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Establishment of *Sorghum bicolor* L. plants under different water regimes

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Abstract: The present study aim to evaluate the effect of water availability on initial growth of sorghum plants, also, identify the tolerance strategy to drought and excess of water. The study was carried out in a greenhouse covered with transparent plastic and shade cloth on the sides that intercept 50% of radiation at the State University of Goiás, Southeast Campus, Ipameri Unit. The *Sorghum bicolor* seeds were sown in 5 liters pots containing a mix of soil, sand and manure 3:1:1, respectively. The experimental design used was completely randomized, with six treatments and six replications. After 20 days of emergence, the plants was irrigated with water volumes according to 0%, 25%, 50%, 100%, 200% and 400% of daily evapotranspiration. The analysis was carried out 40 days after emergence (DAE). The water restriction limit growth of sorghum plants, however, to tolerate stress, the plants increase the root system and decrease the transpiration through the efficient stomatal control and maintain hydrated to support growth, besides that, decrease the concentration of leaf photosynthetic pigments as a photoprotection mechanism. The excess of water between 100% and 400% of evapotranspiration (ET) did not limit growth of *Sorghum bicolor* plants, because the high transpiration rate eliminated to atmosphere the excess of water, however, above 230% of ET, the plants showed smooth signs of decreased leaf initiation and water relative content.

Keywords: off-season, drought, flood.

Estabelecimento de plantas de *Sorghum bicolor* L. sob diferentes regimes hídricos

Resumo: O presente estudo teve como objetivo identificar o efeito da disponibilidade hídrica no crescimento inicial de plantas de sorgo, bem como, identificar a estratégia de tolerância a seca e ao excesso de água. O trabalho foi conduzido sob bancada em casa de vegetação coberta com plástico transparente e laterais com sombrite que interceptam 50% da radiação solar na Universidade Estadual de Goiás, Campus Sudeste, UnU-Ipameri. As sementes de *Sorghum bicolor* foram semeadas em vaso com capacidade de cinco litros contendo uma mistura de solo, areia e esterco na proporção de 3:1:1 respectivamente. Utilizou-se o delineamento inteiramente casualizado com seis tratamentos e seis repetições. Após a emergência e desenvolvimento inicial de 20 dias as plantas foram irrigadas com volumes de água referentes a 0%, 25%, 50%, 100%, 200% e 400% da evapotranspiração diária. As avaliações ocorreram aos 40 dias após emergência. A restrição hídrica limita o crescimento de plantas de sorgo, no entanto, para tolerar o estresse as plantas incrementam o comprimento do sistema radicular e reduzem a transpiração através de eficiente controle estomático e mantem-se hidratada para continuidade do crescimento, além disso, reduz a concentração de pigmentos

fotossintéticos foliares como mecanismo de fotoproteção. O excesso de água entre 100% e 400% da ET não limitou o crescimento de plantas de *Sorghum bicolor*, pois a elevada taxa transpiratória eliminou para atmosfera o excesso de água no solo, no entanto, a partir de 230% da ET as plantas apresentaram acanhados sinais de decréscimo na iniciação de folhas e teor relativo de água.

Palavras-chave: safrinha, seca, inundação.

Introduction

In the last few years was notorious the expressive growth of sorghum in Brazil, especially Goiás state that consolidated the largest producer, with an estimation of 1.09 million tons production (Ibge, 2019). For the 2020 season, Companhia Nacional de Abastecimento (Conab) estimates that Goiás will represent 44% of sorghum production in Brazil (Conab, 2019). According to Ribas et al. (2014), the significant expansion of sorghum area occurred due to an excellent option to replace maize in animal nutrition.

In animal nutrition, sorghum can replace partially maize in poultry and swine diet and fully in ruminants, with advantage of require less production costs and earn 80% of maize price (Duarte, 2012). Besides be considered as a great option for animal nutrition, sorghum has others fundamental importance to economy. According to Conab (2019), sorghum is an essential basic food for more than 500 million people in more than 30 countries, used as a flour, sugar, fermented alcoholic beverage, morning meals, and others. Also can be applied in chemical, petrochemical and pharmaceutical industries (Noerhartati et al., 2017).

The agronomic point of view, the growth in sorghum production is explained, mainly, due to the high potential of grain production, besides the extraordinary capacity of support environmental stresses, as the frequent drought in second crop season (Jordan et al., 2017).

The grain sorghum (*Sorghum bicolor* L.) is a tropical plant species, thus, requires hot weather to maximum genetic potential. The sorghum plant does not handle low temperatures and, by that, in Brazil, sorghum is cultivated in areas with mean temperatures higher than 20 ºC (Wani et al., 2012). In brazilian cerrado, sorghum can be used as an option in the second crop season, aiming grain production or supplying straw for no-till farming (Júnnyior et al., 2015), can be sown after soybean or maize, due to the greater tolerance to water deficit (Choudhary et al., 2019).

According to Bell et al. (2018), there is a linear positive response of grain yield and crop evapotranspiration (ETc). Despite the hardiness of sorghum, is possible to verify in establishment and growth stages, between germination and panicle initiation, the specie may have impaired development due to water deficit in drought periods, frequent in second crop season, or excess of rain, at the beginning of season. In the flowering and grain filling stages, the water deficit, harms the grain yield and the losses depends of a sum of factors as soil water holding capacity, evapotranspiration and cultivar tolerance (Masasi et al., 2019).

The estimates of alternatives water managements systems, in growth and production, begin by knowing crop responses to water availability in soil. Therefore, the present study aimed to evaluate the effect of water availability in the initial growth of sorghum plants, also, identify the tolerance strategy to drought and excess of water.

Material and methods

The study was carried out in a greenhouse covered with transparent plastic and shade cloth on the sides with 50% interception of the light at State University of Goiás, Southeast Campus, Ipameri Unit (Lat. 17° 42’ 59,12 S, Long. 48°08’40,49’’ West, Alt. 773 m) , Ipameri, GO. The region has tropical climate with dry winter and wet summer (Aw) according to Köppen classification and average temperature of 20°C (Alvares et al., 2013). Three seeds of DOW 1G100 grain sorghum were sown per pots with 5 liters capacity full with 5 Kg of substrate containing oxisol, sand and manure with 3:1:1 proportion, respectively. The chemical analysis of the soil showed the following aspects: pH ($CaCl_2$) 5.4; 16 g dm^{-3} of organic matter; 68 mg dm^{-3} of P; 6.81 mmolc dm^{-3} of K (Mehlich-1); 22 mmolc dm^{-3} (SMP Method) of H + Al; 31 mmolc dm^{-3} of Ca; 15 mmolc dm^{-3} of Mg; 53 mmolc dm^{-3} Base Saturation; 75 mmolc dm^{-3} cation exchange capacity and 71% base saturation.

At 20 days after emerging (DAE) the plants were thinned, just one plant remained in each pot. The experiment was set up in a completely randomized design with six treatments and six replications. After emergence and initial development (20 DAE) the plants were irrigated with water volumes according to 0%, 25%, 50%, 100%, 200% and 400% of daily evapotranspiration.

The seedlings were irrigated daily, according to 100% of daily evapotranspiration until 20 DAE. The crop coefficient (kc) of sorghum hasn’t been established for Ipameri region, Go, it was used kc = 1 following estimate from FAO 56 (Allen et al., 1998) for a group of crops in initial development stage.

The water volume provided was estimated establishing the reference evapotranspiration and crop coefficient according to the equation:

$$ETc = ETo \times kc$$

where:

ETc = crop evapotranspiration (mm day^{-1}),

kc = crop coeficiente,

ETo = reference crop evapotranspiration (mm day^{-1}).

The daily ETo calculation was made by Penman-Monteith method recommended by FAO (Smith et al., 1991), using daily data of maximum and minimum temperature, air relative humidity, insolation and wind speed obtained at INMET Meteorological Station in Ipameri, GO.

At 40 DAE the following analysis were carried out: number of leaves, plant height, stem diameter, biomass, root mass ratio (RMR), stem mass ratio (SMR), leaf mass ratio (LMR), photosynthetic pigments, relative water content (RWC), transpiration rate and chlorophyll *a* fluorescence.

Plant height was measured from the root-stem transition region at soil level (crown) to the tip of the stem using graded rule. The stem diameter was measure at the crown with a digital pachymeter. The number of leaves was obtained by counting. The roots, stems and leaves were separated and dried in an oven at 72 ºC until constant dry weight and the weighed. The dry matter data were used to calculate the RMR, LMR and SMR and biomass.

To determine the total chlorophylls and carotenoids concentrations, 0.6 mm diameter leaf discs were removed from completely opened leaves and placed in test tubes containing dimethyl sulfoxide (DMSO). Then extraction was carried out in a water bath at 65 °C for one hour. Aliquots were removed for spectrophotometric reading at 480, 649 and 665 nm. Then contents of chlorophyll a (Cl *a*), chlorophyll b (Cl *b*) and total carotenoids (Car) were determinate according to the equation proposed by Wellburn, (1994).

To obtain the relative water content, ten 12 mm leaf discs were removed, weighed and placed for four hours to saturate in petri dishes with distilled water. The discs were again weighed and placed to dry at 70 °C for 72h to obtain the dry matter weight.

The total daily transpiration of the plant was determined from the difference in the mass of the pots. The set of pots with plants were placed in individual plastic bags fixed with a rubber band at the stem of the plant, leaving the canopy (leaves and stem) exposed. The pots were weighed at 12 o'clock (mass 1) and again 24 hours later (mass 2). The total transpiration was estimated based on the difference between mass 1 and mass 2 (Dos Anjos et al., 2017).

The analysis of chlorophyll *a* fluorescence were performed using a portable fluorometer JUNIOR-PAM (Walz, Germany) at 4 am with light saturation pulse emission of 0,3 seconds, under 0,6 KHz frequency, at 30 days after implement the water regimes. The data of fluorescence were computed using software WinControl-3.

An analysis of variance and a linear and quadratic regression were performed and in the cases of significance of regression by the F test, the coefficient of determination (R^2) was calculated by the ratio of the sum of the squares of the regression over the total sum of squares. Multivariate analysis was carried out by multiple regression using the *forward stepwise* model (Sokal and Rolf, 1995) and principal component analysis was performed using a permutational multivariate analysis of variance (PERMANOVA - Anderson, 2001). The Statsoft (Statistica, 2007) and SigmaPlot 10.0 (SYSTAT Software, 2006) software was used to carry out these analyses.

Results and discussion

Variables with significant regression adjustment are shown below with the equations. In Figure 1 shows that plant height, stem diameter, leaf area, biomass and stem mass ratio (SMR) were 20%, 50%, 51%, 67% and 33% lower in plants under severe water deficit, without irrigation, compared with plants under 400% of evapotranspiration (ET) water volume respectively. These variables had linear increments as the water volume increased.

Cell turgidity represents *sine qua non* condition to expansion of the relaxed cell wall (Matos et al., 2019). In present study, it's observed on *Sorghum bicolor*, drought tolerant species, that the water is crucial to maximum genetic potential. The data corroborates to those found by Osman et al. (2018), which reports the importance of water in vegetative growth of *Sorghum bicolor* plants and identified decreases in plant height mainly in plants under lack of water. The multiple regression analysis showed in Table 1 represents 91% of biomass variation and indicates that the changes of water status and variables related to root and shoot systems are determinants to biomass accumulation in *Sorghum bicolor* plants.

It is critical to assert that the interval between severe water deficit and 100% of ET irrigation, the angle of curve slope of the referred variables is lower than the interval between 100% of ET and 400% of ET. This result indicate that the growth limitation is greater when the plants are under water volumes lower than 100% of ET and, thus, the water deficit in the present study was most limitant than the excess of water. Following Taiz et al. (2017), the cell expansion is limited in flaccid cells or with low water volume.

The number of leaves showed adjustment with quadratic regression, the maximum number was reached in plants irrigated with a volume of water corresponding to 230% of ET. Under severe water deficit, the number of leaves was 20% lower than the peak. The growth is dependent of hydrostatic pressure between turgid protoplast and cell wall, so that the variation of water availability results in changes in cell division and expansion (Imorou et al., 2018; Schulze et al., 2019). However, according to Matos et al. (2019), the leaf initiation is a result of morphophysiological mechanism and biochemical signaling in which the water is the main factor to induce the leaf initiation, so, the present study the increments of water availability increased the number of leaves and the excess of water over 230% of ET compromised the leaf initiation and indicates reasonable negative effect of high water volume provided.

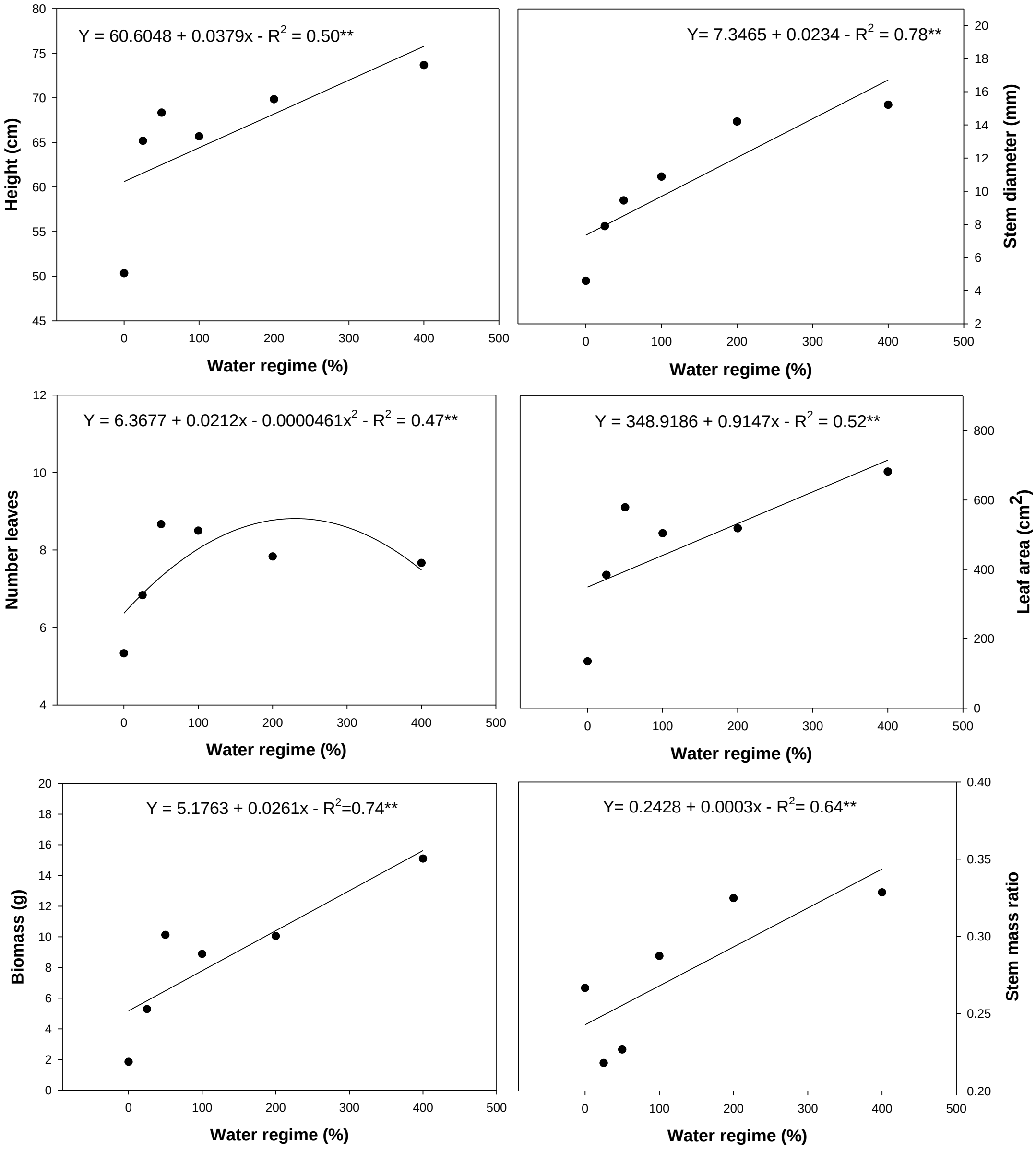

Figure 1. Regression graphics for height, stem diameter, number of leaves, leaf area, biomass and, stem mass ratio for *Sorghum bicolor* plants irrigated with different water regimes. * significant at 5% of probability. ** significant at 1% of probability.

The results in Figure 2 shows that the root length and leaf chlorophyll concentration in plants under sever water deficit were 46% and 42% higher than the plants under water volume corresponding to 400% of ET. These variables fitted to decreasing linear function as the water volumes increased. Under shortage of water is common to increase the root length, thus, increase the possibility to reach more humidity, besides that, the reduction of chlorophyll content represents an important strategy to minimize the light absorption and avoid photosynthesis photoinhibition according to Taiz et al. (2017).

The relative water content (RWC) fitted in the quadratic regression function with maximum RWC in plants irrigated with 244% of ET water volume. The plants under severe water deficit, without water supply, had 46% less RWC compared to maximum RWC. The tissue hydration was critical to growth and the maintaining of biochemical metabolism, required to enzymatic activities, synthesis of essential compounds and cell function. The results shows that the decrease of RWC occurred in plants submitted to water volumes over than 244% of ET and indicates reasonable negative effect of water excess.

Table 1. Multiple regression model to evaluate the importance of the variables analyzed on total biomass in irrigated *Sorghum bicolor* plants with different water volumes.

Biomassa	R^2= 0,91		F (07,28) =45,5		p<0,0000	
	Beta	Std.Err. of Beta	B	Std.Err. of B	t (28)	p-level
Intercept			-8.71	2.31	-3.76	0.0008
Stem diameter	0.82	0.1	1.02	0.12	8.27	0.0000**
Leaf area	0.34	0.09	0.01	0	3.88	0.0006**
Root mass ratio	0.22	0.07	10.96	3.36	3.26	0.0029**
Relative water content	-0.29	0.08	-0.07	0.02	-3.45	0.0018**
Total chlorophylls	0.12	0.07	0.25	0.15	1.66	0.1081
Transpiration	0.18	0.08	0.01	0	2.22	0.0351
Root Length	0.1	0.07	0.03	0.02	1.33	0.1938

**significant at 1% of probability by F test.

The transpiration rate was 75% lower in plants under sever water deficit compared to plants under 400% of ET water volume. The efficient stomatal control limits the excessive water loss, is the main mechanism of tolerance of Sorghum bicolor plants to drought. Following Matos et al. (2014), high stomatal control can allow growth, even though a slower pace, in drought condition. The results suggests that the strong stomatal control allowed the sorghum plants to decrease the transpiration and then keep hydration and preserving growth.

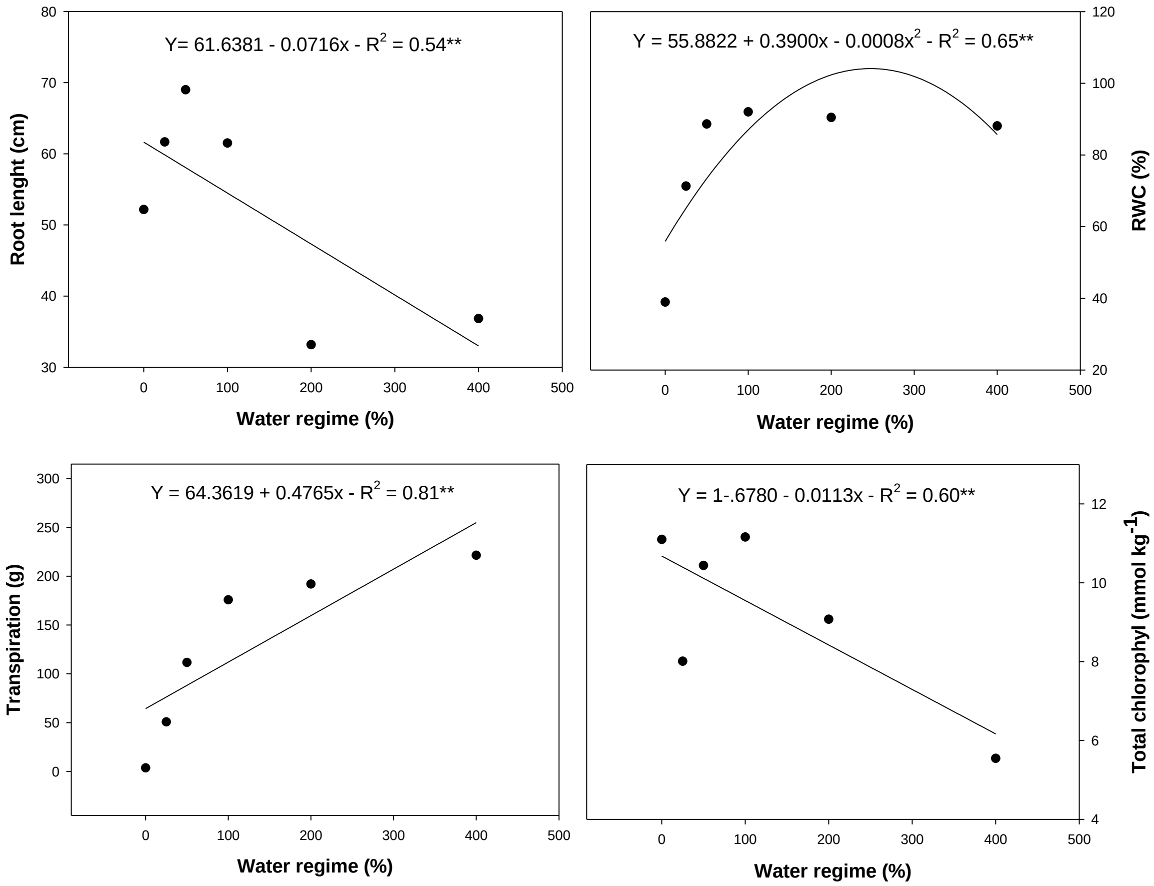

Figure 2. Regression graphics for root length, relative water content (RWC), transpiration and total chlorophyll for *Sorghum bicolor* plants irrigated with different water regimes. * significant at 5% of probability. ** significant at 1% of probability.

The transpiration curve did not show consonance with RWC and indicates that the *Sorghum bicolor* plants under water deficit reduces the water loss to atmosphere and not shows proportional decreases and constancy to RWC, but, smooth decreases of RWC, so that the data points to an intermediary mechanism between isohydric and anisohydric signaling.

The principal component analysis showed in Figure 3 endorse the results presented before and represents 75.46% of data variation. Based on axis 1, that shows the restriction of growth, and the axis 2 shows the relation between root length and leaf mass, it is outstanding that the plants under water deficit showed less growth (axis 1 right) and more investment of resource in root due to positive location to axis 2.

The plants irrigated with 100% of ET water volume showed average values and the plants with greatest water volume (400% of ET) exerted greater plant growth and large investments of resources in shoot (below axis 2). The establishment of *Sorghum bicolor* plants showed strict dependence to tissues hydration by water availability in soil, so that the high stomatal sensitivity limits this resource loss in drought condition and intensifies the transpiration under excess of water.

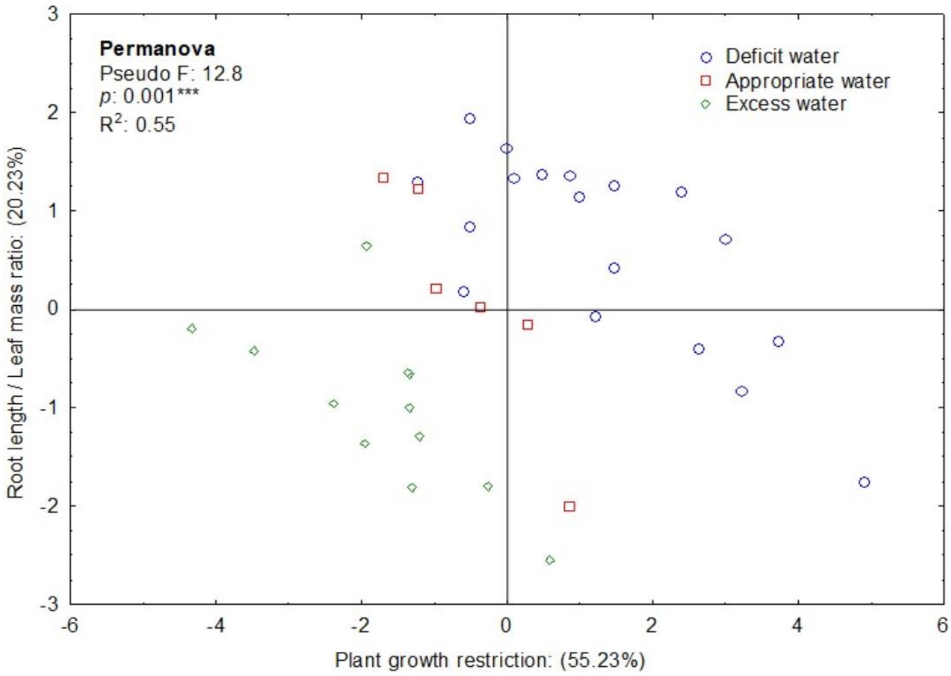

Figure 3. Ranking of the scores of principal components analysis (PCA) for the morphophysiological for *Sorghum bicolor* plants irrigated with different water regimes.

Conclusions

The water restriction limits the growth of sorghum plants, however, to tolerate stress, the plants increase the root length and reduce the transpiration through the efficient stomatal control and maintain hydrated to keep growing, besides that, decrease the concentration of leaf photosynthetic pigments as a photoprotection mechanism.

The excess of water between 100% and 400% of ET did not limit growth of *Sorghum bicolor* plants, due to the high transpiration rate that eliminated to atmosphere the excess of water in soil, however, above 230% of ET, the plants showed smooth signs of reduction in leaf initiation and water relative content.

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