset was subjected to tests of normality and homogeneity of variances, followed by the analysis of variance with the application of the F test, at 5% probability. Once the effects of treatments or interactions between factors were detected, the means were compared by the Tukey's test, at 5% probability.

Results and Discussion

Subjecting plants to water restriction for 5 days (40 days after emergence) negatively affected the physiological traits, which was intensified with the exposition for 10 days (45 days after emergence) (Table 2). At the 5th day under water restriction, there was a single effect of water condition (restriction vs well-watering) on the net photosynthesis (A), transpiration rate (E), stomatal conductance (g_s), and intercellular to atmospheric CO_2 concentration ratio (Ci/Ca) by 11, 15, 41, and 16%, respectively, in the genotypes. Single effects of genotypes were observed only for E and g_s, with generally higher rates in BRB progenies, derived from the slow-wilting PI 471938, compared with the other genotypes. Similarly, Bellaloui et al. (2013) found higher-leaf-water potential in slow-wilting soybean genotypes under drought that was related to higher-stomatal conductance, which is corroborated by our findings. At the 10th day, the effect of water restriction was more intense on A, except for BRB-26, which was 41% higher under drought, in comparison with the average of the nonslow-wilting derived 'BRS 317', R01-581F, and R02-1325. The E decreased significantly in all genotypes under water restriction, but was less affected in BRB-25 (-36%) and BRB-26 (-24%), which significantly differed from 'BRS 317' (-43%) and R02-1325 (-52%). The g_s also followed the same trend as E, but again BRB-26 showed a better performance under water restriction (-51%) than the average of the other five genotypes (-72%). The BRB genotypes also had higher-Ci/Ca under water restriction than 'BRS 317' and R02-1325. These values are in agreement with the g_s, showing that the lower is the g_s, the lower will be the Ci/Ca ratio; however, g_s was more sensitive to discriminate among the genotypes under water restriction (Table 2). Although using different methods, Sadok et al. (2012) also reported the maintenance of E and g_s in PI 471938 subjected to water restriction, suggesting that the BRB progenies acquired traits related to the slow-wilting phenotype from their parental PI 471938. The practical effect of reductions in E is the average increase of leaf temperature by 1°C and 2°C, at 5 and 10 days under water restriction, respectively, with the smallest increase recorded in BRB-26. Decrease in g_s leading to decline in E, A, and increase in leaf temperature are clear plant responses to drought (Salon et al., 2011). Depending on g_s, the drought stress level can be classified as nonstressing ($g_s \geq 0.2$ mol H_2O m^{-2} s^{-1}), moderate (0.1–0.2 mol H_2O m^{-2} s^{-1}) and severe ($\leq$0.1 mol H_2O m^{-2} s^{-1}) (Flexas et al., 2004). Therefore, 10 days of water restriction induced a moderate stress in the BRB genotypes, but a severe stress in the nonslow-wilting genotypes, suggesting a higher capacity of the BRB progenies to cope with drought, a trait probably inherited from the slow-wilting parental PI 471938. However, the slow-wilting phenotype is not related to early decrease of the stomatal conductance under drought to save water (Sadok et al., 2012; Devi &

Sinclair, 2013), and the basis for drought tolerance in PI 471938 remains unknown (Riar et al., 2018).

Plants under water restriction reduced the shoot dry matter by -37% in average, in comparison with the well-watered plants (Figure 1 A), whereas the root dry matter increased, but the increase intensity depended on the genotype (Figure 1 B). Less root increase under drought was recorded in the slow-wilting-

Table 2. Net photosynthetic and transpiration rates, stomatal conductance, intercellular CO$_2$ concentration and temperature of leaves, in six soybean genotypes under water restriction (WR), or well-watered (WW), between 35 and 45 days after emergence$^{(1)}$.

Genotype	40 days after emergence			45 days after emergence		
	WW	WR	Average	WW	WR	Average
Net photosynthetic rate, A (µmol CO$_2$ m^{-2} s^{-1})						
BRS 317	20.3	18.2	19.3^{ns}	17.9Aa	12.1Bbc	15.0
R01-581F	22.7	19.0	20.9	21.8Aa	12.9Bbc	17.4
R02-1325	20.7	17.5	19.1	21.3Aa	11.1Bc	16.2
BRB-25	20.8	21.2	21.0	20.4Aa	15.4Bab	17.9
BRB-26	23.3	20.1	21.7	19.7Aa	17.0Aa	18.4
BRB-27	22.6	19.8	21.2	20.9Aa	14.9Babc	17.9
Average	21.7A	19.3B		20.4	13.9	
Transpitation rate, E (mmol H$_2$O m^{-2} s^{-1})						
BRS 317	3.7	3.3	3.5b	3.3Ab	1.9Bbc	2.6
R01-581F	4.1	3.3	3.7ab	3.9Aab	2.3Babc	3.1
R02-1325	3.7	3.2	3.4b	3.6Aab	1.7Bc	2.7
BRB-25	4.2	3.8	4.0a	4.1Aa	2.6Ba	3.3
BRB-26	4.0	3.7	3.8ab	3.6Aab	2.8Ba	3.2
BRB-27	4.7	3.5	4.1a	4.1Aa	2.5Bab	3.3
Average	4.1A	3.5B		3.8	2.3	
Stomatal conductance, g$_s$ (mol H$_2$O m^{-2} s^{-1})						
BRS 317	0.44	0.24	0.34c	0.29Ab	0.08Bc	0.19
R01-581F	0.46	0.27	0.36bc	0.36Aa	0.10Bbc	0.23
R02-1325	0.43	0.23	0.33c	0.39Aa	0.08Bc	0.23
BRB-25	0.47	0.34	0.41abc	0.40Aa	0.14Bab	0.27
BRB-26	0.54	0.34	0.44ab	0.37Aa	0.18Ba	0.27
BRB-27	0.60	0.31	0.46a	0.40Aa	0.13Bab	0.27
Average	0.49A	0.29B		0.37	0.12	
Intercellular / Atmospheric CO$_2$ concentration (Ci/Ca)						
BRS 317	0.69	0.57	0.63^{ns}	0.62Aa	0.38Bb	0.5
R01-581F	0.66	0.53	0.60	0.61Aa	0.46Ba	0.53
R02-1325	0.65	0.53	0.59	0.61Aa	0.36Bb	0.49
BRB-25	0.67	0.59	0.63	0.64Aa	0.47Ba	0.56
BRB-26	0.67	0.60	0.64	0.65Aa	0.48Ba	0.56
BRB-27	0.68	0.56	0.62	0.64Aa	0.45Ba	0.54
Average	0.67A	0.56B		0.69	0.43	
Leaf temperature (ºC)						
BRS 317	29.1	30.3	29.7^{ns}	29.1	31.2	30.1ab
R01-581F	29.8	30.8	30.3	29.7	32.1	30.9a
R02-1325	29.1	30.4	29.8	29.1	31.4	30.2ab
BRB-25	29.5	30.4	29.9	29.6	31.5	30.6a
BRB-26	29.1	29.8	29.5	29.1	30.0	29.5b
BRB-27	29.7	30.5	30.1	29.5	31.4	30.5ab
Average	29.4B	30.4A		29.3B	31.3A	

$^{(1)}$Means followed by equal letters, uppercases in the lines and lowercases in the columns, do not differ by the Tukey's test, at 5% probability (n = 10).

BRB progenies compared with the other genotypes. Drought-induced reallocation of plant biomass from shoot to roots has been observed (Cerezini et al., 2014, 2017), featuring an adaptive strategy to reduce water loss in the shoots and invest in roots to search for soil water (Eziz et al., 2017). This feature may indicate better adaptation of the progenies derived from PI 471938 to drought, in agreement with generally better gas exchanges as discussed before, resulting in less shoot biomass reallocation into roots. The water restriction quickly reduced the number of nodules in all genotypes. However, higher nodulation was recorded in the BRB progenies under well-watered conditions, and in BRB-25 under drought (Figure 1 C). The mass of dry nodules was reduced only in the genotypes R01-581F and BRB-27 (Figure 1 D). A decrease of nodulation in plants subjected to drought is a negative feature for plant performance, and slows down the BNF retake after rehydration (Mastrodomenico et al., 2013). Thus, the maintenance of the number of nodules and biomass under drought would be helpful for reassuming the BNF after rewetting. Accordingly, the lowest reduction of shoot-N accumulation was recorded in BRB-25 in comparison to the well-watered counterpart.

GS activity and ureides are indicators of N metabolism and transport, respectively (Hungria & Kaschuk, 2014; Baral et al., 2016). The highest GS activity in nodules was recorded in R01-581F and R02-1325, in both water conditions, and, together with BRB-26 and BRB-27 GS showed a stable enzyme activity. In 'BRS 317' and BRB-25 under water restriction, GS showed an increased activity (Figure 2 A). In turn, the concentration of ureides in nodules increased in

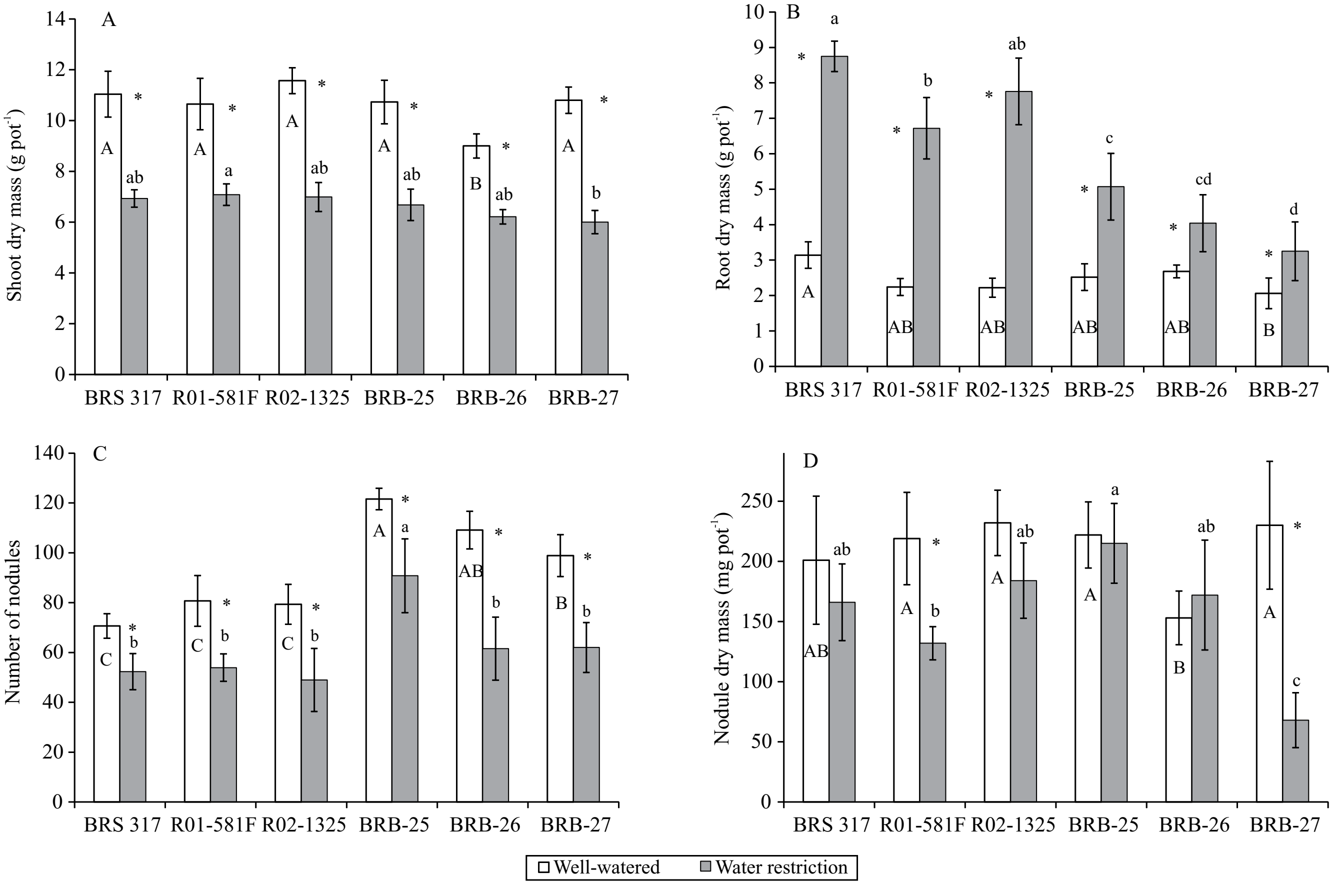

Figure 1. Traits of six soybean genotypes ('BRS 317', R01-581F, R02-1325, B14-207525, BRB14-207526, BRB14-207527) under well-watered (WW) or water restriction (WR) conditions, between, 35 and 45 days after emergence: A, shoot dry mass; B, root dry mass; C, number of nodules; and D, nodule dry mass. Means followed by equal letters, uppercases for WW and lowercases for WR, do not differ by the Tukey's test, at 5% probability. *Significant difference between water conditions for each genotype. Vertical bars represent the standard deviation (SD) (n = 6).

all genotypes under water restriction (Figure 2 B), as a result of the limitation of ureide transportation via xylem to shoots due to water limitation (Baral et al., 2016). The highest ureide concentration was observed in R02-1325, followed by R01-581F, about 60% higher than the average of the other genotypes. The different concentrations of ureides in nodules are probably related to the mechanisms of drought tolerance. While R02-1325 and R01-581F show NFDT (Table 1), that is, they keep the BNF process for longer under drought (Chen et al., 2007), the BRB progenies – derived from the slow-wilting PI 471938 (Devi & Sinclair, 2013) – keep leaf turgidity and water flow for longer, allowing of more transportation of ureides from nodules to shoots, which may explain the lower ureide concentrations nodules of the BRB progenies than in the NFDT genotypes. By turn, the highest-relative increase of ureides in nodules was observed in the drought sensitive 'BRS 317', and it was 15 times higher than the well-watered control (Cerezini et al., 2017). Water restriction also led to an increased concentration of ureides in petioles of 'BRS 317' and BRB progenies, but it did not change in R01-581F and was reduced by -52% in R02-1325 (Figure 2 C). Ureides in petioles have been more sensitive than ureides in leaves to discriminate BNF for drought tolerance in soybean genotypes. In a previous work, we found exactly the same behavior for 'BRS 317' and R01-581F exposed to drought and similar concentrations of ureides (Cerezini et al., 2014). In leaves, ureides increased by 104, 135, and 24% under water restriction in 'BRS 317', BRB-25, and BRB-26, respectively, in comparison

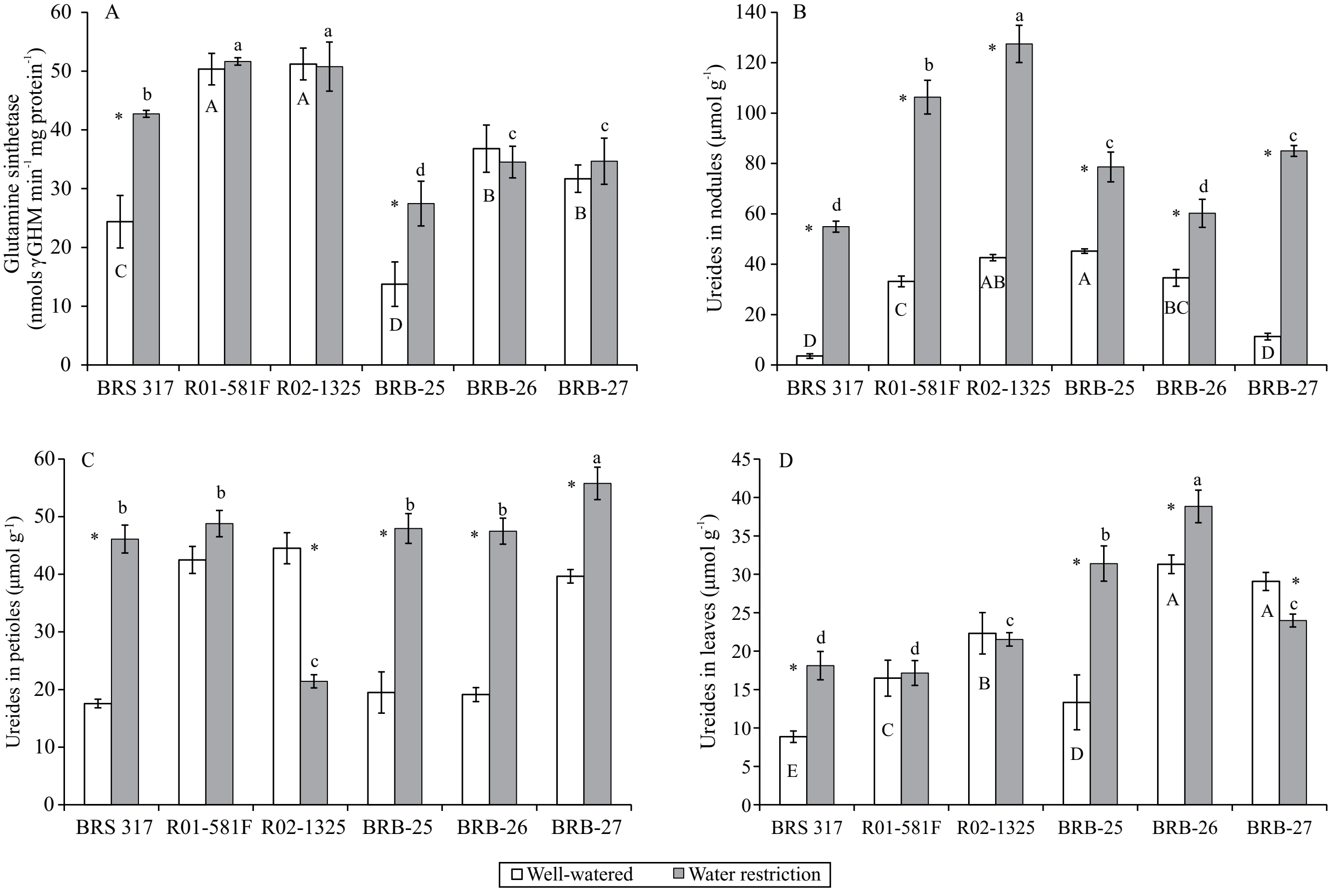

Figure 2. Glutamine synthetase activity and ureide concentrations in six soybean genotypes ('BRS 317', R01-581F, R02-1325, B14-207525, BRB14-207526, BRB14-207527) under well-watered (WW) or water restriction (WR) conditions, between 35 and 45 days after emergence. Glutamine synthetase activity: A, in nodules. Ureide concentrations: B, in nodules; C, in petioles; and D, in leaves. Means followed by equal letters, uppercases for WW and lowercases for WR, do not differ by the Tukey's test, at 5% probability. *Significant difference between water conditions for each genotype. Vertical bars represent the standard deviation (SD) (n = 6).

with the well-watered plants; however, there were no significant differences for R01-581F and R02-1325, while a 17% ureide reduction was recorded in BRB-27 (Figure 2 D). The maintenance of ureide levels in R01-581F and R02-1325 leaves has also been reported for their parental 'Jackson' (Sall & Sinclair, 1991) as an important NFDT trait (Devi & Sinclair, 2013). Similarly, a decreased concentration of ureides in the leaves of BRB-27 is also a trait for tolerance to drought showing that the metabolism of ureides continued even under drought (Baral et al., 2016). As a practical result, we suggest that the use of ureide concentration, in different plant tissues, to discriminate soybean genotypes for sensitivity to drought should take into account both the mechanism related to drought-tolerance (NFDT, or slow-wilting) and the considered tissue (nodules, petioles, or leaves). Otherwise, one might conclude that higher concentrations of ureides are always negative, when it might simply show a more efficient N fixation.

All genotypes showed reductions in the total shoot-N contents after 10 days of exposure to drought (Figure 3 A). Under well-watered conditions, a higher-N content in shoots was recorded in R02-1325, which differed significantly from the BRB progenies. Under drought, higher-N contents were observed in 'BRS 317', R01-581F, and R02-1325 than in BRB-26 and BRB-27, whereas BRB-25 showed medium N contents. This trend is in agreement with the shoot dry mass (Figure 1 A). Considering the plant capacity to recover after rewetting, the number of pods was reduced by 20% in 'BRS 317', R02-1325, BRB-25, and BRB-26,

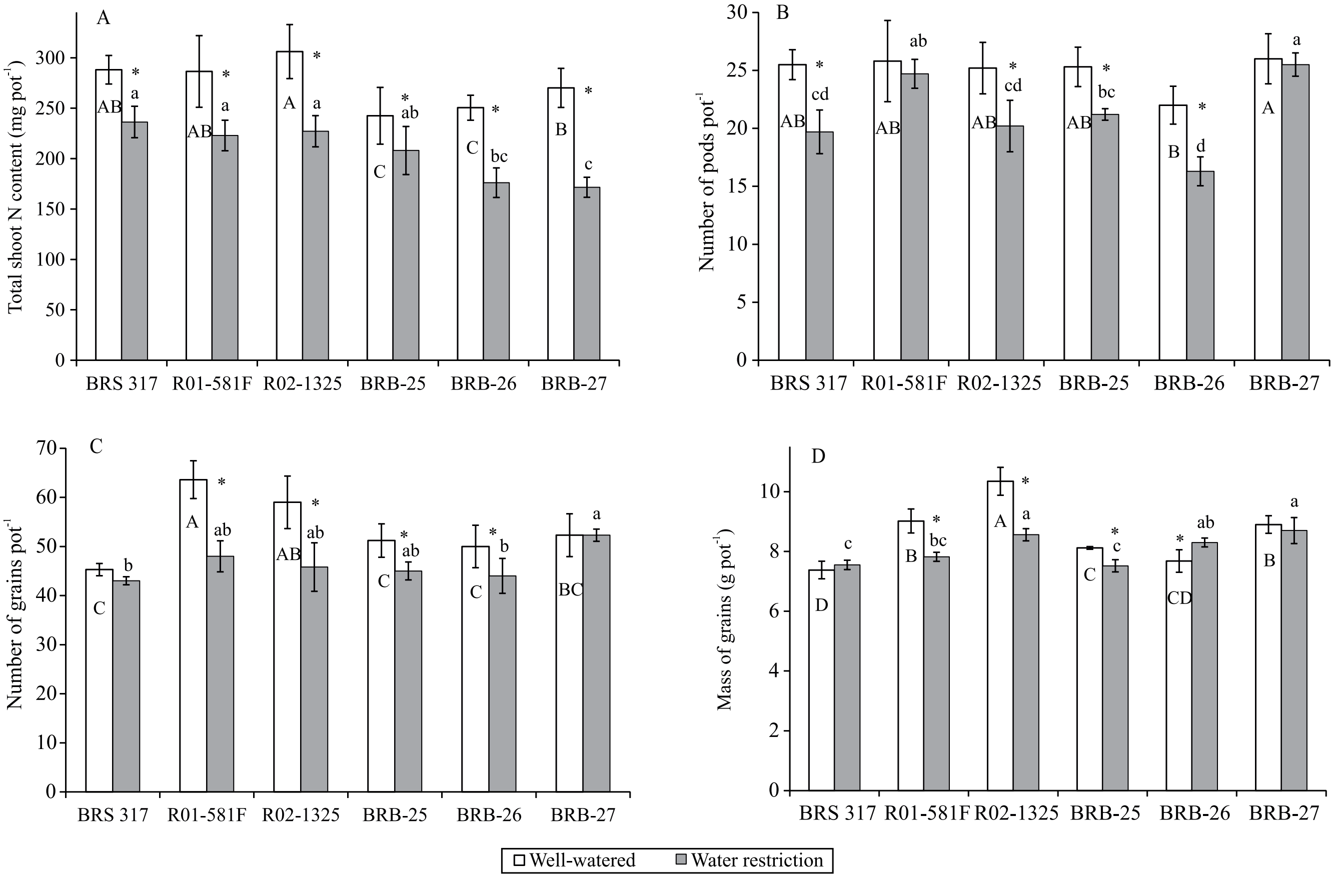

Figure 3. Nitrogen content and yield components in six soybean genotypes ('BRS 317', R01-581F, R02-1325, B14-207525, BRB14-207526, BRB14-207527) under well-watered (WW) or water restriction (WR) conditions, between 35 and 45 days after emergence: A, total shoot-nitrogen content; B, number of pods; C, number of grains; and D, mass of grains. Means followed by equal letters, uppercases for WW and lowercases for WR, do not differ by the Tukey's test, at 5% probability. *Significant difference between water conditions for each genotype. Vertical bars represent the standard deviation (SD) (Figure 3 A, n = 6; Figure 3 B-D, n = 4).

whereas there were no reductions in R01-581F and BRB-27 (Figure 3 B). Water restriction also did not affect the number of grains in 'BRS 317' and BRB-27, in comparison with the well-watered plants, whereas the reductions in the number of grains by 25, 22, 12, and 12% were observed for R01-581F, R02-1325, BRB-25, and BRB-26, respectively (Figure 3 C). The highest number of grains was observed in well-watered R01-581F plants, which did not differ from R02-1325. Under water restriction, more grains were found in BRB-27 than in 'BRS 317' and BRB-26. Water restriction did not affect the mass of grains per pot for 'BRS 317' and BRB-27, while reductions by 13, 17, and 7% were recorded in R01-581F, R02-1325, and BRB-25, respectively. Conversely, the mass of grains increased by 8% in BRB-26 exposed to drought (Figure 3 D). These findings show that two slow-wilting progenies BRB-26 and BRB-27 and the NFDT genotype R02-1325 are resilient to exposure to drought. Yields undergone a reduction under drought exposure in both BRB and NFDT genotypes; notwithstanding, the grain yields of R02-1325 still kept similar to their best performing BRB progenies. Progenies originated from 'Jackson' and PI471938 have also been identified as showing different capacities to sustain the BNF under moderate drought (Chen et al., 2007; Cerezini et al., 2014; Riar et al., 2018), confirming that the slow-wilting trait can be transferred from a parental, as observed in the BRB progenies in this work. Maintenance or recovery of the BNF after drought stress is a desirable trait in soybean that might bring advantages for grain yield. However, although the BNF process in soybean is generally restored three days after moderate drought at the vegetative stage (Silva et al., 2019), the damage is more critical at the reproductive stages (Mastrodomenico et al., 2013).

Our results show a genetic variation in response to drought that can be further explored by breeding programs seeking for tolerance to drought. Evidences of genetic variation in soybean and peanut (*Arachis glabrata* L.) regarding the recovery capacity of transpiration, stomatal conductance, and BNF after soil drying, followed by rehydration, have also been described (Rosas-Anderson et al., 2014). However, other factors like co-inoculation with *Bradyrhizobium* and *Azospirillum* also increase the plant capability to recover from drought (Silva et al., 2019). Therefore, such results should be confirmed under field conditions, since several other environmental factors may interfere in the plant performance under drought.

Conclusions

1. Soybean genotypes have different capabilities to maintain their metabolism under water restriction that results in plant recovery after drought stress relief.
2. BRB progenies from the slow-wilting PI 471938 run moderate stress after ten days of exposure to drought, whereas the nonslow-wilting genotypes run severe stress.
3. The genotype BRB-27 is more resilient to drought and keeps its yield components after exposition to water restriction.

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