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# Biological nitrogen fixation in soybean genotypes with different levels of drought tolerance

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**ABSTRACT:** Periods of drought may cause changes in the physiological condition of soybean [*Glycine max* (L.) Merr.] and the biological nitrogen fixation (BNF) is one of the first processes to be impaired. The objective of this study was to evaluate the effect of water restriction in soybean genotypes on BNF related traits to select genotype(s) with drought-tolerant BNF. The experiment was carried out under greenhouse, in a 5 × 2 factorial arrangement, with five genotypes (BRS 317, Jackson, R02-1325, R01-416F, and R01-581F) at two moisture levels, humid condition (80% of field capacity, FC) and drought condition (30% of FC). Drought induction started 33 days after sowing, at flowering, and was kept for 10, 22, and 42 days. The evaluations were: number and mass of dry nodules; specific mass of nodules; ureide concentrations in leaflets, petioles, and nodules; total nitrogen in the leaves and mass of grains per plant. Water restriction for 10 days reduced nodulation by 30%, grain mass by 35% for R02-1325 and R01-416F, and increased ureide concentration in nodules by 72% and in petioles by 90%. BRS 317, Jackson, and R01-581F stood out for exposure to drought for 10 days. At 22 days, BRS 317 stood out, while the other genotypes had reduction in grain mass by 36%, 40% in nodulation, and ureides in nodules by 34%. Exposure to drought for 42 days reduced nodulation by 46%, grain mass by 44%, leaf N by 15%, ureides in leaflets by 29 and 35% in petioles, with an increase of 136% of ureides in nodules. R02-1325 presented grain yield 30% higher than the other genotypes in wet condition and did not differ from the most productive ones under drought, indicating potential as source of drought tolerance in breeding programs.

**Key words:** *Glycine max*; nitrogen; nodules; ureides; water stress

# Fixação biológica de nitrogênio em soja com diferentes níveis de tolerância à seca

**RESUMO:** Períodos de seca podem causar mudanças na condição fisiológica da soja [*Glycine max* (L.) Merr.] e a fixação biológica do nitrogênio (FBN) é um dos primeiros processos a serem prejudicados. O objetivo deste estudo foi avaliar o efeito da restrição hídrica em genótipos de soja sobre atributos relativos à FBN visando selecionar genótipo(s) com FBN tolerante à seca. O experimento foi conduzido em condições controladas, em arranjo fatorial 5 × 2, com cinco genótipos (BRS 317, Jackson, R02-1325, R01-416F e R01-581F) em dois níveis de disponibilidade hídrica, condição úmida (80% da capacidade de campo, CC) e condição seca (30% da CC). A indução de seca teve início aos 33 dias após a semeadura, na floração, e foi mantida por 10, 22 e 42 dias. As avaliações foram: número e massa de nódulos secos; massa específica de nódulos; teores de ureídeos nos folíolos, pecíolos e nódulos; nitrogênio total nas folhas e a massa de grãos por planta. A indução de restrição hídrica por 10 dias reduziu a nodulação em 30%, a massa de grãos em 35% para R02-1325 e R01-416F, e aumentou o teor de ureídeos nos nódulos em 72% e nos pecíolos em 90%. BRS 317, Jackson e R01-581F se destacaram quanto à exposição à seca por 10 dias; aos 22 dias, BRS 317 se destacou, enquanto os demais genótipos tiveram redução de 36% na massa de grãos, 40% na nodulação e ureídeos nos nódulos em 34%. A exposição à seca por 42 dias reduziu a nodulação em 46%, a massa de grãos em 44%, N foliar em 15%, ureídeos nos folíolos em 29 e em 35% nos pecíolos, com aumento de 136% de ureídeos nos nódulos. R02-1325 apresentou produção de grãos 30% maior que os demais genótipos em condição úmida e não diferiu das mais produtivas sob seca, indicando potencial como fonte de tolerância à seca em programas de melhoramento.

**Palavras-chave:** *Glycine max*; nitrogênio; nódulos; ureídeos, estresse hídrico

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## Introduction

The soybean plant [*Glycine max* (L.) Merr.] has about 90% water in its fresh mass, which acts in practically all physiological and biochemical processes, with an important role in the regulation and distribution of heat in the plant (Salisbury & Ross, 2013; Taiz et al., 2017; Embrapa, 2020). However, prolonged periods of drought can cause changes in the physiological condition of the plant, such as the closure of stomata, and cause premature fall of leaves, flowers, and pods, with consequent decrease in yield potential. One of the first physiological processes affected by water restriction is biological nitrogen fixation (BNF), whose decline is related to decrease in the photosynthetic capacity of the host and/or damage to the nitrogenase complex (Purcell et al., 2004; Taiz et al., 2017; Embrapa, 2020).

Nodulation capacity and efficiency in fixing atmospheric ($N_2$) nitrogen (N) are dependent on the interaction between symbiont bacterial strains and the host plant, which are influenced by environmental conditions (Cerezini et al., 2020). Nodules in the soybean root system can remain active for weeks, constantly forming and renewing during the crop cycle, with maximum nodulation between the reproductive stages of full bloom ($R_2$) and grain filling ($R_5$). From $R_6$ (fully formed green grains) onwards, the gradual process of maturation, plant and nodule senescence occurs ($R_7$ and $R_8$), with a consequent reduction in BNF rate (Purcell et al., 2004; Hungria et al., 2007; Finoto et al., 2009).

To carry out BNF, the bacteroids inside the nodules need photoassimilates provided by the host and oxygen ($O_2$) to generate adenosine triphosphate (ATP) and reduce $N_2$ to ammonia ($NH_3$) through the nitrogenase complex, which then receives protons from the medium and forms ammonium ion ($NH_4^+$). Finally, an organic substrate receives $NH_4^+$ for subsequent incorporation into the plant metabolism in the form of ureides, which correspond to 90% of the N translocated by the xylem towards the aerial part (Taiz et al., 2017). Water restriction may impair the ureide metabolism in leaves, resulting in accumulation in the leaflets, petioles, and possible exportation to nodules via phloem, which may inhibit BNF by a retroinhibitory effect (Purcell et al., 2004; King & Purcell, 2005).

Water restriction also decreases the number and mass of nodules on soybean roots (Cerezini et al., 2020). Nodulation is generally lower under drought, both in genotypes considered tolerant and in those considered sensitive to water deficit, although the effect is lower in tolerant genotypes because they are able to maintain the supply of photoassimilates for longer, greater water flow, and lower ureide concentrations in the tissues (King & Purcell, 2001; Ladrera et al., 2007).

The strain R02-1325 has been characterized for drought tolerance in relation to BNF components, as well as high yield potential (Devi et al., 2014). Similarly, Chen et al. (2007) reported that strains R01-416F and R01-581F have high grain yield potential while exhibiting drought tolerant BNF. These strains are the result of crossing the soybean genotypes Jackson (drought tolerant) and KS4895 (high yielding). Jackson, since its release, has been characterized as having drought tolerant BNF (Serraj & Sinclair, 1996).

With the development of soybean genotypes with drought tolerance traits, there is a need for studies to identify more precisely the factors involved in the limitation of BNF under water restriction. The effects of drought on BNF and soybean yield depend on its duration and intensity. The stage at which water restriction occurs can also influence the effects on the crop, especially in the most critical stages such as flowering and grain filling (Ladrera et al., 2007; Kron et al., 2008).

The objective of this study was to evaluate the effect of water restriction on BNF related traits in soybean genotypes with different levels of drought tolerance, and on grain yield performance, exposed or not to drought, at different times between the reproductive stages of flowering and grain filling.

## Materials and Methods

The experiment was carried out in the agricultural year 2013-2014 in a greenhouse, in a controlled environment with temperature records between 25 and 30 °C, and relative air humidity (RH%) between 70 and 85% on average during the crop cycle at the Agronomy Department of the Universidade Estadual de Londrina (UEL), Londrina, PR, Brazil (latitude 23° 22’ S, longitude 51° 12’ W, and altitude of 585 m). Sowing took place at the end of November and harvesting in early April.

The experimental design was in randomized blocks with five replications, in a 5 × 2 factorial arrangement, with five soybean genotypes: the strains R01-416F, R01-581F, and R02-1325, and the varieties Jackson and BRS 317; under two water conditions: full water supply at 80% of field capacity (FC) (wet condition) during the whole cycle and water restriction at 30% of FC (drought condition) at different stages of reproductive cycle.

BRS 317 is a conventional soybean variety with high yield potential, developed by Embrapa Soja and indicated for cultivation in the states of Paraná, São Paulo, Santa Catarina, and southern Mato Grosso do Sul, Brazil. It has a determinate growth type, maturity group 6.6 (Brazil) and a cycle of 122 to 128 days at an altitude of 500 to 800 m (Embrapa, 2016). There is no disclosure on drought tolerance or sensitivity trait, however some authors reported that this cultivar presents drought sensitive BNF (Cerezini et al., 2014). However, it is indicated as moderately tolerant to water deficit for the southern region of Mato Grosso do Sul, Brazil (Pitol, 2015). The Jackson variety was developed in the United States in 1953, with maturity group VII (USA), and has been characterized as drought tolerant BNF since its release. The strains R02-1325, R01-416F, and R01-581F were developed in the United States at the Arkansas Agricultural Experiment Station, belonging to maturity group V (USA). They have been reported as able to keep the BNF rates under moderate water deficit and have high yield potential (Chen et al., 2007; Devi et al., 2014).

Exposure to water restriction (30% of FC) started at 33 days after sowing (DAS) in half of the experimental plots when the genotypes were between flowering stages $R_1$ and $R_2$, while the other half of the plots continued to receive water at 80% of FC. The evaluations were carried out at three times, i.e. at 43 DAS, after 10 days of exposure to drought, at stage $R_2$ (full flowering); at 55 DAS, after 22 days of drought, at stage $R_4$ (pods formed); and at 75 DAS, after 42 days of drought, at stage $R_6$ (grains formed and green) (Fehr & Caviness, 1977).

The strains were earlier than the varieties. Thus, the times for evaluations were determined when more than 50% of the experimental plots were in the respective reproductive stages, within dry or wet conditions. For statistical analysis, each evaluation time (10, 22, and 42 days of exposure to drought) was considered a separate trial, where evaluation of the treatment effect within one analysis time was independent of the other times.

Each experimental plot was represented by a polyethylene pot with capacity of 9 L in marble color, to minimize the effects of solar radiation on the pot temperature. Each pot received 8 kg of a substrate in the ratio 3:1 (soil:sand), formed by mixing the sample of 'Latossolo Vermelho distroférrico' of clayey texture collected in the layer 0-20 cm in an area of commercial soybean cultivation and washed fine river sand.

Samples of the substrate were analyzed for fertility (Silva, 2009), which results were: pH ($CaCl_2$) = 6.8; $Ca^{2+}$ = 8.2 $cmol_c\ dm^{-3}$; $Mg^{2+}$ = 0.8 $cmol_c\ dm^{-3}$; $K^+$ = 0.64 $cmol_c\ dm^{-3}$; $Al^{3+}$ = 0.0 $cmol_c\ dm^{-3}$; H + Al = 2.5 $cmol_c\ dm^{-3}$; CTC = 12.2 $cmol_c\ dm^{-3}$; V% = 79; OM = 12.1 g $kg^{-1}$; and, P = 47.5 mg $dm^{-3}$.

For pH determination, the $H^+$ ion activity in the substrate suspension was estimated using calcium chloride solution ($CaCl_2$ 0.01 mol $L^{-1}$) and read using a potentiometer. The determination of exchangeable acidity ($Al^{3+}$) was with the potassium chloride (KCl) extractor and the reading performed by titration with sodium hydroxide solution (NaOH). Potential acidity (H + Al) was determined using SMP buffer solution (Shoemaker, McLean, and Pratt method) and read using a potentiometer.

Exchangeable calcium ($Ca^{2+}$) and magnesium ($Mg^{2+}$) concentrations were extracted with KCl and determined by titration with EDTA. Phosphorus (P) and potassium ($K^+$) were determined in Mehlich-1 extractant solution. The P content was obtained by reading in a spectrophotometer at 630 nm , while K concentration was obtained by reading in a flame photometer. Carbon (C) determination was performed according to the Walkley-Black method and the OM was obtained by the multiplication factor 1.724 × C.

Before sowing, soybean seeds were inoculated with commercial peat inoculant containing 7.2 × $10^9$ colony forming units per gram (manufacturer guarantee), containing *Bradyrhizobium* spp. strains SEMIA 5079 and SEMIA 5080. At the $V_2$ stage, plants were thinned to two per pot.

The mass of water in the substrate was determined in the condition of full supply (80% of FC, wet) and water restriction (30% of FC, dry), when applied (Albuquerque, 2010). Water availability was determined daily from the mass of water (kg) contained in each pot using an electronic scale with a capacity of 15 kg, and once a day the values were restored to 80 or 30% of FC by determining and replacing the mass of water lost.

Half of the experimental plots with each soybean genotype were maintained with water supply at 80% of FC throughout the whole crop cycle. However, the other half of the plots was subjected to different periods of water restriction with humidity at 30% of FC, between 33 DAS and 75 DAS, for a total time of 42 days. From 75 DAS until harvest at 127 DAS, all plants received water again to reach 80% of FC and were maintained in this condition until the end of the cycle. At the end of each period under water restriction (10, 22, or 42 days) five replicates of each treatment (five genotypes × two water conditions) within five blocks were randomly sampled for destructive analyses, for a total of 50 experimental plots for each evaluation. At harvest (127 DAS), during the $R_8$ stage (maturity), 100 representative plots of the five genotypes were sampled for yield determination, i.e. 25 plots under 30% of FC for 10 days, 25 for 22 days, 25 for 42 days and 25 under 80% of FC throughout the cycle. A total of 250 experimental plots were used in the experiment.

The effect of the applied water regime (80 or 30% of FC) was evaluated by non-destructive determinations in the aerial part of the plants and in the substrate of the plots during the periods of exposure to water restriction (10, 22, or 42 days) by the variables: stomatal conductance (mmol $m^{-2}$ $s^{-1}$) (Leaf Porometer, Model SC-1, Decagon Devices, Inc); leaf temperature (°C) (Mira Laser Thermometer, Incoterm); substrate water content before daily irrigation (kg) (digital scale, capacity 15 kg, Toledo); water concentration ($m^3$ $m^{-3}$) and substrate temperature (°C) at 5 cm depth (5TE Soil Moisture Content/Procheck, Decagon Devices, Inc); and, substrate water potential (MPa) (WP4-T Dewpoint Potentiameter, Decagon Devices, Inc). Foliar determinations took place on the central leaflet of the 3rd fully developed trefoil from the apex of the main stem of the plants.

In each evaluation, the pots were disassembled, the aerial part was separated from the roots which were washed and the nodules separated. Nodules and aerial part were dried in a forced air circulation oven at 65 °C until they reached constant mass. The number of nodules and the mass of dry nodules per plant (g); the specific mass of nodules (mg/nodule); the concentration of total N in the dry biomass of leaves (g $kg^{-1}$) by the Kjeldahl method; the mass of grains per plant (g); and the concentrations of ureide in the extracts obtained from dry tissues of leaflets, petioles, and nodules (µmol $g^{-1}$) were evaluated.

To obtain the extract for ureide analysis, after the material was dried, ground, and passed through a 60 or 100 mesh sieve, an aliquot was added to a test tube in the amount of 0.1 g (nodules) or 0.3 g (leaflets or petioles), together with 5.0 mL of phosphate buffer, 0.1 mol $L^{-1}$, pH 7.0, and 2.5 mL of ethanol (2:1 ratio, buffer:ethanol). The tube was heated at 80 °C for 5 min and its contents mixed with the aid of a vortex. After 1 h of rest, the material was filtered on layers of sterile hydrophilic cotton, and centrifuged in a microcentrifuge for about 5 min (10,000 *g*). Aliquots of 50 µL of the clean extract free of any suspension were used for the analyses of total ureides (Hungria, 1994).

The total ureide concentrations (allantoin and allantoic acid) in the tissue extract were quantified by the technique of Vogels & Van der Drift (1970), described in Hungria (1994). The technique was based on the principle of selective hydrolysis of ureides to glyoxylate, which was quantified colorimetrically after the Rimini-Scrhyver reaction. Briefly, 50 µL aliquots of the extract in duplicate were subjected to alkaline hydrolysis and acid hydrolysis sequence for quantification of ureides. Once the hydrolysis products were obtained, they were quantified colorimetrically after reaction with phenylhydrazine and potassium ferricyanide which formed a pink chromophore, which intensity was read at 535 nm in a spectrophotometer. The absorbance readings were converted to µmols of total ureides against a standard curve constructed for each set of analyses. Results were expressed as µmols of total ureides per gram of dry biomass.

At $R_8$ (full maturity), the grains of the genotypes exposed to wet (80% of FC) and drought conditions (30% of FC) for three different periods (10, 22, and 42 days) were collected for evaluation of grain mass per plant (g), which was determined from the correction to 13% moisture.

The data were submitted to analysis of variance by the F test and means comparisons by the Tukey test at 5% significance level.

## Results a Discussion

The main route for gas exchange in the plant is the stomatal, whose opening influences the transpiration rate and the energy balance in the photosynthetic process (Kerbauy, 2008). Stomatal closure in response to water deficit or high temperatures influences the whole plant, as it compromises photosynthetic performance and the BNF process, which depends on reducing power generated in the former for the reduction of $N_2$ to $NH_3$ (Salisbury & Ross, 2013). Nascimento et al. (2011) observed that stomatal conductance decreased by 72% and leaf temperature increased by 12% in cowpea (*Vigna unguiculata*) that received 50% of the optimal water supply. Thus, as stomata close, transpiration decreases, leading to an increase in leaf temperature (Cerezini et al., 2014). The results of this study corroborate these authors, as water restriction reduced stomatal conductance by 73% and increased leaf temperature by about 1 °C (Table 1).

The 10 days of drought exposure (30% of FC) between flowering stages $R_1$ and $R_2$ did not influence the nodule number and nodule mass for Jackson, or nodule number for strain R02-1325. For the other genotypes, water restriction decreased both nodule mass and number (Figure 1).

Drought induction for 10 days did not change the ureide concentrations in leaflets, but increased in petioles and nodules in all genotypes, except in nodules of strain R01-416F, which showed the highest concentration even under full water supply. Despite the increase in ureides in petioles and nodules under water restriction, N concentrations were lower in the leaf dry biomass, which indicated a negative effect on BNF.

Water restriction during the $R_1$ and $R_2$ stages for 10 days reduced the grain mass of strains R02-1325 by 32% and R01-416F by 39%, but did not influence BRS 317, Jackson, and strain R01-581F. Among the genotypes, the strain R01-416F showed 39% lower grain yield than the tolerant cultivar Jackson under water restriction (Figure 1).

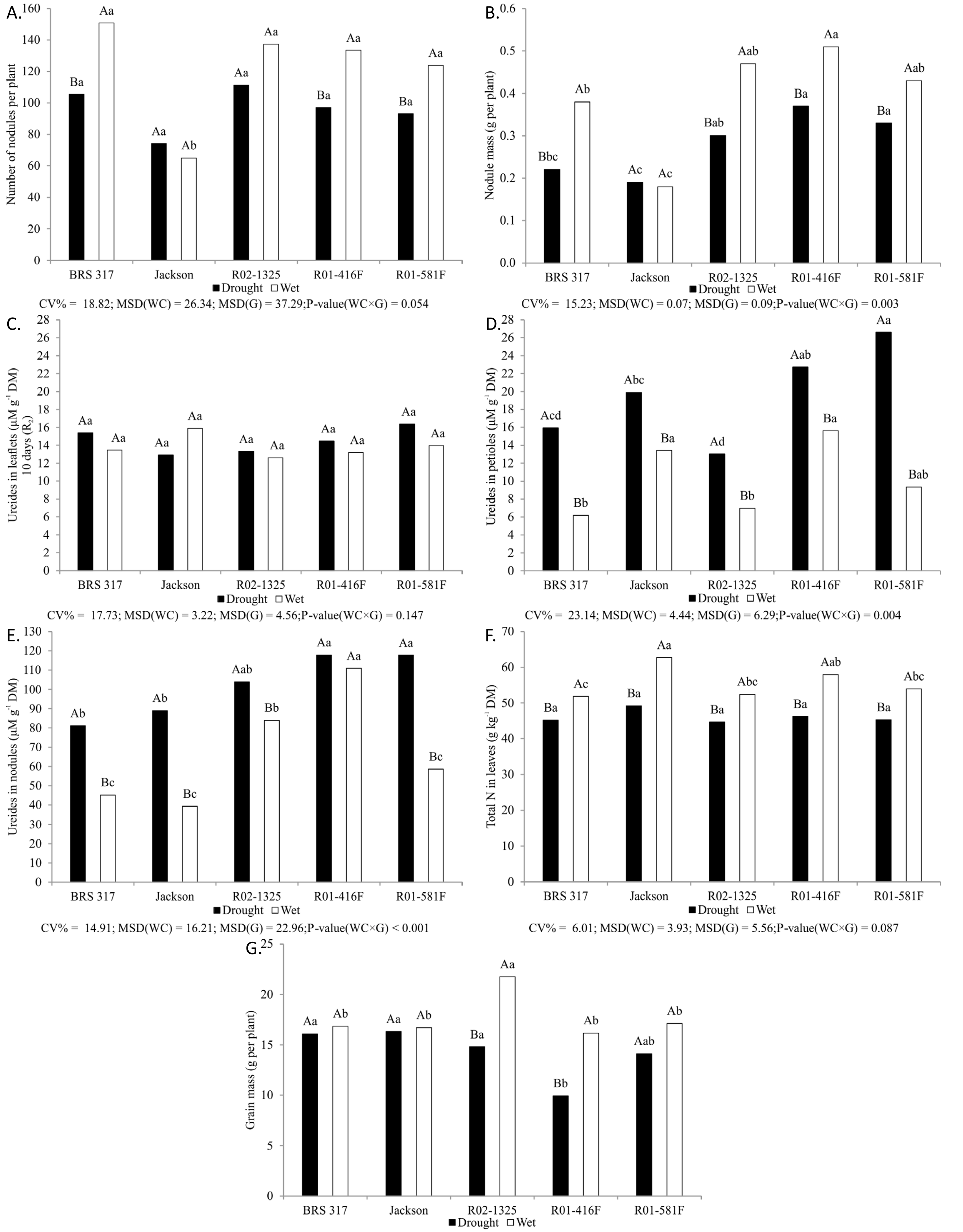
At 22 days of exposure to drought, during the reproductive stage, when the pods were formed ($R_4$), all genotypes submitted to water restriction had the number and dry mass of nodules reduced. Among the genotypes, Jackson presented the lowest number of nodules, corresponding to 44 and 25% of the means of the other genotypes, under water restriction and full water supply, respectively (Figure 2). However, specific nodule mass, which is an indication of nodule size, was higher in percent under drought when compared to with wet control treatment for Jackson (Figure 4).

King & Purcell (2001) suggest that larger nodules help to confer drought tolerance because the fraction of $N_2$ fixing tissues is greater than in small nodules. These authors concluded that the drought tolerance of Jackson is partly

**Table 1.** Average of the determinations carried out in the substrate and in the aerial part of soybean plants (cv. BRS 317, cv. Jackson, R02-1325, R01-416F, and R01-581F) exposed to wet (80% of FC) or drought (30% of FC) conditions at 10, 22, or 42 days between stages $R_1$ and $R_6$.

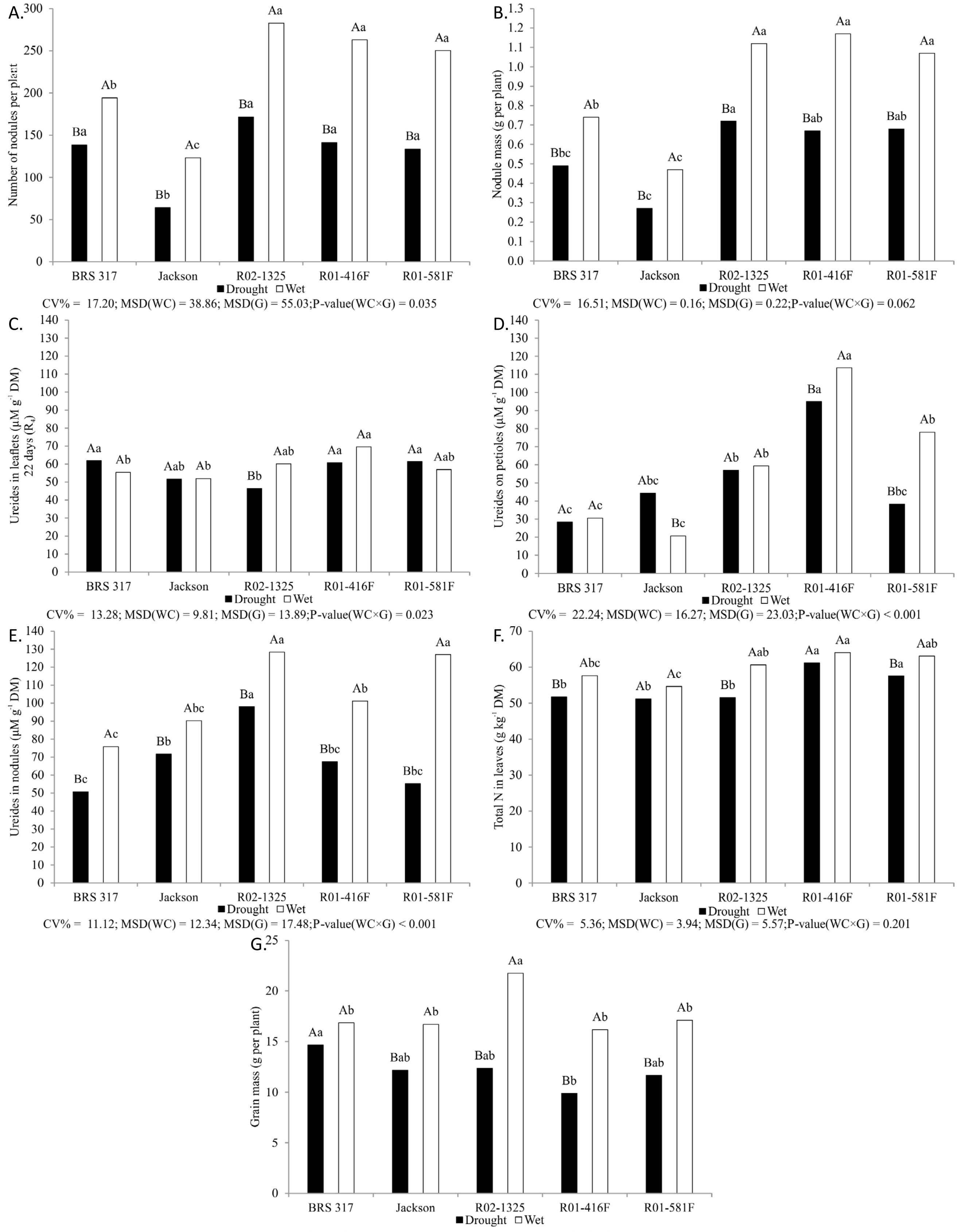
| Treatment | Variables | | | | | |
|---|---|---|---|---|---|---|
| | Leaf temperature (° C) | Stomatal conductance (mmol $m^{-2}$ $s^{-1}$) | Water content* (kg) | Water content** ($m^3$ $m^{-3}$) | Substrate temperature (° C) | Water potential (MPa) |
| Wet | 24.3 | 584.7 | 1.3 | 0.33 | 27.0 | - 0.01 |
| Drought | 25.4 | 156.6 | 0.3 | 0.22 | 28.3 | - 1.20 |

* Water content in kg per pot. ** Water content in $m^3$ $m^{-3}$ in the first 5 cm of depth. Average measurements taken immediately before the daily water replenishments in the pots. FC - Field capacity.




Means followed by the same letter, uppercase (water conditions, within genotype) and lowercase (genotypes, within water condition), do not differ by Tukey test (p ≥ 0.05).
CV - Coefficient of variation; MSD - Minimal significant difference; WC - Water condition; G - Genotype; DM - Dry mass.

**Figure 1.** Number (A) and mass (B) of nodules, ureide concentrations (leaflets - C, petioles - D, and nodules - E), leaf N concentration (F) and grain mass per plant (G) of soybean genotypes (BRS 317, Jackson, R02-1325, R01-416F, and R01-581F), exposed to wet (80% of FC) or drought (30% of FC) conditions for 10 days between flowering stages $R_1$ and $R_2$.

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CV - Coefficient of variation; MSD - Minimal significant difference; WC - Water condition; G - Genotype; DM - Dry mass.

**Figure 2.** Number (A) and mass (B) of nodules, ureide concentrations (leaflets - C, petioles - D, and nodules - E), leaf N concentration (F) and grain mass per plant (G) of soybean genotypes (BRS 317, Jackson, R02-1325, R01-416F, and R01-581F), exposed to wet (80% of FC) or drought (30% of FC) conditions for 22 days between flowering ($R_1$) and pod formation ($R_4$) stages.

driven by the advantage of larger nodules, but also results from more efficient supply of photosynthates to the nodules.

At 22 days of drought exposure (stage $R_4$), leaf ureides decreased only in strain R02-1325 under water restriction. Ureide concentrations in petioles varied among genotypes when exposed to drought, sometimes increasing, sometimes decreasing, or without effect. However, ureide concentrations in nodules decreased in all genotypes exposed to water restriction. The highest ureide concentration in nodules was observed in strain R02-1325 (Figure 2), regardless of water condition. Water restriction did not influence the leaf N concentration for Jackson and R01-416F, but decreased in the other genotypes (Figure 2).

Purcell et al. (2004) and King & Purcell (2005) suggest that the increase in ureide concentration in leaves may cause a retroinhibitory effect on BNF, with re-exportation via phloem and accumulation of ureides in nodules, a behavior that would be associated with drought-sensitive soybean genotypes. However, the higher ureide concentration in nodules of R02-1325 did not result in lower leaf N concentrations or decreased grain yield. However, Coleto et al. (2014) found accumulation of ureides in stems and leaves but not in nodules of *P. vulgaris* under drought. The accumulation of ureides was not associated with impaired translocation of the BNF product under drought, as has been suggested for soybean, but seems to have been a general plant response to stress, so that higher accumulation may correspond to greater sensitivity of the genotype to drought.

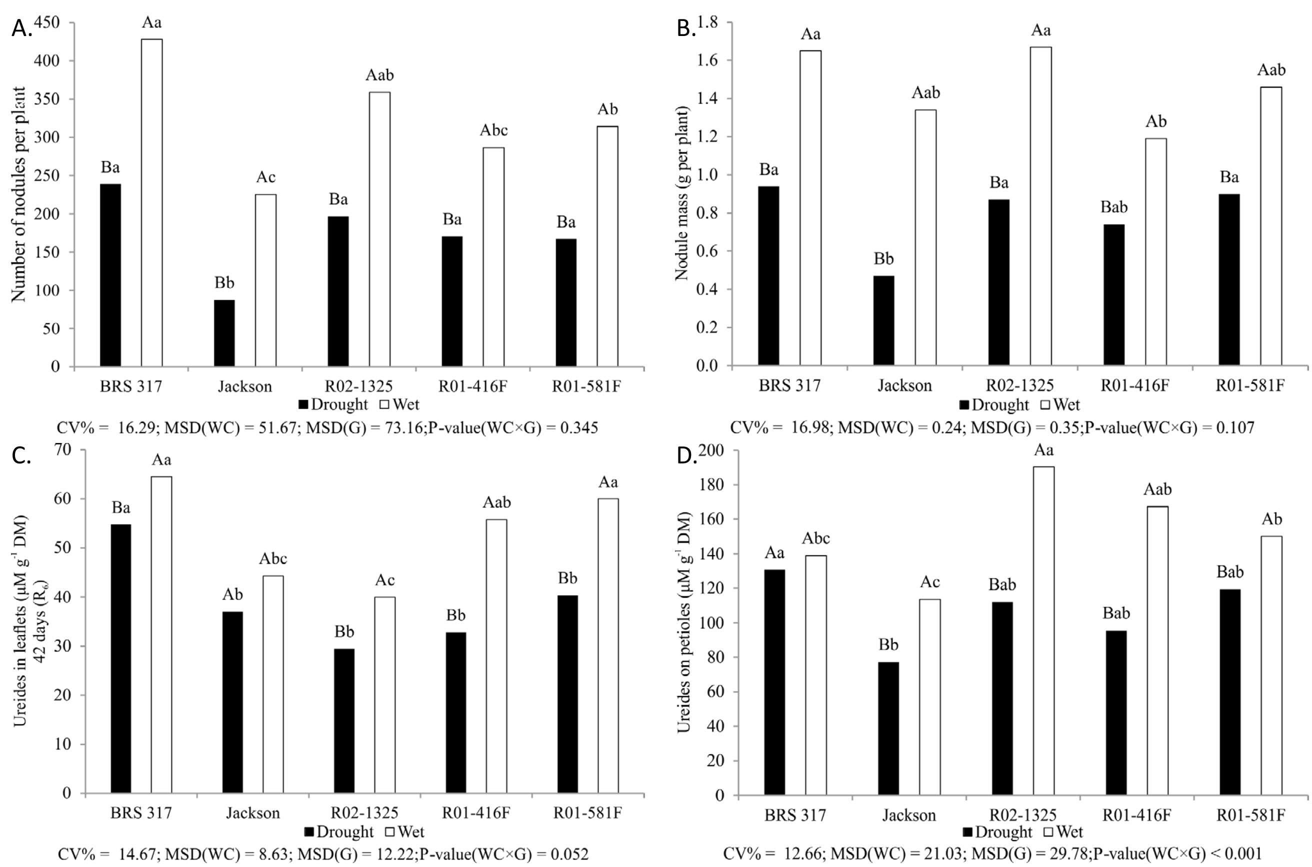
Increased ureide concentration in aerial part tissues also seems to be related to drought-induced senescence, in which nucleic acids are degraded to purines and pyrimidines that are relocated in the plant in the form of ureides (De Luca & Hungria, 2014).

Water restriction for 22 days decreased grain mass, except for BRS 317, which showed drought tolerance for this variable. Under water restriction, strain R01-416F produced 33% less compared with BRS 317, and did not differ from the other genotypes (Figure 2).

At stage $R_6$, after 42 days of water restriction, Jackson showed, in general, the lowest number and mass of nodules (Figure 3), but its specific mass remained close to 100% compared with the wet control plants (Figure 4).

Still at $R_6$, the 42 days of drought reduced ureide contents in leaflets, except Jackson, and in petioles, except BRS 317 that were not influenced by water condition. Leaf N concentration, grain mass, number and mass of dry nodules were reduced in all genotypes exposed to water restriction. However, water restriction increased the ureide concentration in nodules, except in R01-416F, which presented the lowest concentration compared with the other genotypes, especially when subjected to drought (Figure 3).

At $R_6$, BRS 317 showed the highest ureide concentration in leaflets under water restriction, while in petioles the differences were less evident. Ureide concentrations in nodules of strains R01-581F and R01-416F were lower than in the other genotypes under water restriction. Higher leaf

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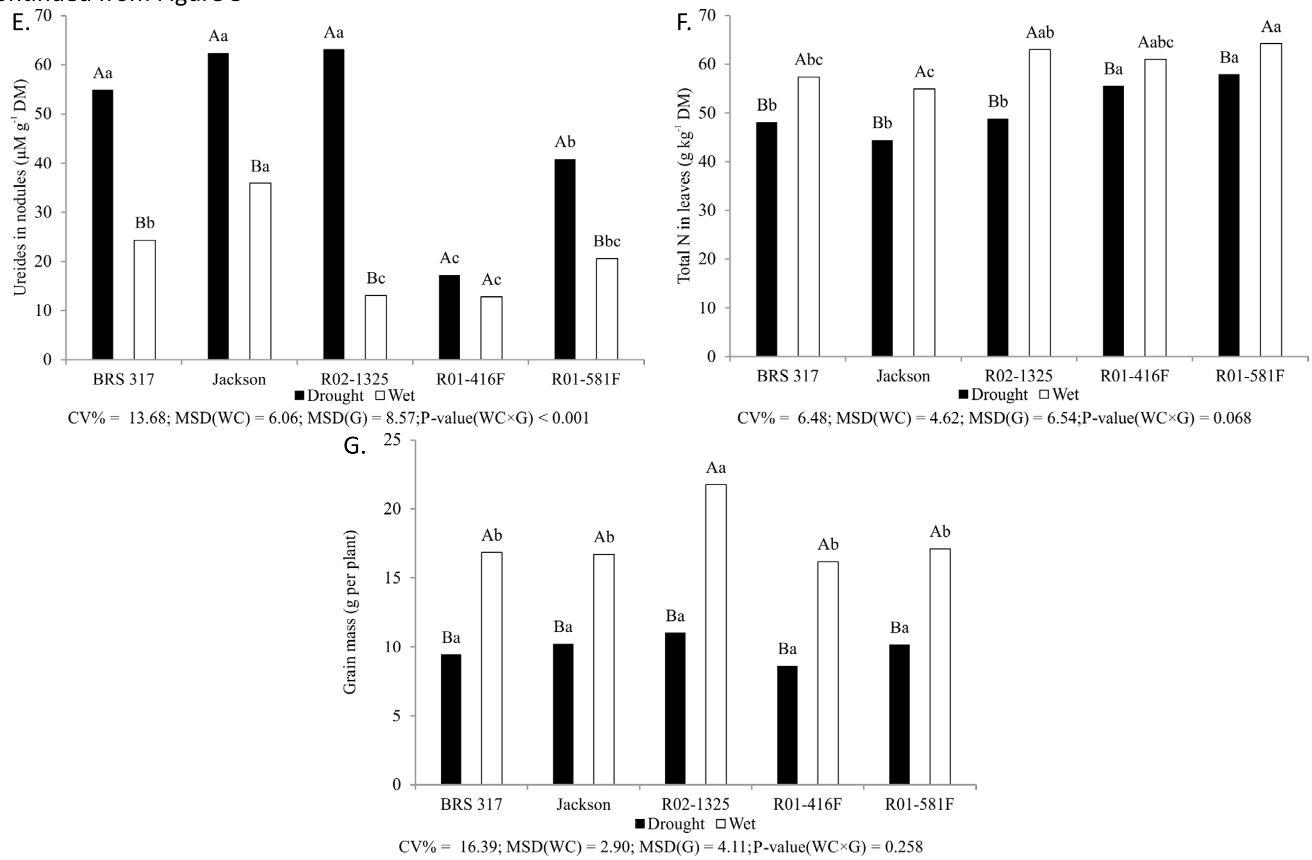


Means followed by the same letter, uppercase (water condition, within genotype) and lowercase (genotypes, within water condition) do not differ by Tukey test (p ≥ 0.05). CV - Coefficient of variation; MSD - Minimal significant difference; WC - Water condition; G - Genotype; DM - Dry mass.

**Figure 3.** Number (A) and mass (B) of nodules, ureide concentration (leaflets - C, petioles - D, and nodules - E), leaf N concentration (F) and grain mass per plant (G) of soybean genotypes (BRS 317, Jackson, R02-1325, R01-416F, and R01-581F), exposed to wet (80% of FC) or drought (30% of FC) conditions for 42 days between the reproductive stages of flowering ($R_1$) and grain filling ($R_6$).

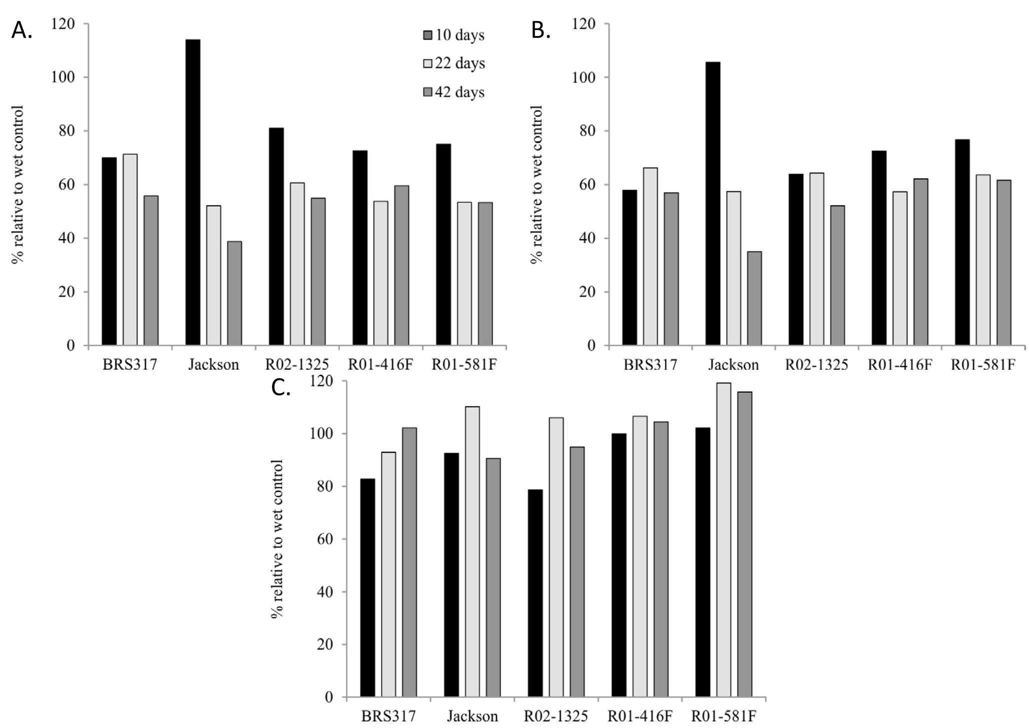


**Figure 4.** Percentage in relation to the wet control of the number (A), mass (B), and specific mass (C) of nodules of soybean genotypes (BRS 317, Jackson, R02-1325, R01-416F, and R01-581F) obtained under drought condition (30% of FC) in relation to the wet condition (80% of FC) at 10 ($R_2$), 22 ($R_4$), and 42 ($R_6$) days of exposure to water restriction from the $R_1$ stage.

N concentrations were observed in strains R01-581F and R01-416F, with more evident effects under water restriction (Figure 3).

Sinclair et al. (2007) observed higher leaf N concentration in the strains R01-416F and R01-581F in relation to the parental Jackson, indicating that the attributes inherited from the parental make the strains more tolerant to drought, and with greater yield potential in this condition. These results corroborate the observations at 22 and 42 days under drought (Figures 2 and 3), where strains R01-416F and R01-581F showed higher leaf N concentrations compared with the genotypes Jackson, BRS 317, and R01-1325. However, higher foliar N concentrations did not result in higher grain yield, especially for R01-416F, indicating that although foliar N concentration is important for yield potential, other factors also interfere with grain yield (Figure 3).

The results for grain mass indicated that the cultivar Jackson and the strain R01-581F showed tolerance to water restriction at 30% of FC for up to 10 days during flowering stages (Figure 1). Likewise, cultivar BRS 317 behaved as drought tolerant, but for up to 22 days under water restriction at 30% of FC between flowering and pod formation stages (Figures 1 and 2). Among the genotypes, strain R01-416F showed the greatest reduction in grain yield when exposed to drought for 10 and 22 days during the flowering ($R_1$/$R_2$) and pod formation ($R_4$). Exposure to water restriction for 42 days ($R_6$) reduced the performance of all genotypes. However, under full water supply, the highest grain mass yield was obtained with strain R02-1325 (Figures 1, 2, and 3). Among the strains, R01-581F and R02-1325 show potential for use in breeding programs for drought tolerance when compared with R01-416F.

## Conclusions

Exposure to drought for 10 days during flowering at 30% of FC reduced nodulation by an average of 30%, except for the Jackson genotype. Yet in this drought phase, leaf N concentration was 17% lower, grain yield reduced for R01-1325 and R02-416F by an average of 35%, and nodule ureide concentration increased by 72%, except for R02-416F. In contrast, ureide concentration increased in petioles by an average of 90% after 10 days under drought at flowering.

The grain mass revealed BRS 317, Jackson, and R01-581F as drought-tolerant under 30% of FC for 10 days during the flowering, while R02-1325 and R01-416F had an average reduction of 35%. BRS 317 was also tolerant for 22 days of exposure to drought between flowering and pod formation, while the other genotypes were susceptible, with an average reduction of 36% in grain yield. At this stage, after 22 days of drought, there was a decrease in the number and mass of nodules by 40% and in the ureide concentration in nodules by 34%.

With the increase of the exposure for 42 days under drought, between stages $R_2$ and $R_6$, all genotypes were susceptible to water restriction at 30% of FC, with an average reduction by 46% in the number and mass of nodules and 44% in grain mass, while the N concentration in leaves decreased by 15% and that of ureides in leaflets and petioles by 29 and 35%, respectively, whereas the ureide concentrations in nodules increased by 136%.

R02-1325 showed 30% higher grain yield compared with the other genotypes under full water supply at 80% of FC and did not differ from the most productive under drought at 30% of FC, showing to be promising for use in breeding programs aiming to increase drought tolerance.

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## Compliance with Ethical Standards

**Author contributions:** Conceptualization: VCNJ, CECP, MAN; Data Curation: VCNJ; Formal Analysis VCNJ; Funding Acquisition: MH, MAN; Investigation: VCNJ, CECP; Methodology: CECP, MH, MAN; Project Administration: VCNJ, CECP; Resources: CECP, MH, MAN; Supervision: VCNJ, CECP; Validation: CECP, MH, MAN; Visualization: VCNJ; Writing-original draft: VCNJ; Writing-review & editing: CECP, MAN.

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