

Biological nitrogen fixation in soybean under water restriction and exposed to 1-methylcyclopropene

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Abstract – The objective of this work was to assess the effects of 1-methylcyclopropene (1-MCP) on traits related with biological nitrogen fixation on 'BRS 268' soybean (*Glycine max*) subjected to water restriction. Plants were either exposed to drought between 32 (R₂) and 47 (R₃) days after sowing (DAS) or kept well-watered, in combination with exposure or not to 1-MCP. On the second day under drought (34 DAS), plants from both water conditions were exposed to 1-MCP in a hermetically sealed chamber for 15 hours. Control plants, dry or well-watered, that were not exposed to 1-MCP were kept in a separate chamber. At 36 (R₂) and 47 (R₃) DAS, shoot and root dry weights, leaf area index, number and dry weight of nodules, total ureides in sap, and N concentration in leaves were assessed. From 47 DAS on, extra plants were well watered until physiological maturity (R₈) and assessed for yield components. Water restriction increased ureides in sap and reduced N in leaves in R₂; reduced the number and mass of nodules, shoot dry weight, and leaf area index in R₃; and reduced the number of pods and seed mass of plants not exposed to 1-MCP. However, when plants are exposed to 1-MCP, there is an attenuation of water restriction effects.

Index terms: *Bradyrhizobium*, *Glycine max*, inhibitor of ethylene, nodulation, water stress.

Fixação biológica do nitrogênio em soja sob restrição hídrica e exposta ao 1-metilciclopropeno

Resumo – O objetivo deste trabalho foi avaliar os efeitos do 1-metilciclopropeno (1-MCP) em parâmetros relacionados à fixação biológica de nitrogênio em soja (*Glycine max*) 'BRS 268' submetida à restrição hídrica. As plantas foram submetidas à seca entre 32 (R₂) e 47 (R₃) dias após a semeadura (DAS) ou mantidas bem irrigadas, em combinação com exposição ou não ao 1-MCP. No segundo dia sob seca (34 DAS), as plantas de ambas as condições hídricas foram expostas ao 1-MCP em câmara hermeticamente fechada por 15 horas. As plantas-controle, sob restrição hídrica ou bem irrigadas, não expostas ao 1-MCP foram mantidas em câmara separada. Aos 36 (R₂) e 47 (R₃) DAS, foram avaliados massa seca da parte aérea e das raízes, índice de área foliar, número e massa seca de nódulos, ureídeos totais na seiva e concentração de N foliar. A partir dos 47 DAS, as plantas remanescentes foram mantidas bem irrigadas até a maturação fisiológica (R₈) e os componentes de produção foram avaliados. A restrição hídrica aumentou os ureídeos na seiva e reduziu o N foliar em R₂; reduziu o número e a massa de nódulos, a massa seca da parte aérea e o índice de área foliar em R₃; e reduziu o número de vagens e a massa de sementes em plantas sem exposição ao 1-MCP. No entanto, quando as plantas são expostas ao 1-MCP, há atenuação do efeito da restrição hídrica.

Termos para indexação: *Bradyrhizobium*, *Glycine max*, inibidores de etileno, nodulação, estresse hídrico.

Introduction

Drought is one of the main concerns in cropping systems, especially at critical stages of the plant cycle, such as the reproductive ones (Nascimento et al., 2011), and may lead to severe losses. Several physiological processes are impaired in soybean (*Glycine max* L.) under drought, among which biological nitrogen fixation (BNF) is one of the most sensitive, even more

than transpiration and photosynthesis (Sinclair et al., 2007; Cerezini et al., 2014).

Ethylene is known for acting in the ripening process, but it is also produced by plants under stressing conditions, including drought, which leads to a cascade of events that result in senescence. Compounds like 1-methylcyclopropene (1-MCP) compete with ethylene for binding sites in cell receptors and block the cascade of events associated to ripening and

senescence (Li et al., 2016). Based on this information, the use of an inhibitor of ethylene might attenuate the effect of drought on plants.

1-MCP is widely used to keep the quality and prolong the shelf life of climacteric and non-climacteric fruits. It acts as an inhibitor of ethylene action, resulting in a delay of the maturation process (Li et al., 2016). Similarly, 1-MCP is also employed to increase the shelf life of flowers after harvesting; the treatment with 1-MCP, for example, delayed the senescence of roses in 19 days (De Pietro et al., 2010).

There are no research results, however, showing the effect of 1-MCP on the attenuation of drought on soybean. In cotton (*Gossypium hirsutum* L.) subjected to drought, the increase of ethylene levels caused early senescence of leaves, which was delayed in plants treated with 1-MCP. As a result, plants increased water use efficiency and chlorophyll concentration, suggesting an attenuation of drought stress (Chen et al., 2015). It should be highlighted that each plant species may have a different response to 1-MCP, requiring an adjustment for each crop condition (Watkins, 2006).

Exposure of soybean to 1-MCP at the flowering stage might attenuate the effects of water restriction on traits related to BNF, as well as the negative effects on plant development and yield components.

The objective of this work was to assess the effects of 1-methylcyclopropene (1-MCP) on traits related to biological nitrogen fixation on 'BRS 268' soybean (*Glycine max*) subjected to water restriction.

Materials and Methods

The experiment was carried out under greenhouse conditions at the department of agronomy of Universidade Estadual de Londrina, in the municipality of Londrina, in the state of Paraná, Brazil.

'BRS 268' soybean plants of determinate growth were grown in 5-L ceramic pots containing 5 kg of substrate (topsoil:compost, 3:1, v/v). The results of chemical analysis (Silva, 2009) showed: 5.8 pH (CaCl₂); 4.6 cmol_c dm⁻³ Ca²⁺; 1.6 cmol_c dm⁻³ Mg²⁺; 0.0 cmol_c dm⁻³ Al³⁺; 2.5 cmol_c dm⁻³ H⁺+Al³⁺; 1.1 cmol_c dm⁻³ K⁺; 13.4 g kg⁻¹ C; 23.0 g kg⁻¹ organic matter; 57.8 mg dm⁻³ P; cation exchange capacity at pH 7.0 of 9.8 cmol_c dm⁻³; and base saturation of 75%.

Soybean seeds were inoculated with peat-based inoculant containing 1x10⁹ cells g⁻¹ of N-fixing bacteria, *Bradyrhizobium* spp., of the Semia 587 and

Semia 5080 strains (Rizo-Liq, Rizobacter do Brasil, Cambé, PR, Brazil). Five seeds were sown per pot and thinned to two plantlets at the V₂ stage (Fehr & Caviness, 1977). The treatments consisted of two water conditions: well-watered or dry, in combination with exposure or not to 1-MCP at the R₂ stage, forming a 2x2 factorial arrangement, in an completely randomized design, with eight replicates.

Ninety-six pots containing soybean plants were mounted to be assessed at 37 and 47 days after sowing (DAS), as well as at physiological maturity. All plants were well watered (water replacement to reach 80% field capacity) up to 32 DAS, corresponding to the R₂ reproductive stage. From 32 DAS on, drought-simulation watering was applied to reach only 40% soil field capacity in the dry treatments, whereas well-watered plants continued to receive water to reach 80% field capacity. The water conditions were kept by weighing each pot daily and replacing the amount of water lost in each water condition. At 34 DAS (two days under water restriction), plants of 48 plots were exposed to 1-MCP, and at 37 DAS (four days under water restriction), 32 plots were analyzed. Water restriction was carried out up to 47 DAS, at the R₃ stage, i.e., 15 days under drought, when another assessment was made in 32 plots. From 48 DAS on, 32 remaining plots were kept under well-watered conditions up to physiological maturity (R₈), when they were evaluated for the following yield components: number of pods and seeds per plants, and mass of seeds.

The trial was performed from February to June 2010, using 'BRS 268' soybean, tolerant to photoperiod. Average air temperatures in the period were: maximum of 26.7°C, average of 21.4°C, and minimum of 16.1°C. The average relative humidity was 74%.

The exposure to 1-MCP occurred in a 1.50-m³ hermetically sealed plastic chamber, in which 6.4 g of the formulated product AFxRD-014 (SmartFresh, Agrofresh, Inc., Collegeville, PA, USA) were dissolved in 75 mL deionized water and kept in an open beaker. Considering the amount of 1-MCP and its vapor pressure, the air-solution surface, and the chamber volume, the estimated concentration in the atmosphere was 1,000 ppb. To assure a homogeneous distribution, fans were placed within the chamber. Control plants not exposed to 1-MCP were kept in an identical chamber in another greenhouse to avoid contamination. After 15 hours, plants from both treatments were maintained

in the same greenhouse. Green tomatoes (*Solanum lycopersicum* L.) were kept in the hermetically sealed chambers along with the evaluated plants to check the effect of 1-MCP application, which caused late ripening of the tomato fruits.

At the R₂ and R₃ stages, shoots were cut just above the cotyledonal node, early in the morning. A 200- μ L pipette tip was fit onto the stump to collect the flowing sap coming from the roots. To make sap exudation easier, all pots received 1.2 L of water the night before. Sap samples were collected with a micropipette and stored in microtubes at -15°C until analysis for ureides (Vogels & Van der Drift, 1970; Hungria, 1994). Briefly, 5 μ L of sap, in duplicate, were subjected to a sequence of alkaline and acid hydrolysis and to colorimetric quantification at 535 nm after reaction with phenylhydrazine and potassium ferricyanide. Data were expressed as nmol of ureides per millimeter of sap.

Plants collected at the R₂ and R₃ stages were also assessed for number and mass of root nodules. Leaf area was determined in a leaf area meter, model LI-3100 (LI-COR, Lincoln, NE, USA) and used to calculate the leaf area index (LAI) (Adami et al., 2008). Shoot and root dry weights were obtained after forced-air oven-drying, at 65°C, until constant weight. The concentration of N in shoots was based on the Kjeldahl method in sulfuric-acid digested extracts (Tedesco et al., 1995). Plants remaining up to the R₈ stage were evaluated for number of pods and seeds, and mass of seeds per plant.

The dataset was subjected to a two-way analysis of variance for each evaluation date, following Tukey's test, at 5% probability.

Results and Discussion

The exposure to 1-MCP at R₂, that is, four days under water restriction and three days after exposure to 1-MCP, resulted in higher nodule dry weight and higher ureide contents in the sap of plants. Considering the effect of water condition, water restriction increased the concentration of ureides in the sap of plants exposed to 1-MCP, but decreased it in plants not exposed to the compound; however, under well-watered conditions, the exposure to 1-MCP decreased the concentration of ureides. Under dry conditions, the concentration of N in leaves decreased, regardless of the exposure to 1-MCP (Table 1), whereas the other variables related to plant growth (shoot and root dry weights)

and the LAI were not affected. Although no effect was observed on plant growth-related parameters, water restriction for only four days showed negative effects on BNF-related parameters. According to the literature, an increase in ureides can be attributed to an impairment of assimilation and transportation of N compounds caused by drought, which may lead to a feedback inhibition of the BNF process (King &

Table 1. Assessments of 'BRS 268' soybean (*Glycine max*) plants at the R₂ stage, on the fourth day under water restriction and three days after exposure to 1-methylcyclopropene (1-MCP), and their respective controls⁽¹⁾.

| Variables/ treatments | Exposed to 1-MCP | Not exposed to 1-MCP | Average |
|---|---------------------------------------|-------------------------|---------|
| Number of nodules per plant | | | |
| Dry | 45.68Aa | 35.62Aa | 40.65A |
| Well watered | 42.43Aa | 38.06Aa | 40.25A |
| Average | 44.06a | 36.84a | - |
| CV and p-value ⁽²⁾ | 26.3%; W=0.9148; M=0.0652; WxM=0.4560 | | |
| Nodule dry weight (mg) | | | |
| Dry | 71.62Aa | 50.25Ab | 60.93A |
| Well watered | 58.69Aa | 67.02Aa | 62.87A |
| Average | 65.15a | 58.65a | - |
| CV and p-value | 29.1%; W=0.7630; M=0.3162; WxM=0.0270 | | |
| Root dry weight (g) | | | |
| Dry | 0.92Aa | 0.77Aa | 0.85A |
| Well watered | 0.84Aa | 0.70Aa | 0.77A |
| Average | 0.88a | 0.73a | - |
| CV and p-value | 32.9%; W=0.4005; M=0.1343; WxM=0.9843 | | |
| Shoot dry weight (g) | | | |
| Dry | 5.38Aa | 4.28Aa | 4.83A |
| Well watered | 4.80Aa | 4.45Aa | 4.63A |
| Average | 5.09a | 4.37a | - |
| CV and p-value | 25.4%; W=0.6408; M=0.0996; WxM=0.3909 | | |
| Total ureides in sap (nmol mL ⁻¹) | | | |
| Dry | 7.42Aa | 4.50Bb | 5.96A |
| Well watered | 3.50Bb | 6.15Aa | 4.82B |
| Average | 5.46a | 5.33a | - |
| CV and p-value | 24.9%; W=0.0237; M=0.790; WxM<0.0001 | | |
| Leaf area index (cm ² cm ⁻²) | | | |
| Dry | 3.79Aa | 3.09Aa | 3.44A |
| Well watered | 3.43Aa | 3.20Aa | 3.31A |
| Average | 3.61a | 3.15a | - |
| CV and p-value | 22.9%; W=0.6422; M=0.1009; WxM=0.3961 | | |
| N concentrations in leaves (g kg ⁻¹) | | | |
| Dry | 48.15Ba | 47.76Aa | 47.95B |
| Well watered | 53.59Aa | 50.46Aa | 52.02A |
| Average | 50.87a | 49.11a | - |
| CV and p-value | 7.7%; W=0.0058; M=0.2076; WxM=0.3228 | | |

⁽¹⁾Means followed by equal letters, uppercase in the columns and lowercase in the lines, do not differ by Tukey's test, at 5% probability. ⁽²⁾CV (%), coefficient of variation and p-value for water condition (W=), 1-MCP (M=), and WxM=.

Purcell, 2005). Sinclair et al. (2007) found differences between genotypes exposed to drought regarding N concentration in leaves. However, there have been no reports on the effect of 1-MCP on N compounds or even on N concentration in leaves of soybean exposed to drought. The present study, therefore, is apparently the first known work on 1-MCP effect on soybean exposed to drought.

Table 2. Assessments of 'BRS 268' soybean (*Glycine max*) plants at the R₃ stage, on the fifteenth day under water restriction and 12 days after exposure to 1-methylcyclopropene (1-MCP), and their respective controls⁽¹⁾.

| Variables/ treatments | Exposed to 1-MCP | Not exposed to 1-MCP | Average |
|---|---------------------------------------|-------------------------|---------|
| Number of nodules per plant | | | |
| Dry | 50.00Ba | 31.50Bb | 40.75B |
| Well watered | 75.12Aa | 59.62Ab | 67.37A |
| Average | 62.56a | 45.56b | - |
| CV and p-value ⁽²⁾ | 24.2%; W<0.0001; M=0.001; WxM=0.7479 | | |
| Nodule dry weight (mg) | | | |
| Dry | 127.75Ba | 102.25Ba | 115.00B |
| Well watered | 246.87Aa | 155.37Ab | 201.12A |
| Average | 187.31a | 128.81b | - |
| CV and p-value | W=<0.0001; M=0.0012; WxM=0.051 | | |
| Root dry weight (g) | | | |
| Dry | 1.20Aa | 1.29Aa | 1.25A |
| Well watered | 1.56Aa | 1.37Aa | 1.46A |
| Average | 1.38a | 1.33a | - |
| CV and p-value | 29.4%; W=0.1371; M=0.7326; WxM=0.3302 | | |
| Shoot dry weight (g) | | | |
| Dry | 5.97Ba | 5.37Ba | 5.67B |
| Well watered | 7.38Aa | 7.39Aa | 7.39A |
| Average | 6.67a | 6.38a | - |
| CV and p-value | 20.3%; W=0.0010; M=0.537; WxM=0.5131 | | |
| Total ureides in sap (nmol mL ⁻¹) | | | |
| Dry | 8.90Aa | 9.40Aa | 9.15A |
| Well watered | 10.42Aa | 5.42Bb | 7.92A |
| Average | 9.66a | 7.41b | - |
| CV and p-value | 32.1%; W=0.2147; M=0.028; WxM=0.0085 | | |
| Leaf area index (cm ² cm ⁻²) | | | |
| Dry | 4.18Ba | 3.79Ba | 3.99B |
| Well watered | 5.09Aa | 5.10Aa | 5.09A |
| Average | 4.63a | 4.44a | - |
| CV and p-value | 18.7%; W=0.001; M=0.5347; WxM=0.5109 | | |
| N concentrations in leaves (g kg ⁻¹) | | | |
| Dry | 38.97Aa | 40.60Aa | 39.78A |
| Well watered | 36.05Aa | 34.42Ba | 35.24B |
| Average | 37.51a | 37.51a | - |
| CV and p-value | 11.1%; W=0.0045; M=0.9975; WxM=0.2775 | | |

⁽¹⁾Means followed by equal letters, uppercase in the columns and lowercase in the lines, do not differ by Tukey's test, at 5% probability. ⁽²⁾CV (%), coefficient of variation and p-value for water condition (W=), 1-MCP (M=), and WxM=.

The assessments at the R₃ stage showed a more evident effect of water restriction in reducing nodulation, i.e., both number and mass of nodules, than at R₂ (Table 2). In that stage, the exposure to 1-MCP stimulated nodulation, regardless of the water condition. With the exposure to 1-MCP, the concentration of ureides in the sap increased under well-watered conditions, but remained the same under dry ones; however, when plants were not exposed to 1-MCP, dry conditions resulted in an increase of ureides in the sap. The concentration of N in leaves did not change with the exposure to 1-MCP, but increased with drought in plants not exposed to 1-MCP.

Root dry weight was not affected by treatments, whereas shoot dry weight and the LAI decreased under water restriction (Table 2). This decrease in shoots under dry conditions can be responsible for higher N concentrations in plants, considering the concentration effect on smaller plants. An increase in N concentration in soybean with restricted growth due to drought has also been reported in another study (Streeter, 2003).

Water restriction reduced the number of pods by 14.5% and the mass of seeds by 12.2% in plants not exposed to 1-MCP (Table 3). It should be noted that shoot dry weight is highly related to the LAI, which

Table 3. Assessments relative to yield components of 'BRS 268' soybean (*Glycine max*) plants at the R₈ stage, after exposure to water restriction and/or 1-methylcyclopropene (1-MCP) at the R₂ stage, and their respective controls⁽¹⁾.

| Variables/ treatments | Exposed to 1-MCP | Not exposed to 1-MCP | Average |
|-----------------------------|---------------------------------------|-------------------------|---------|
| Number of pods per plant | | | |
| Dry | 19.06Aa | 18.31Ba | 18.68B |
| Well watered | 21.75Aa | 21.93Aa | 21.84A |
| Average | 20.40a | 20.12a | - |
| CV (%) and p-value | 13.9%; W=0.003; M=0.7781; WxM=0.6391 | | |
| Number of seeds per plant | | | |
| Dry | 40.43Aa | 39.43Aa | 39.93B |
| Well watered | 46.12Aa | 44.81Aa | 45.46A |
| Average | 43.28a | 42.15a | - |
| CV (%) and p-value | 13.4%; W=0.0107; M=0.5722; WxM=0.939 | | |
| Mass of seeds per plant (g) | | | |
| Dry | 8.98Aa | 8.54Ba | 8.76B |
| Well watered | 10.06Aa | 9.90Aa | 9.98A |
| Average | 9.52a | 9.22a | - |
| CV (%) and p-value | 13.1%; W=0.0091; M=0.4997; WxM=0.7527 | | |

⁽¹⁾Means followed by equal letters, uppercase in the columns and lowercase in the lines, do not differ by Tukey's test, at 5% probability. CV (%), coefficient of variation and p-value for water condition (W=), 1-MCP (M=), and WxM=.

may correlate with crop yield (Sartori et al., 2015). The assessment in R₃ showed a reduction of the LAI under water restriction. Therefore, a reduction in yield caused by water restriction was also observed at the R₈ stage (Table 3). Soybean plants subjected to water stress at early reproductive stages had 13.7% reduction in yield, but the exposure to 1-MCP at R₂ attenuated losses. Even though the exposure to 1-MCP attenuated the effect of water restriction on nodulation, there was no significant effect on yield in this trial.

The effects of drought on traits related to BNF have been widely studied (King & Purcell, 2005; Hungria et al., 2006; Sinclair et al., 2007; Cerezini et al., 2014). However, despite some works on cotton, flowers, and fruits (Serek et al., 2006; De Pietro et al., 2010; Chen et al., 2015; Li et al., 2016), there is no knowledge of researches on the effect of 1-MCP on soybean, aiming to the attenuate negative effects of ethylene induced by drought stress on plant senescence, including nodules. This strategy could help plants to cope with periods under water restriction and reduce the negative effects on BNF. However, more studies are needed to validate the technical and economic viability of 1-MCP application on commercial soybean crops exposed to drought.

Conclusion

The exposure to 1-methylcyclopropene at the beginning of water restriction attenuates the effects of drought on soybean (*Glycine max*) nodulation.

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