Journal of Crop Science and Biotechnology (2023) 26:27–37
https://doi.org/10.1007/s12892-022-00157-1

ORIGINAL RESEARCH

Physiological and yield responses of soybean under water deficit

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Accepted: 8 May 2022 / Published online: 31 May 2022

Abstract

Water deficit during grain filling is a key factor on soybean (*Glycine max* (L.) Merrill) production, but plant response to different water stress levels should be better understood. This study evaluated soybean plant response to different soil water levels during grain filling. It was assessed the gravimetrical humidity and soil matric potential, leaf relative water content, leaf water potential, proline, and yield components of plants under a range of days without irrigation (0, 3, 6, 9, and 12) during grain filling phase. Until soil matric potential was around −0.8 atm soybean water deficit tolerance mechanisms were enough to maintain leaf relative water content and leaf water potential at acceptable levels, which proline concentration was a key factor in this mechanism. Leaf relative water content and leaf water potential showed to be related in maintaining soybean yield under water deficit and they may be used in studies of soybean tolerant cultivars to water restriction. From 9 days on, critical yield losses were observed due to water stress. The information presented in this study supports soybean producers in decision-making in irrigated systems to minimize productivity losses due to water deficit during the critical period of the crop–grain filling.

Keywords *Glycine max* · Soil matric potential · Proline · Water deficit tolerance · Crop responses

Introduction

Water availability affects the growth and development of the soybean (*Glycine max* (L.) Merrill) crop, especially during the reproductive period, a phase of high physiological activity. Water stress is considered one of the most important factors limiting plant performance and yield worldwide (Boyer 1982). Characterize soybean response to different water stress levels should be better understood and could help growers in decision-making to carry out irrigation.

The ideal water status is always sought for the plant to develop and exploit its productive potential. However, models referring to the water transfer processes in this system treat water stress in an empirical manner due to several environmental factors, such as water content in the soil and the vapor pressure in the atmosphere. The quantification of transpiration reduction due to water stress, is necessary in hydrological and plant growth models and for irrigation management. It depends on understanding the mechanisms that cause stress.

Water stress, here defined as the condition in which a plant partially closes stomata increasing leaf resistance, occurs due to the physical conditions of the environment for crop production and to physiological conditions. The decrease in the soil water content triggers a series of reactions in the plant to optimize its use as much as possible; however, this decrease in the transpiratory flow can also affect, depending on its intensity, the entry of nutrients through the root system and consequent drop in productivity due to nutritional deficiencies. In addition to nutritional issues, the soil dryness causes physiological changes in plants. The accumulation of the amino acid Proline in

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the leaves is characteristic in stressed plants (Nguyen et al. 2020).

Weather conditions during the growing season are the main cause of soybean yield variability between cropping seasons and regions, where water deficit accounts on average between 46% (Battisti et al. 2018) and 74% (Sentelhas et al. 2015) of the yield gap in the field. Water is one of the main factors that most contribute to the reduction of productivity, since the water deficit limits the growth of the crop (Boyer 1982), due to the stomatal closure in response to the low water content in the soil, reducing the entry CO_2 with consequent reduction of photosynthesis (Bailey-Serres et al. 2019). Reductions in crop productivity due to water stress have been reported varying from 20% to 46% (Zhang et al. 2007), but also up to 70% (Assad et al. 2007).

The loss of water from the plant by evaporation, mainly from the leaves via open stomata to the atmosphere is transpiration, which is the driving force that generates the stresses for the translocation of water in the plant, and the passive movement of water from the soil to the plant (absorption). When the soil water availability decreases, the passage of water to the atmosphere through the plant decreases, causing a new metabolic adjustment. The impediments that the plant develops that oppose resistances to the flow of water, and consequently its loss, are adaptations to overcome the water deficit (Ferrari et al. 2015).

The amount of water evaporated from the plant through leaves is controlled by weather conditions and by the crop, and therefore, crop productivity is related to the ability of the plant to extract water from the soil, especially when it is under water deficit conditions (Procópio et al. 2004). The best indicators of a water deficit are the total soil water available to plants and the fraction of water available, which is the ratio between the current amount and the potential amount of water in the soil. However, visually, when the soybean plant suffers from lack of water, morphophysiological changes also occur, with leaf curl and wilting being indicators of severe water scarcity.

Plants under water stress also present biochemical changes. Under water deficit, proline accumulates in the cell vacuole and promotes osmotic regulation. This increases the plant ability to extract water from the soil, to protect cell integrity, or even to participate in the constitution of N and C stocks that could be used after water stress periods (Taiz and Zeiger 2013). Proline is an amino acid belonging to the class of small molecules called solute-compatible, which promote osmotic adjustment in cells, such as inorganic ions, without the harmful effect of the latter on enzymes or other macromolecules of the cytoplasm, even in high concentrations (Taiz and Zeiger 2013).

Studies with proline in soybean plants under water deficit were conducted by researchers (El-Sabagh et al. 2017; Nguyen et al. 2020). The accumulation of proline in response to water deficit is a specific mechanism to each cultivar (Burle and Rodrigues 1990). Apparently, there is also an inversely proportional relationship between the osmotic potential and the length of the root system (Moraes and Menezes 2003). However, it was also reported in soybean seedlings that lower osmotic potential provided by water deficit could increase root system in soybean seedlings (Umburanas et al. 2019). Regardless of the type of metabolism, water stress causes a reduction in photosynthesis and an increase in respiration, promoting an increase in the production of reactive oxygen species (Pereira et al. 2012).

In this study, we hypothesized that soybean plants, when subjected to water deficit, show an increase in proline contents, as a strategy to minimize damage to the plants. Furthermore, we also hypothesized that the yield components are affected by the water deficit according to the duration of the deficit. Therefore, the objective of this study was to evaluate soybean mechanisms of tolerance to water stress during the grain filling period, as well as study yield and yield components when affected by changes in the matrix potential of the soil.

Materials and methods

Estudy area and experimental design

The experiment was carried out in a greenhouse, located in Piracicaba, SP, Brazil (22° 42′ S, 47° 30′ W), under a Cwa climate according to the Köppen's climatic classification. The soybean cultivar used was 'Anta 82 RR', with a semi-determined growth habit, belonging to the 7.4 maturity group, presenting a semi-early cycle in the region.

The experiment consisted of a completely randomized design with five replicates. Treatments consisted of four increasing levels of water stress, established through days without irrigation after the R5 growth stage of the soybean (grain filling phase). Black cylindric polypropylene pots (model 'Citropote') were used, with diameter of 0.15 m and height of 0.35 m (4 dm^3). Before filling, pots were lined with a permeable fabric. To prepare the substrate, soil was collected from a subsurface layer of an area not used for agriculture. After standardization of the material and removal of impurities, the soil was mixed with medium sand and commercial substrate, in a proportion of 1:1:1. The physical and chemical characteristics of the substrate are shown in Table 1.

The substrate had its pH corrected to 6.5 applying an amount proportional to 1300 kg ha^{-1} of limestone (80% PRNT). The fertilizer used was calculated based on the volume of each pot and applied in the form of a nutrient solution, adding 50 kg ha^{-1} of P_2O_5 and 80 kg ha^{-1} of K_2O, with the potassium fertilization being divided in two,

Table 1 Chemical and physical analysis of substrate used for the water stress study carried out in Piracicaba, SP, Brazil

pH	O.M.	P	K	Ca	Mg	H + Al	Al	S.B	CEC	V	m	SO_4
($CaCl_2$)	g dm^{-3}	mg dm^{-3}	$mmol_c$ dm^{-3}							%		mg dm^{-3}
5.2	7	26	0.5	12	5	22	0	18	40	44	0	11
Cu	Fe	Zn	Mn	B	Clay	Silt	Sand					
mg dm^{-3}					g kg^{-1}		Total	Coarse	Fine			
0.5	14	0.4	3.2	0.25	179	21	800	470	330			

O.M., organic matter; P, evaluated through resin; S.B., sum of bases; CEC, cation exchange capacity; V, base saturation; Cu, Fe, Zn and Mn, evaluated through DTPA; B, evaluated through hot water; Clay, < 0.002 mm; Silt, 0.053–0.002 mm; Total sand, 2.00–0.053 mm; Coarse sand, 2.00–0.210 mm; Fine sand, 0.210–0.053 mm; Texture, sandy substrate

with first and second fertilizer installments applied 15 and 30 days after sowing. The substrate was placed in the pots, accommodating the same mass of substrate in each. Seeds were inoculated with *Bradyrhizobium japonicum*, via liquid inoculant (Concentration: 5.0 × 109 viable cells per mL) and treated with pyraclostrobin + fipronil + thiophanate-methyl. The sowing was carried out on March 26, 2015 with 5 seeds per pot and then thinned to 1 plant per pot. During the crop cycle, 3 applications of beta-cyfluthrin + imidacloprid were made in V4, V8 and R2 (Fehr and Caviness 1977) to control *Bemisia tabaci*.

The soil (substrate) water retention curve was obtained through a Richards pressure chamber. Samples were placed on porous plates previously saturated. Subsequently, they were subjected to six matrix potentials: −60, −100, −330, −1000, −3000 and −15,000 cm of H_2O, using five replicates.

The set of data pairs (u and ψ) was adjusted to the model proposed by van Genuchten (1980), presented below:

$$u = u_r + \frac{(u_s - u_r)}{\left[(1 + \alpha \cdot |\psi|)^n\right]^m} \quad (1)$$

where u refers to soil water content (kg kg^{-1}), ur to residual water content (kg kg^{-1}), us to saturation water content (kg kg^{-1}), ψ to matrix potential (cm H_2O), α (cm^{-1}), n and m belong to the empirical model. The following restriction was adopted for the calculation:

$$m = \left[1 - \left(\frac{1}{n}\right)\right] \quad (2)$$

The water retention curve obtained for the substrate by adjustment of the experimental data to Eq. (1), minimizing the deviations, as follows:

$$u = 0.05178 + \frac{(0.25146 - 0.05178)}{\left[(1 + 0.00478 \cdot |\psi|)^{1.96007}\right]^{0.4898}} \quad (3)$$

which is represented in Fig. 1 together with the experimental points.

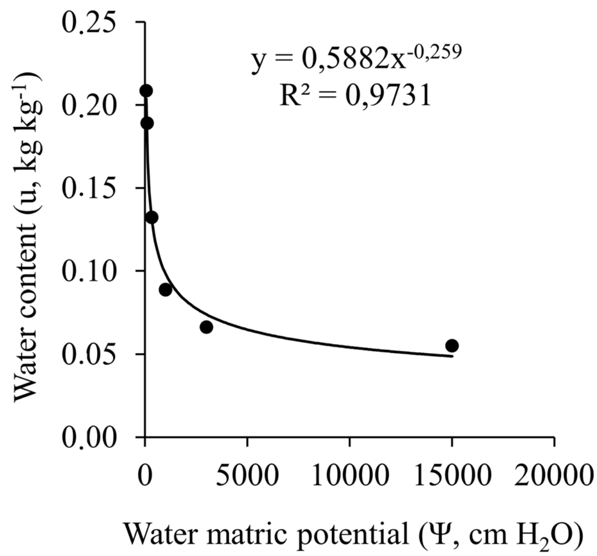

Fig. 1 Substrate water retention curve. The ordinate u is the water content on weight basis (kg kg^{-1}) and the abscissa the water matric potential (Ψ, cm H_2O)

With the results of the substrate water content evaluated after each water stress period, and using Eq. (3), treatments were established according to Table 2.

During the establishment of the different levels of stress due to water deficit applied during the grain filling growth period (R5), the greenhouse remained under mild atmospheric demand (the average actual evapotranspiration, estimated from the loss of pot weight, was 2.33 mm day^{-1}). The control treatments (one for each water stress treatment, see Table 2) maintained 100% of the available water capacity (water content corresponding to the theoretical field capacity FC, obtained from the soil water retention curve). After water stress periods, pots returned to 100% available water until harvest.

Table 2 Substrate average water content (u, kg kg^{-1}) and respective soil water potentials, obtained after each water deficit period imposed at grain filling (the R5 soybean growth stage)

Description		u (kg kg^{-1})	Matric potential	
			cm H_2O	kPa
EV_1 (1st)	WD-0	0.308 a*	− 60	− 6.0
	WD-3	0.206 b	− 170	− 16.96
EV_2 (2nd)	WD-0	0.326 a	− 60	− 6.0
	WD-6	0.104 b	− 800	− 81.00
EV_3 (3rd)	WD-0	0.320 a	− 60	− 60
	WD-9	0.080 b	− 1540	− 155.88
EV_4 (4th)	WD-0	0.314 a	− 60	− 6.0
	WD-12	0.056 b	12,430	− 1259.71
	PWP		15,000	− 1500.00
	FC		− 60	− 6.0

EVi are evaluations of soil water content; Treatments: Control with 0 days of water deficit (WD); WD-3 with 3 days of water deficit (WD); WD-6 with 6 days; WD-9 with 9 days; and WD-12 with 12 days. Permanent wilting point (PWP) and Field capacity (FC)

*Averages followed by the same letter do not differ by the Duncan test at 5% probability

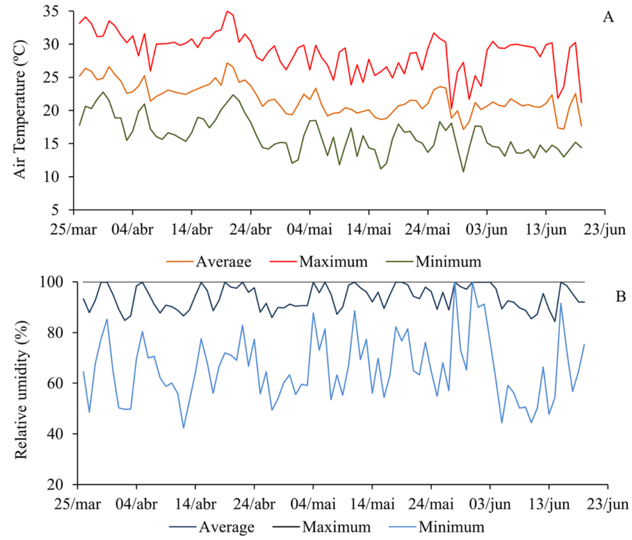

Fig. 2 Daily average, maximum and minimum temperature (**A**), relative average, maximum and minimum humidity (**B**), global and net radiation (**C**) and potential evapotranspiration (**D**) during soybean growth. Sowing at 26-Mar, grain filling at 30-Apr and maturity at 10-June

Yield and physiological evaluations

For substrate water content evaluation at field capacity and after WD treatments, status of the plant water content in leaf and free proline leaf content were evaluated on three pots from each treatment, according to the sequence indicated in Table 2.

To evaluate the leaf water potential of the plants, a SAPS II 3115 equipment (Soil moisture, Santa Barbara, CA, USA) (Scholander et al. 1965) was used. Three replicates of each treatment were chosen at random, of which two trifoliolate leaves were collected from each plant, resulting in a total of six samples. The water potentials of the soybean leaves were measured according to the sequence indicated in Table 2, with readings taken before sunrise.

For the determination of the relative water content (RWC,%), six leaf discs with an approximate diameter of 8 mm were removed and immediately weighed (Mf, g disk^{-1}) on a scale with precision of 1 mg. Then, they were placed in glass bottles, filled with distilled water, and taken to the refrigerator (±2 °C). After 6 h, the surfaces of the leaf discs were dried on paper towels and weighed again (Mst, g disk^{-1}). After this operation, the material was placed in an oven with forced air circulation (80 °C) for 24 h, thus obtaining the dry matter mass (Ms, g disk^{-1}), according to the methodology proposed by Barrs and Weatherley (1962):

$$RWC = \left[\frac{(Mf\text{-}Ms)}{(Mst\text{-}Ms)}\right] \cdot 100 \quad (4)$$

To determine the levels of proline, one trifoliate collected in each evaluation was used according to the method described by Bates et al. (1973). Results obtained were expressed in μmol g^{-1} of fresh matter.

The following yield parameters were evaluated at the end of the cycle: number of aborted pods (PA, pods plant^{-1}); number of productive pods (NPP, pods plant^{-1}); total number of pods (TNP, pods plant^{-1}); pod fixation (PF,%); grain number (GN, grains plant^{-1}); number of grains per pod (GP, grains pod^{-1}); grain mass (GM, mg grain^{-1}) and yield (Y, g plant^{-1}).

Meteorological data

Meteorological data were obtained through a meteorological station located inside the greenhouse. During the experiment, the average temperature was 21.8 °C (Fig. 2a) and the average relative humidity was 93.8% (Fig. 2b).

Statistical analysis

Statistical analyzes of the variables were performed through analysis of variance and Duncan's test (α = 0.05). The parameters substrate water content, leaf water potential, relative water content in the leaf and proline content were always analyzed compared to the control. The yield components were compared at harvest and the analyzes were performed using the SAS software. In addition, regression analysis was used to observe yield responses according to the days without irrigation.

Results and discussion

Considering that water deficit is the main factor that reduces soybean yield, this study presents alternatives to be used to reduce the yield gap due to water deficit. The water stress variables evaluated in this study, such as relative water content, leaf water potential and leaf proline content, were also related to the maintenance of soybean yield under water deficiency, presenting adequacy for soybean cultivars tolerance to water restriction.

For the averages of leaf water potentials (Table 3) evaluated in relation to different periods of water stress, no statistically significant difference was found in EV1 between the control and WD-3, and in EV2 between the control and WD-6. For the later evaluations EV3 and EV4 great differences were observed.

According to studies by Fioreze et al. (2011), soil water content correlates with values of leaf relative water content, and it is possible to observe a pattern of leaf dehydration, depending on the dryness of the soil. However, these authors draw attention to the fact that the coefficients of this correlation vary according to the soybean genotype, thus indicating a differential sensitivity between them to the decrease in available water in the soil.

For a water deficiency stress to be considered moderate/severe, the leaf water potential and RLWC (%) must be considered, and severe water stress occurs when the plant has its relative water content reduced by more than 20% (Hsiao 1973). Leaf water potential tends to increase with increased water stress duration, whereas water content in the leaves tends to decrease (Table 3). It can be said that the soybean plants, at the end of the 12th day without irrigation, are in a state of severe water stress (Hsiao 1973). Only in EV4 the RLWC was lower in WD relative to control (Table 3).

Table 3 Average values of relative leaf water content (RLWC), proline leaf water content (PRO) and leaf water potential (ψl) as a function stress duration

Description		RLWC (%)	PRO (μmol g^{-1})	ψ_l (cm H_2O)
EV_1 (1st)	WD-0	52.93* ns[1]	1.04 b[2]	2,549.29 ns
	WD-3	50.52 ns	1.44 a	1,917.07 ns
EV_2 (2nd)	WD-0	70.57 ns	1.11 ns	2,590.08 ns
	WD-6	73.25 ns	1.25 ns	2,467,71 ns
EV_3 (3rd)	WD-0	51.93 ns	1.29 b	1,101.29 b
	WD-9	54.83 ns	2.70 a	7,729.45 a
EV_4 (4th)	WD-0	80.74 a	0.92 b	2,335.15 b
	WD-12	57.33 b	52.78 a	16,397.04 a

EVi are evaluations and Treatments WD-i are described in the text and Table 2

*Means were compared between treatments belonging to the same evaluation

[1]Non-significant

[2]Means followed by the same letter do not differ by Duncan's test at the level of 5% probability

There are two basic strategies by which plants resist drought: avoiding and/or tolerating dehydration (Levitt 1980). Characteristics of avoiding dehydration act in the maintenance of the relative water content (RLWC) in plant tissues during the water deficit period, while plants that present the tolerance strategy have tissues that can tolerate dehydration to a certain extent (low relative critical water content).

The use of the methodology to determine RLWC is considered efficient in the study of the adaptation of plants to drought (Jones 2007). Fioreze et al. (2011), studying the behavior of soybean genotypes subjected to intense water deficit, found a reduction in the RLWC, in comparison to the treatment maintained under irrigation, for all studied genotypes, but in a different way. However, this decrease occurred on the third day after the irrigation was discontinued, a fact that was not observed in the present study, where, compared to the irrigated treatment, a decrease was observed only in 12th day after the irrigation was discontinued (Table 3). This fact probably explained by the differences between the demands for water (evapotranspiration), resulting from the different times of days without irrigation.

The maintenance of the RLWC, observed in the case of the present study, had no difference from control until the 9th day without irrigation, was also reported by James et al. (2008), where the authors classified this characteristic as efficient in differentiating soybean genotypes, in many of the studies with water deficit. Maia et al. (2007), studying the behavior of two corn cultivars subject to 5-day water stress levels in summer cultivation found a rapid reduction in RLWC. Sinclair and Ludlow (1986) suggested that, in conditions of severe water deficit, in which the stomata remain closed most of the day, the low stomatal conductance values are of great importance for the maintenance of the relative water content.

Lobato et al. (2008), studying the biochemical behavior of soybean subjected to 6 days of water stress, at the beginning of the reproductive phase, observe a 67% increase in proline levels, among other biochemical changes. Under water stress, the concentration of free amino acids such as proline and glycine betaine are strongly altered and, consequently, more rapidly accumulated (Ramos et al. 2005).

At 9 days without irrigation the leaf proline content almost doubled and at 12 days a significant increase was detected, in the order of fifty times in relation to the control (Fig. 3). This high concentration of proline in the leaves was, however, not sufficient to maintain the RLWC in EV4, since there was a 30% decrease for this parameter during evaluation.

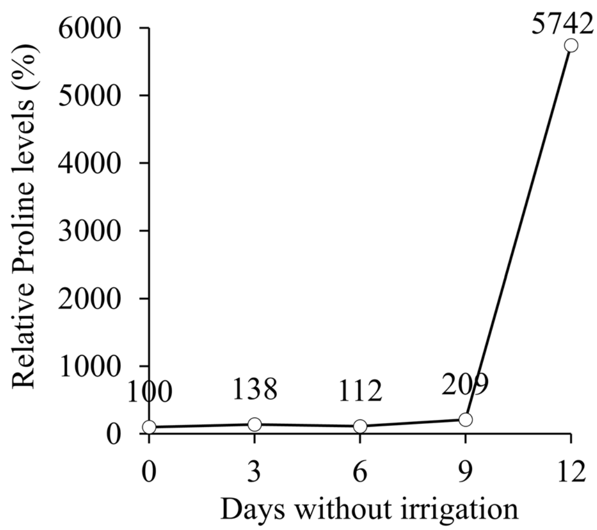

Fig. 3 Levels of proline in soybean leaf as a function of the duration of the water stress treatments in the interval from 0 to 12 days without irrigation

There is a tendency of decrease in pod abortion, while the intensity of the water deficit increases (Table 4). This results agree with the hypothesis of Casagrande et al. (2001): the plant under water stress directs its photoassimilates, preferably, to structures with a higher probability of reproductive success, aiming to guarantee the production of offsprings. According to the observed results, it can also be observed that there was a decrease in the number of productive pods (NPP) as the water stress was intensified (Table 4).

Treatment without water restriction was superior in terms of NPP compared to the plants submitted to water restriction (Table 4). Plants submitted to 9 and 12 days without irrigation showed the lowest averages, 6.79 and 4.68, respectively.

To better visualize data of Table 4, Fig. 4 was constructed in a relative form, making the value of the control (WD-0), with no water stress, as 100%. The yield data corroborate results found in other studies (Garcia et al. 2010; Masoumi et al. 2010; Sincik et al. 2008), reaffirming the concept of plasticity and sensitivity of this parameter to water stress. In Fig. 4b, there is a tendency to decrease the pods number (PN) while increasing the intensity of the water deficit. The treatment under more severe water restriction presented an average PN 30% less when compared to the plants that were irrigated throughout the cycle. Fioreze et al. (2011), comparing in greenhouse soybean cultivars submitted to different periods of water deficit at the beginning of the reproductive period of the crop, also reported a decrease in the average PN.

In studies carried out in different years with different irrigation depths and soybean cultivars, Comlekcioglu and Simsek (2011) reported a significant decrease in the average PN, observing values ranging between 54 and 111 pods per plant, varying according to genotype and water regime. These authors state that among the components of soybean production, this is the most important parameter for crop productivity, being the most sensitive parameter to water restriction and, therefore, indicating that an adequate supply of water is necessary to produce pods for cultivars of high productivity.

Isoda et al. (2006) report that, under favorable cultivation conditions, soybean cultivars with high yields may produce more than 60 pods per plant. Demirtaş et al. (2010) observed a similar result in which different irrigation deficits significantly affected the total number of pods per plant, interfering in crop productivity. In Table 4, it can be noted that the treatments submitted to greater water deficits had significantly smaller values of PF.

In plants submitted to 9 days without irrigation, a greater PN is observed; however, in contrast, they presented a greater PA. In the case of plants subject to the most severe water deficit, they have a smaller number of total pods, but with more productive pods. However, this difference in behavior did not result in a significant difference in PS.

Table 4 Average of yield components with results per plant

WD-i[1]	PA		PPN		TNP		PF		NG		GP		GM		Y[2]	
0	10.42	a*	9.03	a	19.45	a	46.69	a	17.06	a	1.90	bc	121.21	a	2.06	a
3	8.83	a	7.60	ab	16.14	b	45.84	a	13,71	b	1.81	c	134.10	a	1.81	a
6	8.47	b	7.75	ab	16.22	b	48.05	a	14.33	b	1.87	bc	126.93	a	1.84	a
9	10.21	a	6.79	b	17.23	ab	39.13	b	13.76	b	2.17	ba	99.31	b	1.38	b
12	7.88	b	4.68	c	12.56	c	36.78	b	9.32	c	2.39	a	83.90	b	0.79	c

Pod abortion (PA, pods aborted plant^{-1}), Productive pod number (PPN, pods plant^{-1}), total number of pods (TNP, pods plant^{-1}), Pod fixation (PF, %), Number of grains (NG, grains plant^{-1}), Grains per pod (GP, grains pod^{-1}), Grain mass (MG, mg grain^{-1}), and Yield (Y, g plant^{-1}) as a function of days without irrigation

*Means followed by the same letter do not differ by Duncan's test at the level of 5% probability

[1]Treatments

[2]Y: yield per plant corrected to 13% grain water content

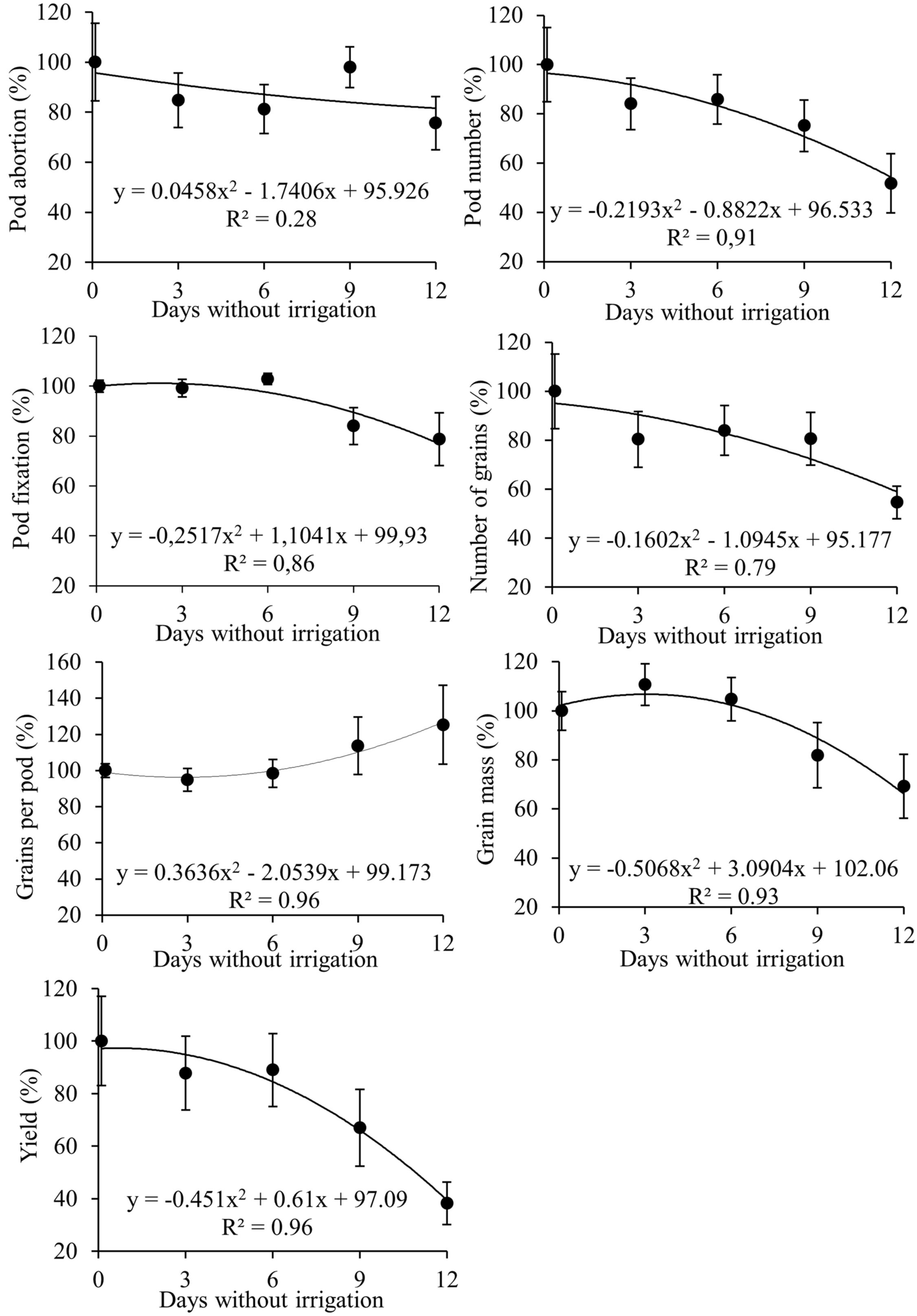

Fig. 4 Yield components under water deficit. **A**—Pod abortion, **B**—pod number (PN), **C**—pod fixation (PF), **D**—number of grains (NG), **E**—grains per pod (GP), **F**—grain mass (MG), and **G**—yield (Y) as a function of days without irrigation

In this study we observed a tendency of a decrease in PF as the soil loses water (Fig. 4c), possibly due to the decrease in the PN. Brevedan and Egli (2003) and Egli et al. (1983) observed, in their studies when evaluating the effects of water deficit at different intensities, during the grain filling period, an increase in the abortion of pods and grains concomitantly with a decrease in the number of pods per plant, which results in lower rates of pod filling in treatments subject to greater water stress.

According to the results presented in Table 4, significant differences were observed in terms of the NG, depending on the number of days without irrigation. There is, on average, an increasing trend for this characteristic (Fig. 4a) as the water deficit is intensified. Possibly plants under more restrictive water conditions prioritize the fixation of pods with a greater number of grains. The NG, among the other components, had the least variation between different stress situations. This demonstrates a uniformity of genetic improvement in the search for plants with an average production of two grains per pod (Navarro Júnior and Costa 2002). In the literature, several studies relating to water deficit and soybean production do not verify significant interactions between these factors. Comlekcioglu and Simsek (2011), Fioreze et al. (2011) and Demirtaş et al. (2010) conclude that the number of seeds per pod is related to the egg fertilization rate and that water stress does not affect this process, being related to the plant genotype. Possibly this disagreement is due to the 'intensity of water stress', since in the present study the treatment submitted to 12 days without irrigation showed a drastic reduction in the substrate water content, subjecting the plants to extremely negative tensions in the soil (− 12.43 atm), even approaching the permanent wilt point, adopted as −15 atm.

Soybean plants without water deficit presented higher NG (Table 4 and Fig. 4d). The treatments with 3, 6 and 9 days without irrigation were statistically equal, only the treatment not irrigated for 12 days presented the lowest value. Sincik et al. (2008), studying water deficit levels on soybeans observed a significant reduction in yield, with a reduction of approximately 50% in the treatment submitted to greater water deficit intensity compared to the control. Rosadi et al. (2005) also found significant effects of water stress on the yield components of soybean, observing the same tendency of decrease in the number of grain per plant as the water stress is intensified. However, Fioreze et al. (2011) report that there were no significant differences in relation to different water regimes on yield.

In relation to grain mass (GM), there were no statistical differences for treatments with water restriction of up to 6 days. The treatments with 9 and 12 days without irrigation, did not differ significantly, and were inferior to the others, presenting means equal to 99.31 and 83.90 mg, respectively (Table 4). Despite the downward trend observed in the present study (Fig. 4), Navarro Júnior and Costa (2002) say that the grain mass (GM) has a characteristic value for each cultivar. However, the authors admit that this characteristic may vary according to environmental conditions and management.

Salinas et al. (1996) found that, among the yield components, grain size and mass were the most sensitive to water deficit. These authors further argue that the lack of water during grain filling reduces grain size and mass, due to the decrease in the supply of photoassimilates produced by the plant and/or inhibition of the grain metabolism itself. Rambo et al. (2003), verifying the responses of soybeans to irrigation, found that there was a lower filling rate of soybeans in non-irrigated treatments. On the other hand, Kuss et al. (2008) found that the average grain mass was higher in treatments that did not receive irrigation during the crop cycle. These authors argue that due to water deficit, which occurred during flowering and final grain filling periods, there was a greater abortion of flowers and pods, causing those pods that remained on the plant to accumulate more dry mass in the grains in relation to plants with a greater number of pods and grains, which consequently would require more photoassimilates.

For Casagrande et al. (2001), this behavior is a mechanism of tolerance to lack of water, aiming to direct the flow of photoassimilates to the pods that are more advanced in the development process and that, theoretically, would have greater chances of producing viable seeds.

No statistically significant differences were found between the first three water deficit intensities in relation to yield. The treatment with 9 days without irrigation showed an average yield of 1.38 g plant^{-1}, whereas the treatment with greater water restriction (12 days without irrigation) differed from all the others, presenting an average of 0.79 g plant^{-1} (Table 4). Figure 4d illustrates the expressive reduction in the average production of grains per plant. For treatments under severe water stress, a reduction of approximately 30 and 60% were observed for the 9- and 12-day regimes without irrigation, respectively.

The behavior and interaction of some evaluated parameters can be observed side by side (Fig. 5). In the case of the relative water content (RLWC,%), its maintenance is noteworthy until the ninth day without irrigation, resulting in a substrate water content of 0.08 kg kg^{-1} (ψsoil = −1.54 atm). Most likely the effect of leaf concentration of proline is largely responsible for this maintenance, since in that same period (up to 9 days without irrigation), the levels doubled, compared to the control. According to Fioreze et al. (2011), the maintenance of water status and leaf area under conditions of water deficit deserves to be highlighted, due to its relationship with the accumulation of assimilates and with the maintenance of the productive potential.

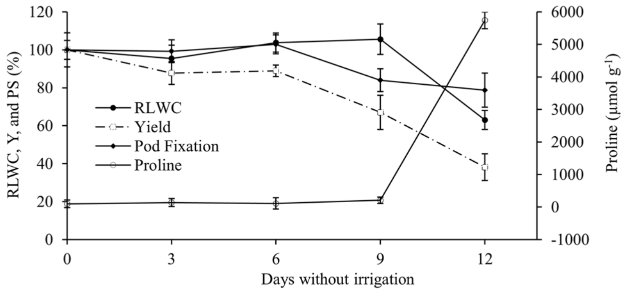

Fig. 5 Relative values of yield per plant (Y%—water content corrected to 13%), relative leaf water content (RLWC), pod fixation (PF) and absolute values of leaf proline contents (PRO), related to different water stress regimes

The accumulation of proline in plants represents a compensatory mechanism for survival, and under normal conditions these levels can vary, in soy, between 1 to 5 µmol g^{-1} and under stress conditions this value can be 20–100 times higher (Nogueira et al. 2001). From this point on, it can be said that the plant has its natural defense against dehydration overcome, there is a rapid, significant increase in leaf water potential and some parameters are significantly impaired, as pod setting, grain mass, number of grains and grain production per plant.

The subject on water stress of plants cultivated with economic interest is frequently addressed by researchers from all over the world; however, even due to the complexity of the subject and the methodological difficulties to carry out studies of this nature, researchers tend to explore the theme from a single branch of science, which can be, for example, plant physiology, agrometeorology, soil physics or crop production. Thus, it ends up generating a difficulty in comparing results and even in interpreting results, caused by different approaches to the same subject.

This study was developed to approximate and try to correlate this research. For this we evaluated parameters related to soil, plant biochemistry, and, finally, components related to soybean productivity. It is known that the soil undergoes, according to environmental conditions, wetting and drying cycles, and these variations occur during the crop cycle. To survive such events, plants have, over time, adapted strategies to tolerate or avoid water stress, several times related to high temperatures. In some specific cases, these mechanisms can cause the plant to tolerate and overcome periods of water deficit, without significant losses to production. Such adaptation mechanisms vary according to plant species and even more to the stage of development in which they are. The presence and efficiency of such mechanisms are increasingly important for the development of new genotypes, more adapted to adverse situations.

For soybeans, there is information that these mechanisms are efficient to a certain extent, but when they are overcome, the plant suffers serious losses with reduced yield. The results observed in the present study suggested that for soil matrix potential values of up to approximately −100 cm H_2O (6 days without irrigation), the plant protection mechanisms were sufficiently efficient in maintaining the leaf relative water content and water potential adequate. These effects were attributed to proline (osmoprotective amino acid) concentration. From that point on, at the end of the ninth day without irrigation, the soil reaches a matrix potential of −1540 cm H_2O, the proline content doubles its concentration (in relation to the control). However, the water content and leaf water potential were already beginning to point to the dehydration of plant tissues, which was reflected in the yield components. On the 12th day without irrigation (ψm = −12,430 cm H_2O), proline levels increased very much. However, the water parameters of the plant already point to a water deficit classified as severe resulting in drastic reductions in the yield components. Finally, with the behavioral tendencies of the evaluated characters and their probable relationships with each other, the need for further studies on the subject is evident, seeking to integrate the knowledge of the different areas to have a more comprehensive view of the problem and how to handle it.

Some strategies can be included to avoid water deficit, such as: physiological traits selection (Gilbert et al. 2011; Battisti et al. 2017; Sinclair et al. 2010); irrigation (Almeida et al. 2018; Hatfield and Walthall 2015); deep soil preparation (Battisti and Sentelhas 2017; Rodrigues et al. 2017); choice of the sowing date based on the ENSO phase (Nóia Júnior and Sentelhas 2019) and crop cycle and sowing dates interaction (Battisti and Sentelhas 2014).

Conclusions

Soybean crop is very sensitive to water stress. This conclusion was reached after submitting soybean plants to 3, 6, 9 and 12 days under water stress conditions. That plant mechanisms of tolerance to the water deficit were active up to 9 days of water stress, maintaining the water status of the plant and its productivity until the soil matrix potential of −800 cm H_2O (−0.8 atm) and up to this value the leaf proline content presented itself adequate in its osmoprotective role. From 9 days on, serious yield losses were observed due to water stress.

Yield and yield components were affected by changes in the matrix potential of the soil. In periods of water stress, the productivity of the soybean plant was correlated with the maintenance of the relative water content and leaf water potential. The parameters of relative water content in the leaf and leaf water potential proved to be adequate for evaluation studies regarding tolerance to water deficit.

The information presented in this study supports soybean producers in decision-making in irrigated systems to minimize productivity losses due to water deficit during the critical period of the crop–grain filling.

Acknowledgements The authors are grateful to the National Council for Scientific and Technological Development (CNPq-Brazil). In addition, the authors are grateful to the applied physiology and production systems group (GFASP), which helped in the implementation and conduct of this project.

Author contributions GF: methodology, formal analysis, data curation, writing—original draft, writing—review and editing; FS and RCU: conceptualization, methodology, writing—review and editing; DD-N and KR: conceptualization, writing—review and editing, supervision, project administration, funding acquisition. All authors contributed in interpretation and discussion of results and approved the final version of this manuscript.

Declarations

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

References

Almeida V, Alves Júnior J, Mesquita M, Evangelista AWP, Casaroli D, Battisti R (2018) Comparison of the economic viability of agriculture irrigated by central pivot in conventional and no-tillage systems with soybean, maize and industrial tomato crops. Glob Sci Technol 11:256–273

Assad ED, Marin FR, Evangelista SR, Pilau FG, Farias JRB, Pinto HS, Júnior JZ (2007) Forecast system of soybean crop yield for Brazil. Pesqui Agropecu Bras 42:615–625

Bailey-Serres J, Parker JE, Ainsworth EA, Oldroyd GE, Schroeder JI (2019) Genetic strategies for improving crop yields. Nature 575:109–118

Barrs HD, Weatherley PE (1962) A re-examination of the relative turgidity technique for estimating water deficit in leaves. Aust J Biol Sci 15:413–428

Bates LS, Waldren RPE, Teare ID (1973) Rapid determination of free proline for water stress studies: short communication. Plant Soil 39:205–207

Battisti R, Sentelhas PC (2014) New agroclimatic approach for soybean dates recommendation: a case study. Rev Bras Eng Agric Ambient 18:1149–1156

Battisti R, Sentelhas PC (2017) Improvement of soybean resilience to drought through deep root system in Brazil. Agron J 109:1612–1622

Battisti R, Sentelhas PC, Boote KJ, Câmara GMS, Farias JRB, Basso CJ (2017) Assessment of soybean yield with altered water-related genetic improvement traits under climate change in Southern Brazil. Eur J Agron 83:1–14

Battisti R, Sentelhas PC, Pascoalino JAL, Sako H, Dantas JPS, Moraes MF (2018) Soybean yield gap in the areas of yield contest in Brazil. Int J Plant Prod 12:159–168

Boyer JS (1982) Plant productivity and environment. Science 218:443–448

Brevedan RE, Egli DB (2003) Short periods of water stress during seed filing, leaf senescence and yield of soybean. Crop Sci 43:2083–2088

Burle ML, Rodrigues GC (1990) Relações hídricas internas da soja sob deficit hídrico em condições de campo. Pesqui Agropecu Bras 25:905–913

Casagrande EC, Farias JRB, Neumaier N, Oya T, Pedroso J, Martins PK, Breton MC, Nepomuceno AL (2001) Expressão gênica diferencial durante deficit hídrico em soja. Rev Bras Fisiol Veg 13:168–184

Comlekcioglu N, Simsek M (2011) Effects of deficit irrigation on yield and yield components or vegetable soybean (*Glycine max* L. (Merr.)) in semi-arid conditions. Afr J Biotechnol 10:6227–6234

Demirtaş Ç, Yazgan S, Candogan BN, Sincik M, Buyukcangaz H, Goksoy AT (2010) Quality and yield response of soybean (*Glycine max* L. Merril.) to drought stress in sub-humid environment. Afr J Biotechnol 9:6873–6881

Egli DBL, Meckel L, Phillips RE, Radcliffe D, Leggett JE (1983) Moisture stress and N redistribution in soybean. Agron J 75:1027–1031

Fehr WR, Caviness CE (1977) Stages of soybean development. Agricultural Experimental Station, Ames, p 80

Ferrari E, Paz A, Silva AC (2015) Deficit hídrico no metabolismo da soja em semeaduras antecipadas no Mato Grosso. Nativa 3:67–77

Fioreze SL, Pivetta LG, Fano A, Machado FR, Guimarães VF (2011) Comportamento de genótipos de soja submetidos a deficit hídrico intenso em casa de vegetação. Rev Ceres 58:342–349

Garcia GA, Persson T, Guerra LC, Hoogenboom G (2010) Response of soybean genotypes to different irrigation regimes in a humid region of the southeastern USA. Agric Water Manage 97:981–987

Gilbert ME, Holbrook NM, Zwieniecki MA, Sadok W, Sinclair TR (2011) Field confirmation of genetic variation in soybean transpiration response to vapor pressure deficit and photosynthetic compensation. Field Crops Res 124:85–92

Hatfield JL, Walthall CL (2015) Meeting global food needs: realizing the potential via genetics x environment x management interactions. Agron J 107:1215–1226

Hsiao TC (1973) Plant responses to water stress. Annu Rev Plant Physiol 24:519–570

Isoda A, Mori M, Matsumoto S, Li Z, Wang P (2006) High yielding performance of soybean in northern Xinjiang, China. Plant Prod Sci 9:401–407

James AT, Lawn RJ, Cooper M (2008) Genotypic variation for drought stress response traits in soybean. I. Variation in soybean and wild glycine for epidermal conductance, osmotic potential and relative water content. Aust J Crop Sci 59:656–669

Jones HG (2007) Monitoring plant and soil water status: established and novel methods revisited and their relevance to studies of drought tolerance. J Exp Bot 58:119–130

Kuss RCR, Kônig O, Dutra LMC, Bellé RA, Roggia S, Sturmer GR (2008) Populações de plantas e estratégias de manejo de irrigação na cultura da soja. Ciênc Rural 38:1133–1137

Levitt J (1980) (1980) Response of plants to environmental stress II: walter radiations, salt and other stress. Academic Press, New York, p 606

Lobato AKS, Oliveira Neto CF, Santos Filho BG, Costa RCL, Cruz FJR, Neves HKB, Lopes MJS (2008) Physiological and biochemical behavior in soybean (*Glycine max* cv. Sambaiba) plants under water deficit. Aust J Crop Sci 2:25–32

Maia PSP, Ferreira Neto C, Castro DS, Freitas JMN, Lobato AKS, Costa RCL (2007) Conteúdo relativo de água, teor de prolina e carboidratos solúveis totais em folhas de duas cultivares de milho submetidas a estresse hídrico. Rev Bras Biociênc 5:918–920

Masoumi H, Masoumi M, Darvish F, Daneshian J, Nourmohammad G, Habibi D (2010) Change in several antioxidant enzymes activity and seed yield by water deficit stress in soybean (*Glycine max* L.) cultivars. Not Bot Horti Agrobotan 38:86–94

Moraes GAF, Menezes NL (2003) Desempenho de sementes de soja sob condições diferentes de potencial osmótico. Ciênc Rural 33:219–226

Navarro Júnior HM, Costa JA (2002) Contribuição relativa dos componentes do rendimento para a produção de grãos em soja. Pesqui Agropecu Bras 37:269–274

Nguyen TTQ, Pham HBV, Le TV, Phung TKH, Lee SH, Cheong JJ (2020) Evaluation of proline, soluble sugar and ABA content in soybean *Glycine max* (L.) under drought stress memory. AIMS Bioeng 7:114–123

Nogueira RJMC, Moraes JAPV, Burity HA, Bezerra EN (2001) Alterações na resistência à difusão de vapor das folhas e relações hídricas em aceroleiras submetidas a deficit de água. Braz J Plant Physiol 13:75–87

Nóia Júnior RS, Sentelhas PC (2019) Soybean-maize succession in Brazil: impacts of sowing dates on climate variability, yields and economic profitability. Eur J Agron 103:140–151

Pereira JWL, Melo Filho PA, Albuquerque MB, Nogueira RJMC, Santos RC (2012) Mudanças bioquímicas em genótipos de amendoim submetidos a deficit hídrico moderado. Rev Ciênc Agron 43:766–773

Procópio SO, Santos JB, Silva AA, Donagemma GK, Mendonça ES (2004) Ponto de murcha permanente de soja, feijão e plantas daninhas. Planta Daninha 22:35–41

Rambo L, Costa JÁ, Pires JLF, Parcianello G, Ferreira FG (2003) Rendimento de grãos da soja em função do arranjo de plantas. Ciênc Rural 33:405–411

Ramos MLG, Parsons R, Sprent JI (2005) Differences in ureide and amino acid content of water stressed soybean inoculated with *Bradyrhizobium japonicum* and *B. elkanii*. Pesqui Agropecu Bras 40:453–458

Rodrigues RR, Casaroli D, Evangelista AWP, Alves Júnior J (2017) Water availability to soybean crop as a function of the least limitng water range and evapotranspiration. Pesqui Agropecu Trop 47:161–167

Rosadi RAB, Afandi MS, Senge M, Ito K, Adomako JT (2005) Critical water content and water stress coefficient of soybean (*Glycine max* (L.) Merr.) under deficit irrigation. Paddy Water Environ 3:219–223

Sabagh AE, Sorour S, Ragab A, Saneoka H, Islam MS (2017) The effect of exogenous application of proline and glycine betaineon the nodule activity of soybean under saline condition. J Agric Biotechnol 2:1–5

Salinas APR, Zelener N, Craviotto RM, Bisaro Z (1996) Respuestas fisiológicas que caracterizan el comportamiento de diferentes cultivares de soja a la diferencia hídrica en el suelo. Pesqui Agropecu Bras 31:331–338

Scholander PF, Hammel HT, Brastreet ED, Hemmingsen EA (1965) Sappressure in vascular plants. Science 148:339–346

Sentelhas PC, Battisti R, Câmara GMS, Farias JRB, Hampf A, Nendel C (2015) The soybean yield gap in Brazil—magnitude, causes and possible solution. J Agric Sci 158:1394–1411

Sincik M, Candogan BN, Demirtaş C, Buyukcangaz H, Yazgan S, Goksoy AT (2008) Deficit irrigation of soya bean [*Glycine max* (L.) Merr.] in a sub-humid climate. J Agron Crop Sci 2250:200–205

Sinclair TR, Ludlow MM (1986) Influence of soil water supply on the plant water balance of four tropical grains legumes. Aust J Plant Physiol 13:329–341

Sinclair TR, Messina CD, Beatty A, Samples M (2010) Assessment across the United States of the benefits of altered soybean drought traits. Agron J 102:475–482

Taiz L, Zeiger E (2013) Fisiologia vegetal, 5th edn. Artmed, Porto Alegre, p 954

Umburanas C, Donegá VC, Queiroz VM, Fontana DC, Bampi D, Dourado-Neto D, Reichardt K (2019) Root attributes and seedling biomass of old and modern soybean cultivars under water deficit. Emir J Food Agric 31:688–696

Van Genuchten MT (1980) A closed form equation for predicting the hydraulic conductivity of unsaturated soils. Soil Sci Soc Am J 44:892–898

Zhang M, Duan L, Tian X, He Z, Li J, Wang B, Li Z (2007) Uniconazole-induced tolerance of soybeans to water deficit in relation to changes in photosynthesis, hormones and antioxidant system. J Plant Physiol 164:709–717