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Physiological and biochemical responses of soybean to drought as represented by the fraction of transpirable soil water

Respostas fisiológicas e bioquímicas da soja à seca representadas pela fração de água transpirável do solo

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Highlights

The FTSW threshold for transpiration can be used for the indication of cultivars.
Cultivars DM 66I68 RSF IPRO and NA 5909 RG showed a more efficient stomatal control.
The antioxidant defense mechanisms were activated in plants under rainfed conditions.

Abstract

The influence of water deficit on plant physiological and biochemical responses as measured by the fraction of transpirable soil water (FTSW) has not been investigated in cultivars developed by the world's largest soybean producer. This information can help obtain plants with improved tolerance to the abiotic stress that most affects soybean production in Brazil, enabling adaptation to edaphoclimatic conditions to enhance the crop's yield potential. We aim to determine the FTSW threshold for transpiration and evaluate changes in the growth, physiological activities, and biochemical and antioxidant responses of soybean cultivars. Three trials were sown on 11/19/2018 (T1), 12/28/2018 (T2), and 9/9/2019 (T3), representing

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almost the entire soybean sowing window in Brazil. The estimated FTSW threshold values were 0.33, 0.29, and 0.31 in T1; 0.35, 0.41, and 0.43 in T2; and 0.31, 0.49, and 0.45 in T3 for cultivars BMX GARRA IPRO, DM 66I68 RSF IPRO, and NA 5909 RG, respectively. In the three trials, NA 5909 RG showed the greatest height. The POD enzyme was activated in non-irrigated plants in T2 only in cvs. DM 66I68 RSF IPRO and NA 5909 RG. We conclude that cvs. DM 66I68 RSF IPRO and NA 5909 RG showed a more efficient stomatal control, conserving soil water for a longer time, which indicates greater tolerance to water deficit.

Key words: Antioxidant enzymes. FTSW threshold. *Glycine max* L. Growth. Transpiration.

Resumo

A influência do déficit hídrico nas respostas fisiológicas e bioquímicas das plantas pelo estudo da fração de água transpirável do solo (FATS) não foi investigada para cultivares produzidas pelo maior produtor mundial de soja. Essas informações podem auxiliar na obtenção de plantas com maior tolerância ao estresse abiótico que mais afeta a produção de soja no Brasil, possibilitando a adaptação às condições edafoclimáticas para explorar o potencial produtivo da cultura. Nosso objetivo é determinar a FATS crítica para a transpiração, avaliar mudanças no crescimento, atividades fisiológicas e respostas bioquímicas e antioxidantes em cultivares de soja. Três ensaios foram semeados em 19/11/2018 (E1), 28/12/2018 (E2) e 9/9/2019 (E3), representando quase toda a janela de semeadura da soja no Brasil. Os valores de FATS crítica estimados foram 0,33, 0,29 e 0,31 em E1, 0,35, 0,41 e 0,43 em E2 e 0,31, 0,49 e 0,45 em E3 para os cultivares BMX GARRA IPRO, DM 66I68 RSF IPRO e NA 5909 RG, respectivamente. Nos três ensaios, NA 5909 RG apresentou uma estatura superior. A enzima POD foi ativada em plantas não irrigadas em E2 apenas em DM 66I68 RSF IPRO e NA 5909 RG. Concluímos que DM 66I68 RSF IPRO e NA 5909 RG mostraram um controle estomático mais eficiente, conservando a água do solo por mais tempo, o que indica maior tolerância ao déficit hídrico.

Palavras-chave: Crescimento. Enzimas antioxidantes. FATS crítica. *Glycine max* L. Transpiração.

Introduction

Soybean (*Glycine max* L.) is one of the most important protein sources for both human and animal nutrition, in addition to being responsible for 60% of oil production for consumption and biofuel (Tagliapietra et al., 2018; Wang et al., 2018). Brazil is the world's largest producer and exporter of soybean, with a cultivated area of 39 million hectares, an average production of 123 million tons in three years (2019-2021), an average yield of 3.4 Mg ha^{-1}, and an average grain export of 76 million tons (Companhia Nacional de Abastecimento [CONAB], 2022).

The primary factor that causes yield losses in soybean in the south of Brazil is water deficit. More than 90% of the Brazilian soybean crops are rainfed, which leads to frequent water deficit periods during the growing season (Sentelhas et al., 2015; Zanon et al., 2016). The state of Rio Grande do Sul (RS) is the third-largest national soybean producer. Losses due to water stress are frequent in RS, having reduced 30% to 50% of the soybean yield in the 2004/2005, 2005/2006, and 2011/2012 harvests (CONAB, 2022).

Furthermore, soybean has become significant in the "lowland cultivation" system in the last ten years in the southern half of Rio Grande do Sul (Rocha et al., 2017; Ribas et al., 2021a). Soybean is grown in crop rotation with irrigated rice, a practice that reduces the weed seed bank in rice fields and constitutes income diversification for producers (Theisen et al., 2017; Ribas et al., 2021b). However, soils in lowland areas are shallow, having low drainage capacity, and thus denominated hydromorphic soils, which may cause both water excess and deficit that lead to significant reductions in yield. Plants produce reactive oxygen species (ROS) under water deficit conditions, which are regulated by antioxidative defense mechanisms that can be enzymatic or non-enzymatic (Darmanti et al., 2016). Plant tolerance to water deficit can be a function of antioxidant capacity in response to drought (Laxa et al., 2019). Another important aspect is the influence of water deficit on biological nitrogen fixation in soybean since the accumulation of nitrogen to achieve high productivity is essential to support protein synthesis (Sinclair et al., 2015).

The fraction of transpirable soil water (FTSW) methodology was the concept that most closely indicated the amount of water extracted by plants by transpiration (Sinclair & Ludlow, 1986; Lago et al., 2011, 2012; Souza et al., 2014; Kelling et al., 2015). By using FTSW, it is possible to control the availability of water to plants and the stage of the plant cycle at which one wishes to impose the water deficit to examine the plants' response. Studies using this methodology have also been conducted in soybean, but in trials outside Brazil and considering genotypes, growth habits, and relative maturity groups different from those of cultivars currently sown in Brazil (Sinclair & Ludlow, 1986; Bagherzadi et al., 2017).

The influence of water deficit on physiological plant responses as measured by FTSW has not been investigated in cultivars used by producers in Brazil. This information can help to obtain plants with enhanced tolerance to abiotic stresses that most cause yield losses in soybean in Brazil. This type of study is important since the FTSW threshold value can be used as a parameter to identify cultivars more tolerant to water deficit (Sinclair et al., 2015). Additionally, the Intergovernmental Panel on Climate Change indicates that drought frequency will increase in the south of Brazil. This study aims to fill the gap in research on the water deficit tolerance of soybean cultivars, determined at the vegetative stage using the FTSW threshold methodology for transpiration; and evaluate changes in the growth, physiological activities, and biochemical and antioxidant responses of soybean cultivars.

Material and Methods

Experimental location and design

The trials were sown on 11/19/2018 (T1), 12/28/2018 (T2), and 9/9/2019 (T3) and represent almost the entire soybean sowing window in Brazil. They were conducted inside a shelter covered with 200-μm low-density screen, located in the experimental area of the Crop Science Department of the Federal University of Santa Maria, Santa Maria, Rio Grande do Sul, Brazil (29°43′ S, 53°43 W, 95 m). The climate of the region is the Cfa type, according to the Köppen classification, i.e., humid subtropical with no defined dry season and hot summers (Kuinchtner & Buriol, 2001).

A completely randomized design was used in each trial, with six treatments, adopting a 3×2 bifactorial arrangement. Factor A represented the three soybean cultivars (three levels), and factor B, the water regimes (two levels: irrigated [IR] and non-irrigated [NI]), with one plant per pot as an experimental unit. The number of replicates in TI, T2, and T3 was eight, totaling 48 experimental units.

Plant material and cultivation conditions

The soybean cultivars used were BMX GARRA IPRO, with a relative maturity group (RMG) of 6.3, considered tolerant to water deficit; DM 66I68 RSF IPRO, RMG 6.6, considered tolerant to water excess and indicated for lowland soils; and NA 5909 RG, with a RMG of 6.2, one of the most cultivated in southern Brazil in the last years.

Plastic pots with a capacity of eight liters were used. The outer walls of the pots were painted white to reduce the absorption of solar radiation, which would increase the temperature of the soil and be a source of experimental error. The pots were filled with previously crushed soil of the frank textural class (horizon A), classified as a typic alitic Bruno-gray Argisol (Streck et al., 2018). Acidity corrections and soil fertilization were performed according to the results of soil analysis and the technical recommendations for soybean crops (Comissão de Química e de Fertilidade do Solo [CQFS RS/SC], 2016). The plants were selected according to their height, number of nodes, and leaf area before imposing the deficit, choosing the most uniform to avoid variations between the plants, which could influence the transpiration values.

Water deficit imposition

There was no restriction on water supplementation during initial plant growth and development. Water deficit in T1 began on 01/03/2019, when the plants were between stages V7 and V9; in T2, on 02/02/2019, when they were between V6 and V8; and in T3 on 11/19/2019, between V6 and V7, according to the scale described by Fehr and Cavines (1977). The plants were exposed to water deficit following the methodology proposed by Sinclair and Ludlow (1986), whereby the FTSW considers that the soil water content absorbed by the plant and released by transpiration varies between maximum transpiration, under field capacity, to 10% of maximum transpiration.

All the pots were saturated with water and allowed to drain for 20 to 24 h at the beginning of each trial to reach their field capacity, after which they were covered with white plastic film to minimize water loss through soil evaporation, thus ensuring that the water removed from the soil is only due to plant transpiration. Subsequently, the initial weight of each pot was determined, after which the water deficit was imposed.

The weight of all pots was determined at the end of the afternoon of every day using an electronic scale with 30-kg capacity and 5 g accuracy. Soon after, each pot without water deficit (irrigated [IR]) was irrigated with the amount of water lost by transpiration, determined as the difference between the weight of the pot and the initial weight. The plants with water deficit (non-irrigated [NI]) received no water from the imposition of water deficit until the end of the trial. Each trial began 24 h after pot saturation and ended when all plants with water deficit showed relative transpiration (RT) ≤ 10% of plant transpiration in pots without water deficit. This was when the final weight was determined. The limit of 0.1 RT (10%) was imposed, considering that, below this rate, the stomata are closed and loss of water is due only to epidermal conductance (Sinclair & Ludlow, 1986).

The data were analyzed by comparing the RT and FTSW values of each pot. Relative transpiration was calculated using the following equation:

$$RT = \frac{\text{Water loss NI plants (each pot)}}{\text{Mean water loss IR plants}} \qquad (1)$$

Daily water loss by the non-irrigated plants was calculated as the difference between the weight of each pot and its weight on the previous day. Daily water loss by the irrigated plants was determined as the difference between the weight of each pot and its weight on the day of the beginning of the trial (initial weight). After the end of each trial, FTSW was calculated daily for each non-irrigated pot using the Sinclair and Ludlow (1986) equation:

$$FTSW = \frac{\text{(Weight of each pot − Final weight)}}{\text{(Initial weight of each pot − Final weight)}} \qquad (2)$$

Climate conditions

The minimum (Min) and maximum (Max) daily air temperatures were measured during each trial using a thermo-hygrograph temperature and humidity datalogger/recorder installed inside a wooden weather shelter painted white and positioned inside the screened shelter. Daily global solar radiation was collected from the automatic weather station located approximately 300 m from the screened shelter where the trials were conducted. To consider the solar radiation inside the shelter, 80% plastic cover transmissivity was assumed, subtracting 20% to calculate the daily global solar radiation of the plants (Buriol et al., 1995). The air vapor pressure deficit (VPD) at 15h00 (close to the maximum daily VPD) was calculated.

Analyzed variables

The number of nodes (NN), plant height (PH; length, in cm, from the stem at ground level to the base of the last visible node), and leaf area (LA; in cm^2) of each leaf was evaluated in each trial using the estimation equation proposed by Richter et al. (2014). At T2, gas exchange measurements were performed on the last expanded leaf of the plants using an IRGA gas exchange analyzer (Portable Photosynthesis System LICOR, Nebraska, USA), which revealed the maximum photosynthetic rate (A_{max}; in μmol of CO_2 m^{-2} s^{-1}), stomatal conductance (gs; in mol of H_2O m^{-2} s^{-1}), and leaf transpiration (E; in mmol of H_2O m^{-2} s^{-1}).

At the end of each trial, the leaves were separated and dried in a forced-air oven at 65 °C until constant weight. The oven-dried T1 and T2 leaves were ground and reserved for analysis to determine nitrogen content (total N). A total of 0.2 g of the sample of dried and ground leaves was weighed and subjected to sulfuric digestion in the presence of catalysts, after which total N was determined by titration using the Kjeldahl method (Tedesco et al., 1995).

The biochemical and antioxidant enzyme activities of T1 and T2 leaves were analyzed. The hydrogen peroxide (H_2O_2) content was determined according to Loreto and Velikova (2001). Peroxidation of membrane lipids was estimated following the method described by El-Moshaty et al. (1993). The superoxide dismutase (SOD) enzyme activity was determined according to the spectrophotometric method described by Giannopolitis and Ries (1977). The guaiacol peroxidase (POD) enzyme activity was determined following Zeraik et al. (2008).

Normalization of RT variables, adjustment of data to the logistic equation, and statistical analysis

The RT variable was subjected to two normalizations. The first (RT1) was the application of equation 1, so that RT varied from zero to one and allowed large daily environmental variations during the experimental periods to be minimized. The second normalization (RT2) aimed to reduce variations between plants caused mainly by differences in plant size. For the second normalization, an FTSW value was found above which the RT was constant in all plants, which in this study was 0.6. Subsequently, the mean of the RT values with FTSW equal to or greater than 0.6 was calculated for each plant, and all initial estimates of RT were divided by these means (Sinclair & Ludlow, 1986; Lecoeur & Sinclair, 1996). The RT data were plotted according to the FTSW after undergoing the second normalization, adjusting the logistic equation to them:

$$Y = \frac{1}{\{1 + exp[-a(X-b)]\}} \qquad (3)$$

in which Y is the dependent variable (RT); X is the FTSW; and "a" and "b" are empirical coefficients, which were estimated through non-linear regression analysis using SAS (Statistical Analysis System). The FTSW threshold values for RT were estimated by the logistic equation as the value of FTSW when RT is 0.95 (Sinclair & Ludlow, 1986; Lecoeur & Sinclair, 1996, Lago et al., 2012).

The trials were analyzed in isolation. The data of the plant variables were analyzed using ANOVA by SISVAR software version 5.6. Means were compared using the Scott Knott test at 5% of error significance ($p<0.05$).

Results and Discussion

The three trials were conducted on sowing dates that provided different meteorological conditions for water deficiency (Figure 1). The average minimum and maximum air temperatures were, respectively, 21.7 and 32.6 ºC in T1; 20.0 and 36.0 ºC in T2; and 16.8 and 36.8 °C in T3 (Figure 1 A). The daily global solar radiation flux density incident inside the screened shelter was higher in T3 and T2 than in T1, averaging 19.5, 18.7, and 12.7 MJ m^{-2} day^{-1}, respectively (Figure 1 B). Vapor pressure deficit (VPD) at 15h00 showed an average value of 13.3 hPa in T1, which was lower than observed in T3 and T2, whose mean values were 23.0 and 19.6 hPa, respectively (Figure 1 C).

The differences in meteorological conditions during each trial resulted in a shorter duration of T3 (19 days) and T2 (19 days) than T1 (21 days), attributed to the higher values of air temperature and VPD. The latter is a meteorological variable that should be considered in water deficit trials since it highly influences plant transpiration flux (Riar et al., 2018).

The estimated FTSW threshold values (Figure 2), at which transpiration begins to be affected and indicates the beginning of stomatal closure, were 0.33, 0.29, and 0.31 for cultivars BMX GARRA IPRO, DM 66I68 RSF IPRO, and NA 5909 RG, respectively, in T1. The values obtained for the respective cultivars in T2 were 0.35, 0.41, and 0.43; and in T3, 0.31, 0.49, and 0.45.

Cultivar BMX GARRA IPRO, considered tolerant to water deficit, showed little difference in FTSW threshold values between the three studies, indicating that it maintains its stomata open longer regardless of the weather condition. Cultivars DM 66I68 RSF IPRO and NA 5909 RG were more sensitive to stomatal closure, showing a more efficient stomatal control mechanism, conserving water in the soil for longer since the FTSW threshold was higher. According to Bagherzadi et al. (2017), this may be a strategy to conserve soil water and withstand a more prolonged water deficiency, mainly in T2 and T3, which were the periods with the highest VPD. This could explain why DM 66I68 RSF IPRO is suitable for lowlands since it performs well in these soils, which quickly shift from excess to deficient water conditions. Cultivar NA 5909 RG is largely cultivated in southern Brazil, exhibiting good production stability even under frequent climatic variability.

Cultivars with higher FTSW thresholds indicate a more efficient stomatal mechanism through earlier stomatal closure, conserving soil water, an adaptive criterion to support more prolonged water deficiencies (Sinclair et al., 2015). Physiologists have widely used the FTSW threshold values to indicate water deficit tolerance in soybean breeding programs in the USA (Sadok & Sinclair, 2009). Furthermore, a study of transpiration due to different VPD conditions revealed slow wilt in the field in a plant introduced from Japan, PI 416937, which showed constant RT limitation above a VPD of 21 hPa, emphasizing the influence of VPD on plant transpiration (Fletcher et al., 2007).

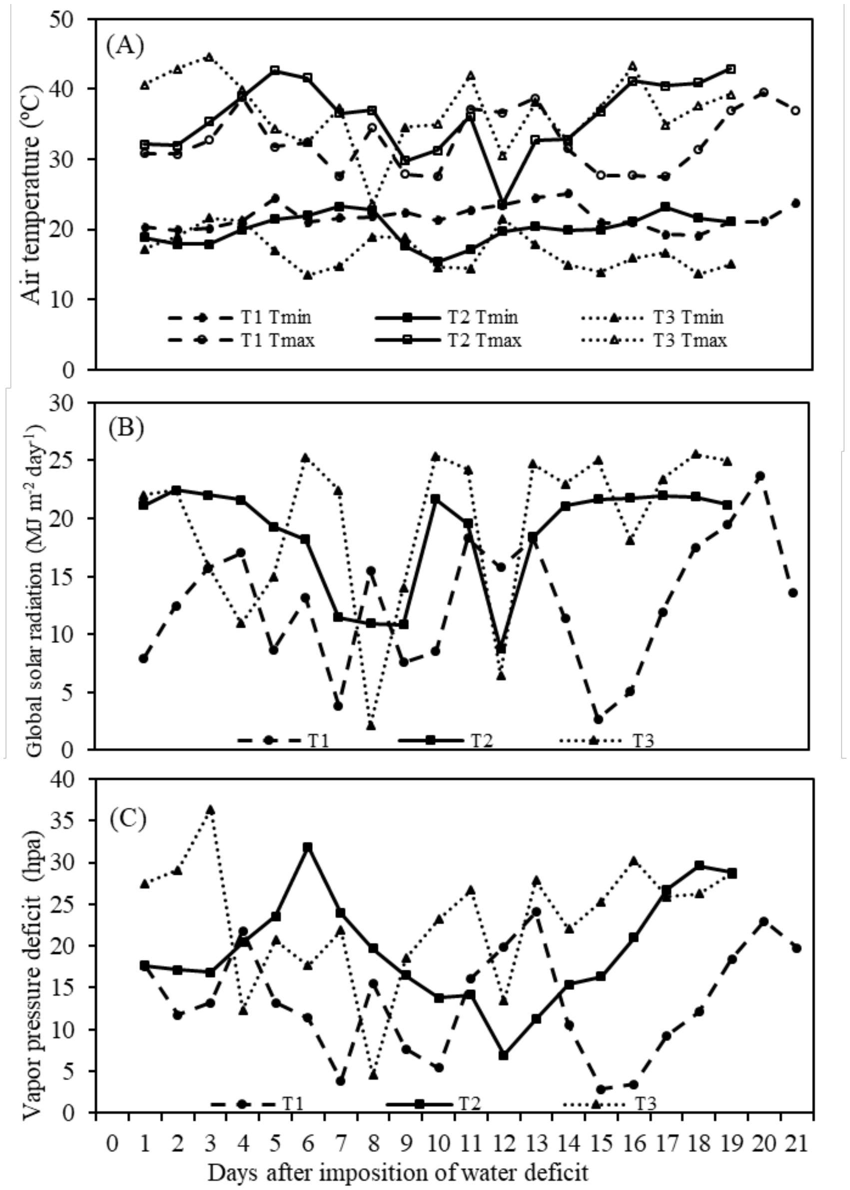

Figure 1. Minimum (min) and maximum (Max) daily air temperatures (A), estimated daily global incident solar radiation estimated (B), and daily air vapor pressure deficit (VPD) at 15 h (C) in three trials with soybean.

The FTSW threshold values obtained in this study are close to those found in the literature for soybean. Sinclair and Ludlow (1986), for instance, found the value of 0.40. During trials with soybean, King et al. (2009) and Sadok et al. (2012) reported a pattern of decline in RT rate with an FTSW threshold for all genotypes of 0.27 to 0.43.

A study conducted by Riar et al. (2018) showed an FTSW value of 0.51 for the N05-UGA-889 genotype, which is significantly higher than those of all others analyzed. The authors emphasized that such a high threshold was not previously identified in soybean, which offers the possibility of water conservation with the drying of the soil and the phenotypic expression of slow wilting. However, a repeated test was not performed for this genotype, which would be necessary before considering it more tolerant to water deficit than the others. It is worth noting that cultivar DM 66I68 RSF IPRO showed an FTSW threshold of 0.49 in T3, which is higher than those shown by the others, indicating the need for further research testing the behavior of this cultivar. The search for characteristics that improve tolerance through a physiological mechanism that allows adaptation to edaphoclimatic conditions is essential to exploit the yield potential and minimize significant socioeconomic losses to producers.

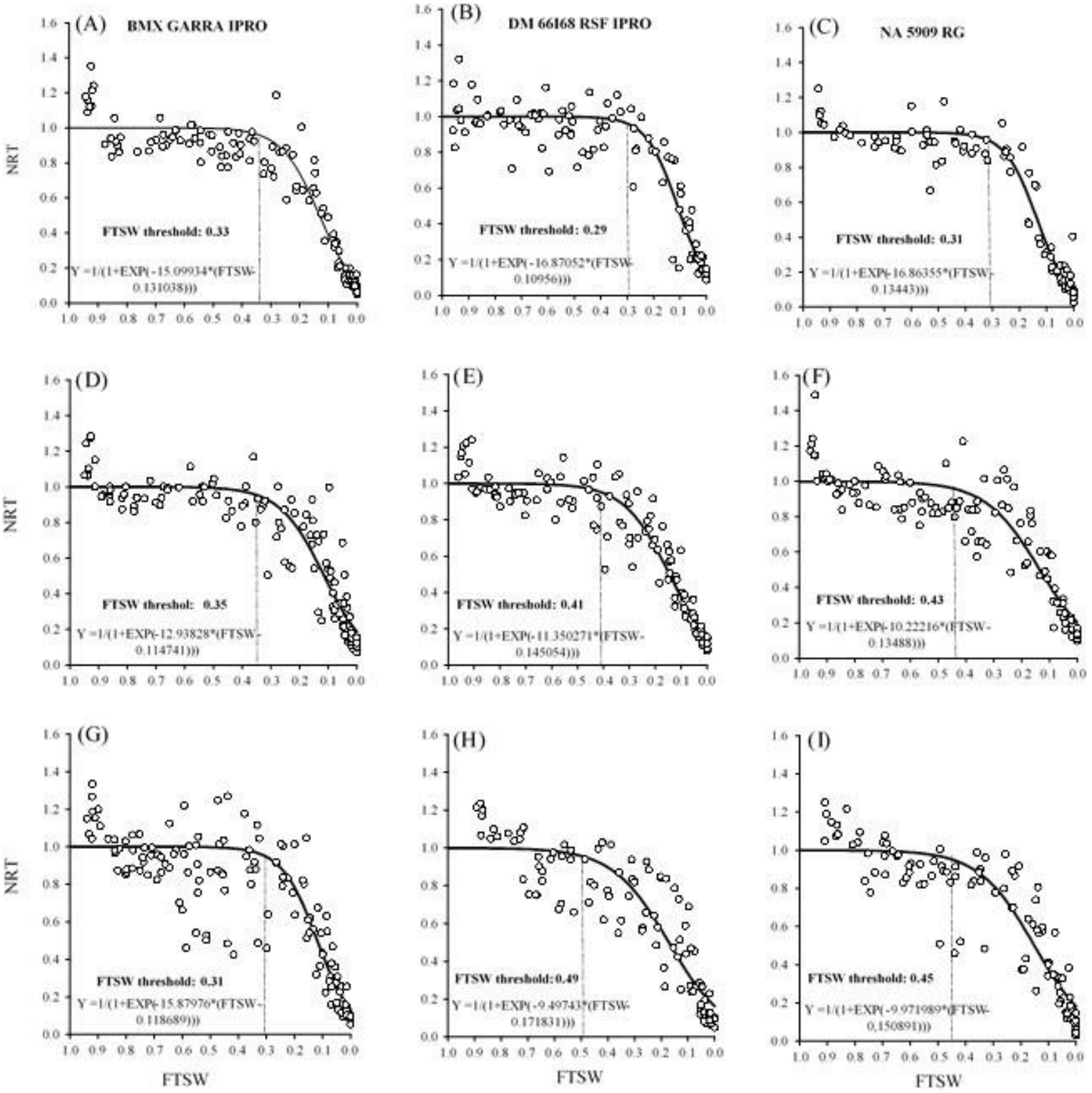

Figure 2. Normalized relative transpiration (NRT) as a function of the fraction of transpirable soil water (FTSW) for soybean cultivars BMX GARRA IPRO, DM 66I68 RSF IPRO, and NA 5909 RG. (A), (B), and (C) refer to T1; (D), (E), and (F) refer to T2; and (G), (H), and (I) refer to T3.

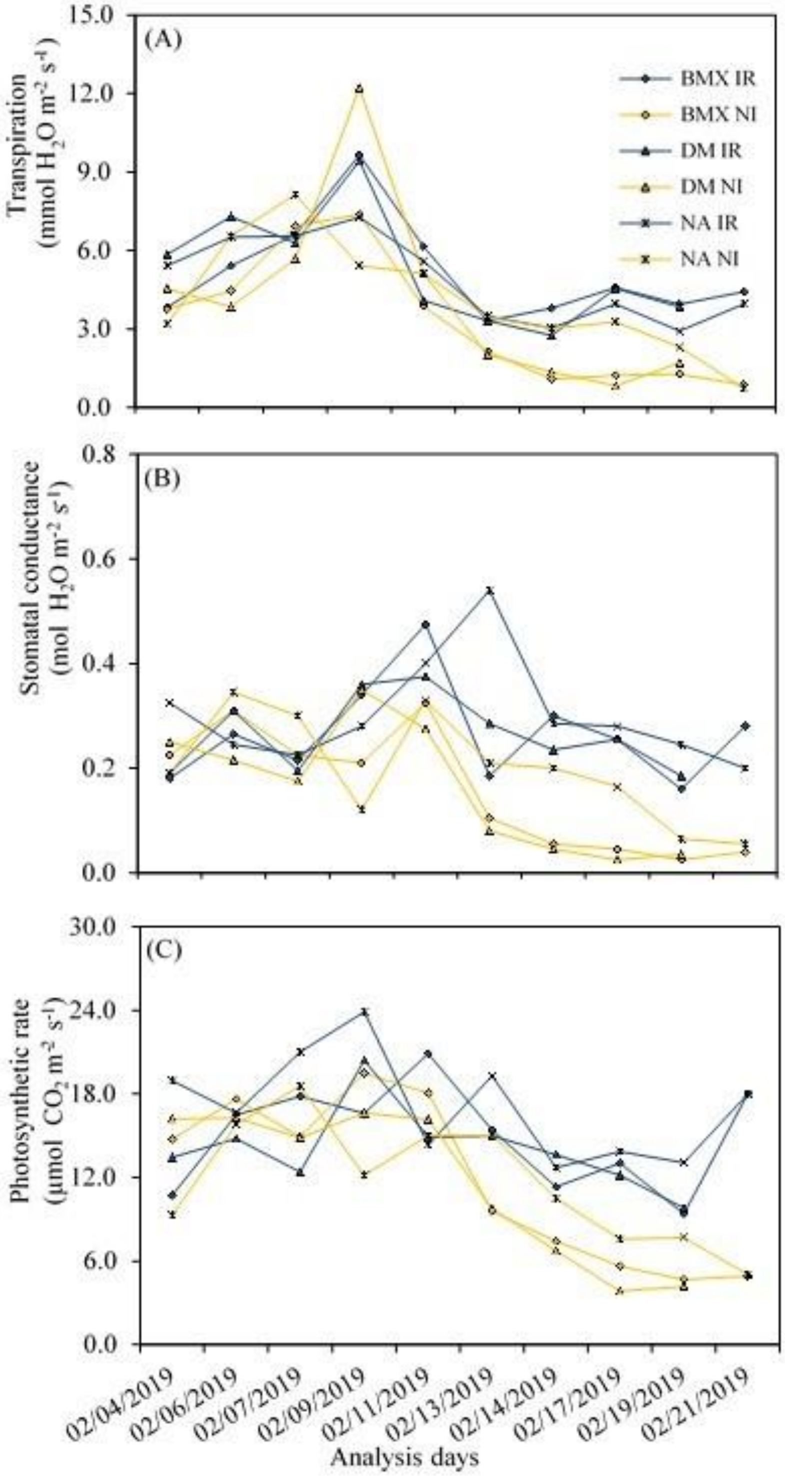

Figure 3. Transpiration (A), stomatal conductance (B), and photosynthetic rate (C) evaluated in T2. BMX= Cultivar BMX GARRA IPRO. DM= Cultivar DM 66I68 RSF IPRO. NA= Cultivar NA 5909 RG. IR= irrigated. NI= non-irrigated.

The gas exchange measurements (Figure 3) performed in T2 indicated that the NI plants of the three cultivars showed a reduction in stomatal conductance (gs), transpiration (E), and photosynthetic rate (Amax) seven days after water deficit was imposed (02/11/2019), demonstrating the negative effect of water deficit on plant physiological variables. The NI plants of cultivar BMX GARRA IPRO showed a decrease of approximately 80% compared with the IR plants on the last day of transpiration evaluation (Figure 3 A), whereas cultivars DM 66I68 RSF IPRO and NA 5909 RG exhibited reductions of approximately 55% and 82%, respectively. On the last day of reading (Figure 3 B), the gs values were very close to zero in the NI plants of the three cultivars evaluated, decreasing by 85% in cultivar BMX GARRA IPRO, 78% in DM 66I68 RSF IPRO, and 70% in NA 5909 RG.

The A_{max} rates (Figure 3 C) of NI plants remained between 4.0 and 6.0 μmol of CO_2 m^{-2} s^{-1} on the last day of reading. The deficit was not as severe as to zero their photosynthetic rate, while the IR plants maintained their physiological activities at higher rates with percentages of 73%, 57%, and 72% for cultivars BMX GARRA, DM 66I68 RSF IPRO, and NA 5909 RG, respectively. The reduction in gas exchange observed in this study, mainly in stomatal closure, is a response mechanism of plants to water deficit since it reduced transpiration. This response aims to minimize the loss of water in the leaves. However, in addition to affecting the diffusion of atmospheric carbon dioxide used in photosynthesis, this stomatal closure can also induce excessive heating of the leaves due to the reduction of evaporative cooling (Taiz et al., 2017).

There was no significant difference in the interaction of factors (cultivars × water regimes) for the variables of plant height and number of nodes (Table 1) at the end of each trial. In other words, the behavior of a factor does not depend on the variation (absence or presence) of another factor. However, there was significance ($p < 0.05$) of the isolated factors (Table 1), with the NI plants showing a reduction in plant height compared with the IR plants in the three trials (Table 2). The number of nodes in NI plants was lower than that of IR plants (Table 2). The interaction was not significant for leaf area, but the isolated factor of water regimes showed significance ($p < 0.05$) in the three studies (Table 1), with the NI plants exhibiting reductions of 45.0%, 37.8%, and 38.1% in leaf area in T1, T2, and T3, respectively, compared with IR plants (Table 2).

Table 1
Analysis of variance for plant height (PH), node number (NN), leaf area (LA), leaf dry matter (LDM), and leaf nitrogen content (LNC) of three soybean cultivars: BMX GARRA IPRO, DM 66I68 RSF IPRO, and NA 5909 RG

	Source of variation	DF	Mean square				
			PH (cm)	NN	LA (cm^2)	LDM (g)	LNC (g.kg^{-1})
T1	Cultivar (C)	2	164.26*	3.27*	168779.46^{ns}	1.93^{ns}	5.96^{ns}
	Water regime (WR)	1	1328.25*	52.08*	14195866.40*	226.92*	169.64*
	C x WR	2	20.15^{ns}	0.27^{ns}	24720.77^{ns}	0.41^{ns}	1.13^{ns}
	Error	42	20.36	0.25	130027.72	1.03	6.91
	Total	47	-	-	-	-	-
	CV (%)	-	9.8	3.8	19.3	14.9	9.5
T2	Cultivar (C)	2	93.89*	1.86*	60594.17^{ns}	2.87*	34.44*
	Water regime (WR)	1	600.66*	24.36*	3598213.89*	88.93*	531.38*
	C x WR	2	18.68^{ns}	0.28^{ns}	2996.21^{ns}	2.86*	11.08^{ns}
	Error	42	12.10	0.30	26935.10	0.38	7.51
	Total	47	-	-	-	-	-
	CV (%)	-	8.2	4.3	14.0	12.4	9.6
T3	Cultivar (C)	2	21.39*	2.61*	74063.84^{ns}	1.04^{ns}	---
	Water regime (WR)	1	461.90*	16.01*	3371875.98*	99.50*	---
	C x WR	2	1.86^{ns}	0.01^{ns}	32347.96^{ns}	0.43^{ns}	---
	Erro	42	4.65	0.17	54917.80	0.75	---
	Total	47	-	-	-	-	-
	CV (%)	-	5.1	4.0	20.8	16.7	---

DF = degrees of freedom, CV = coefficient of variation, * = significant at the 5% level and ns = not significant, --- Total N content was not determined for the leaves in T3.

The number of nodes is an important agronomic parameter in soybean and indicates correlations with flowering and maturity characteristics. That is, they directly reflect the yield and adaptability of the species. This study revealed that of the three cultivars to be cultivated, NA 5909 RG showed the greatest height. However, the number of nodes was not higher than those of the other cultivars, which can be attributed to the genetic characteristic of the plant of having longer internodes. Non-irrigated plants showed decreases in height and number of nodes, which are consistent with the results obtained by Castro et al. (2019).

Table 2

Plant height, node number, leaf area, leaf dry matter, and total N content in the leaves of three soybean cultivars: BMX GARRA IPRO (BMX), DM 66I68 RSF IPRO (DM), and NA 5909 RG (NA)

Cultivar	T1		Mean	T2		Mean	T3		
	Water regime			Water regime			Water regime		
	IR	NI		IR	NI		IR	NI	Mean
Plant height (cm)									
BMX	47.6	39.6	43.6 B	45.6	36.9	41.3 B	45.1	38.1	41.6 B
DM	49.7	38.4	44.0 B	43.0	38.4	40.6 B	44.3	38.7	41.5 B
NA	55.5	43.2	49.4 A	49.1	41.2	45.1 A	46.6	40.5	43.5 A
Mean	50.9 a	40.4 b		45.9 a	38.9 b		45.3 a	39.1 b	
Node number									
BMX	14.9	12.5	13.7 A	14.0	12.4	13.2 A	11.6	10.6	11.1 A
DM	14.8	12.8	13.8 A	13.1	12.0	12.6 B	11	10.0	10.5 B
NA	13.9	12.0	12.9 B	13.4	11.9	12.6 B	11	9.9	10.5 B
Mean	14.9 a	12.5 b		13.5 a	12.1 b		11.2 a	10.2 b	
Leaf area (cm^2)									
BMX	2488.4	1487.0	1987.7	1523.7	963.1	1243.4	1265.9	838.8	1052.4
DM	2380.7	1274.5	1827.6	1409.6	893.5	1151.5	1470.1	900.3	1185.2
NA	2373.9	1218.5	1796.2	1409.5	843.6	1126.5	1440.9	847.6	1144.3
Mean	2414.3 a	1326.7 b		1447.6 a	900.0 b		1392.3 a	862.2 b	
Leaf dry matter (g)									
BMX	9.2	4.8	7.0	7.0 aA	3.8 bA	5.4	6.2	3.6	4.9
DM	8.4	4.4	6.4	5.4 aB	3.7 bA	4.6	6.7	4.0	5.3
NA	9.3	4.6	6.9	6.6 aA	3.4 bA	5.0	7.0	3.7	5.4
Mean	8.9 a	4.6 b		6.3	3.6		6.6 a	3.8 b	
Leaf nitrogen content ($g.kg^{-1}$)									
BMX	32.1	25.3	28.7	33.6	21.1	26.7 B	---	---	---
DM	30.8	24.3	27.6	30.6	22.9	27.3 B	---	---	---
NA	29.3	24.1	26.7	37.3	25.0	31.1 A	---	---	---
Mean	30.7 a	24.5 b		33.8 a	23.0 b				

Means followed by the same lowercase letters in the row and uppercase letters in the columns do not differ (Scott Knott test, $p < 0.05$). IR= irrigated. NI= non-irrigated. --- Total N content was not determined for the leaves in T3.

The reduction of leaf area in NI plants shows the negative effect of water deficit. Water deficit is the result of a decrease in the plant's water content, which occurs when water absorption is lower than transpiration. In this condition, the cells contract, making the walls flaccid, reducing cell turgor pressure, and consequently affecting the processes that depend on cell turgor, such as leaf expansion (Taiz et al., 2017).

Leaf dry matter (Table 1) in T1 and T3 differed significantly only for the isolated factor of water regime, decreasing by 48.3% and 43.3%, respectively, in NI plants, and showing no significant difference between cultivars for this parameter. Analys of variance (Table 1) indicated significance ($p < 0.05$) for the water regime × cultivar interaction in T2, with the NI plants of cultivar NA 5909 RG exhibiting the greatest reduction in leaf dry weight (48.4%), while BMX GARRA IPRO and DM 66I68 RSF IPRO decreased 45.7% and 31.4%, respectively (Table 2). Water deficit affects the accumulation and partition of dry matter, decreasing plant yield (Zou et al., 2019). These results agree with the study conducted by El-Mageed et al. (2017) using soybean under the combined effect of water deficit and potassium fertilization, where dry matter and leaf area were significantly affected.

The total nitrogen content in the leaf tissues (Table 1) showed significance between the isolated factors in T1 and T2. In the first, there was only a statistical difference between water regimes (IR and NI), where the total nitrogen accumulation in the leaves of non-irrigated plants decreased by 20.2% (Table 2). In T2, in addition to the statistical difference between water regimes, cultivar NA 5909 RG showed a total N content in the leaf tissue 22.2% higher compared with cultivar BMX GARRA IPRO and 13.9% higher than that of DM 66I68 RSF IPRO (Table 2), which may indicate that cultivar NA 5909 RG has more significant activity in biological nitrogen fixation in dry conditions.

Biological nitrogen fixation is very sensitive to a decrease in the fraction of transpirable soil water. Sinclair et al. (2010) found that tolerance to water deficit due to N_2 fixation resulted in a yield increase of 85% or more when using modeling to simulate soybean yield in the United States. Additionally, soybean genotypes, including PI 471938, have been identified as expressing substantial tolerance to N_2 fixation in soil drying (Devi & Sinclair, 2013).

Breeding programs in the USA aimed at the development of drought-tolerant cultivars have identified drought-tolerant genotypes with high N_2 fixation capacity for commercial use (Devi et al., 2014). Riar et al. (2018) studied the sensitivity, transpiration rate (RT), and N_2 fixation activity of lines derived from the crossing of the PI 471938 genotype and a high-yielding commercial cultivar called Hutcheson using acetylene reduction activity (ARA) in water deficit conditions. The authors found little genetic variation of the FTSW threshold for RT. However, the FTSW threshold values were significantly different between parental genotypes for N_2 fixation.

There was no variation in the H_2O_2 content in the leaves of cultivar DM 66I68 RSF IPRO, regardless of the water regime (IR and NI), in T1 (Figure 4 A). However, BMX GARRA IPRO and NA 5909 RG showed an increase of 10.8% and 28.6% in H_2O_2 concentration, respectively, in NI plants. These results indicate that water deficit caused a higher production of this compound in plants of cultivar NA 5909 RG compared with other cultivars under the same water regime. Plants in T2 (Figure 4 B) showed a behavior similar to those in T1, in which cultivar DM 66I68 RSF IPRO displayed no increase in H_2O_2 production in the leaves in either water regime. In contrast, H_2O_2 increased in the leaf tissue of the NI plants of cultivars BMX GARRA IPRO and NA 5909 RG (24.8% and 24.1%, respectively). Overall, there was a higher production of H2O2 in plants under water deficiency, mainly in cultivars BMX GARRA IPRO and NA 5909 RG.

The main reactive oxygen species (ROS) are $O^{2\bullet-}$ and H_2O_2, which accumulate in cells and cause oxidative stress (Deng et al., 2012). The increase in H_2O_2 content in the leaves of NI plants in this study, especially in cultivars BMX GARRA IPRO and NA 5909 RG, shows the effect of water deficit in the formation of ROS. Quan et al. (2018) observed similar results in a study with Arabidopsis, in which the water stress treatment mostly induced the accumulation of H_2O_2 in transgenic plants.

Cultivars BMX GARRA IPRO and NA 5909 RG in T1 (Figure 4 C) showed no increase in leaf malondialdehyde (MDA) content regardless of the water regime (IR and NI). However, cultivar DM 66I68 RSF IPRO exhibited a higher concentration of this compound in NI plants. Cultivars DM 66I68 RSF IPRO and NA 5909 RG in T2 (Figure 4 D) showed increased leaf MDA content in NI plants. In other words, the water deficit caused oxidative stress, generating lipid oxidation products, with MDA as a secondary product of this oxidation. As in T1, the concentration of this compound was more expressive in DM 66I68 RSF IPRO.

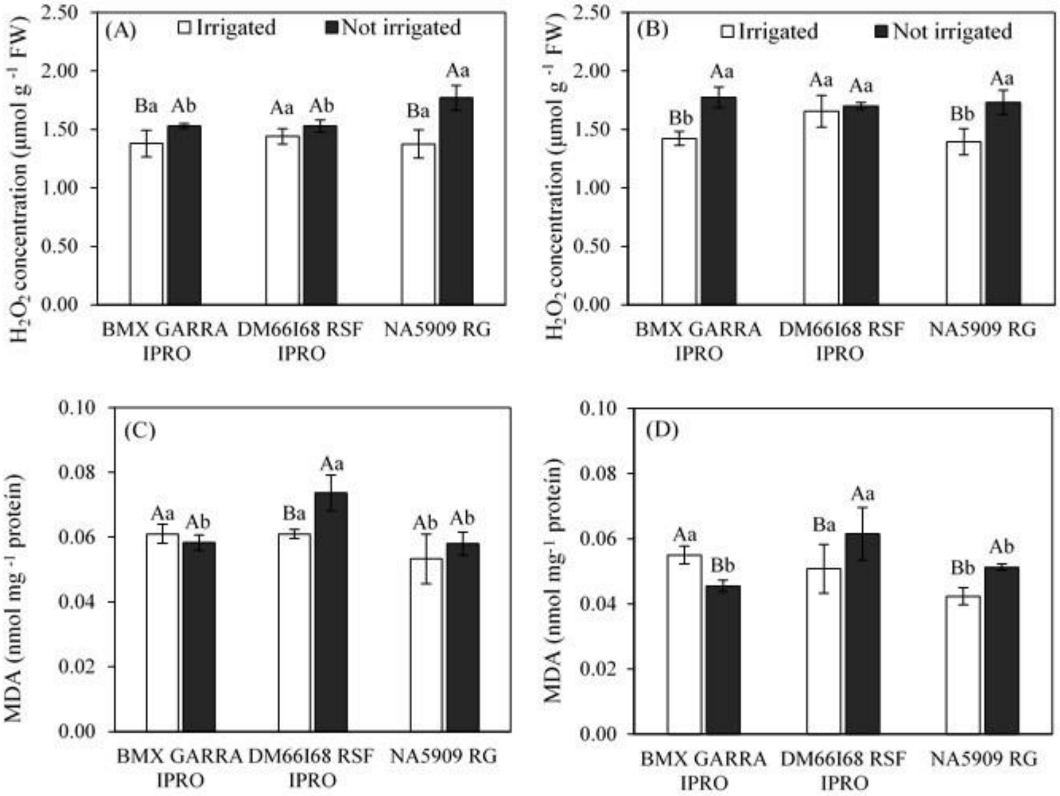

Figure 4. Hydrogen peroxide content (H2O2) and malondialdehyde concentration (MDA) in the leaves of three soybean cultivars. (A) and (C) refer to T1; and (B) and (D) refer to T2. Lowercase letters indicate a difference between cultivars within each water condition. Uppercase letters indicate the difference of each cultivar between water conditions.

Maintaining the integrity and stability of cell membranes under water deficit conditions is essential since they are the primary targets of plant stress. One of the products of cell membrane lipid peroxidation is MDA, considered a fundamental indicator in the analysis of cell membrane damage (Noori et al., 2018). In the present study, the MDA concentration in the leaves in all trials conducted with cultivar DM 66I68 RSF IPRO in NI plants was significantly higher, indicating that lipid peroxidation increased for this cultivar. These results denote that exposure to water deficit negatively affected cultivar DM 66I68 RSF IPRO, which displayed a higher MDA content in NI plants in both T1 and T2. There was no significant difference in H_2O_2 concentration between NI and IR plants, indicating that other ROS were formed in NI plants, causing damage to membrane lipids. A study conducted in Indonesia using soybean and wild soybean cultivars exposed to drought and the action of the Paraquat herbicide showed an increase in the concentration of MDA with increasing water deficit (Hamim et al., 2017).

SOD and POD are antioxidant enzymes that play vital roles in eliminating excessive ROS from the cell and maintainining homeostasis and tolerance to stress, including water deficit (Deng et al., 2012; Cao et al., 2019). The NI plants of cultivar NA 5909 RG showed higher activity of the SOD enzyme, both in T1 (Figure 5 A) and T2 (Figure 5 B). This activity is directly related to the content of H_2O_2, which was increased in NI plants of this cultivar. The SOD enzyme belongs to a class of metalloenzymes that catalyze the disproportionation of $O^{2\bullet-}$ molecules into molecular oxygen (O_2) and H_2O_2. The activation and increase in this enzyme activity are interpreted as a measure to neutralize the $O^{2\bullet-}$ accumulated in several cellular compartments of the plants under water deficit conditions, releasing H_2O_2 to be disproportioned by the peroxidase enzymes (Laxa et al., 2019). Guaiacol peroxidases are oxide reductases that catalyze the oxidation of various electron-donating substances, such as phenols, aromatic amines, and other organic compounds, using hydrogen peroxide as an electron acceptor (Hamid & Rehman, 2009).

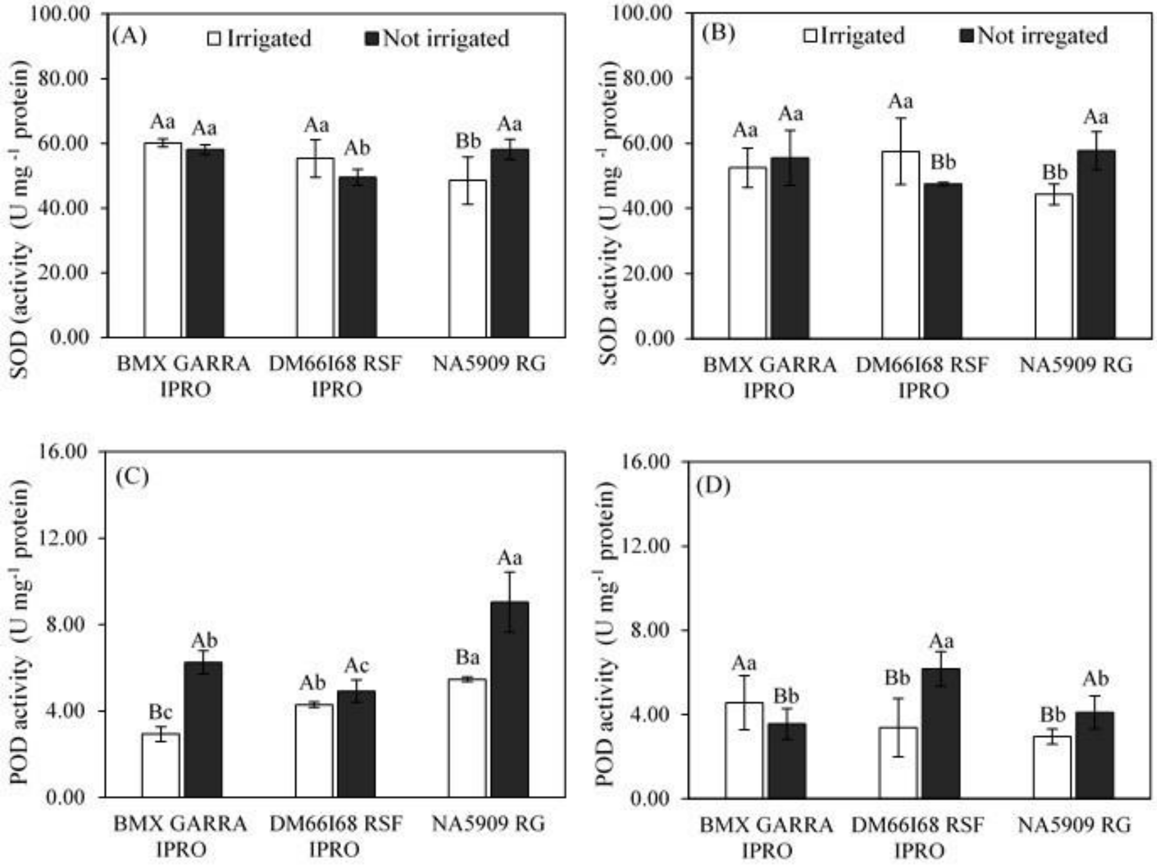

Figure 5. SOD and POD enzyme activities in the leaves of three soybean cultivars, (A) and (C) refer to T1; and (B) and (D) refer to T2. Lowercase letters indicate a difference between cultivars within each water condition. Uppercase letters indicate the difference of each cultivar between water conditions.

Cultivars BMX GARRA IPRO and NA 5909 RG, both in T1 and T2, showed higher production of H_2O_2 in NI plants, even with the activation of the POD enzyme in T1 (Figure 5 C). This indicates that POD enzyme activation was not enough to disproportionate all the H_2O_2 formed due to water deficiency. The POD enzyme was activated in NI plants in T2 (Figure 5 D) only in DM 66I68 RSF IPRO and NA 5909 RG, which exhibited a higher concentration of MDA, indicating that water deficiency induced oxidative stress in these cultivars and that the action of antioxidant enzymes was not effective in reducing this stress.

This study showed an increase in the activity of these antioxidant enzymes in NI plants, which is consistent with the findings of Cao et al. (2019), who studied soybean in China and found an increase in the activity of these enzymes in plants subjected to water deficit. According to the physiological parameters of gas exchange evaluated in T2, stomatal closure in NI plants led to the formation of ROS, which caused lipid degradation of cell membranes and triggered a series of enzymatic reactions to combat the formation and negative effects caused by ROS. Assessing physiological and biochemical responses is essential to obtain genotypes with water deficit tolerance.

Conclusions

Cultivars DM 66I68 RSF IPRO and NA 5909 RG have a more efficient stomatal closure control and greater tolerance to water deficit. These results suggest that these cultivars can be indicated for environments with long drought periods and high VPD. Cultivar BMX GARRA IPRO can be indicated for regions with short drought periods.

The decrease in available soil water reduced plant growth and physiological changes, reducing the total N content in the leaves. Water deficit causes oxidative stress by inducing the production of ROS and activation of the superoxide dismutase (SOD) and guaiacol peroxidase (POD) enzymes as a self-defense mechanism.

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