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SEEDLING SELECTION OF SORGHUM FOR DROUGHT TOLERANCE BASED ON ROOT MORPHOLOGY

Deyvisson Rodrigues Pinto*
Universidade Estadual de Montes Claros – Campus Janaúba, Av. Reinaldo Viana, 2630, Janaúba - MG, 39440-000.
E-mail: deyvisson.rp@gmail.com,
https://orcid.org/0000-0002-9269-3398
***Corresponding author**

Cicero Beserra de Menezes
Embrapa Milho e Sorgo, Rod MG 424 Km 45, Zona Rural, Sete Lagoas - MG, 35701-970.
E-mail: cicero.menezes@embrapa.br
http://orcid.org/0000-0001-9437-6946

Abner José de Carvalho
Universidade Estadual de Montes Claros – Campus Janaúba, Av. Reinaldo Viana, 2630, Janaúba - MG, 39440-000
E-mail: abner.carvalho@unimontes.br
https://orcid.org/0000-0002-6644-5307

Arley Figueiredo Portugal
Universidade Estadual de Montes Claros – Campus Janaúba, Av. Reinaldo Viana, 2630, Janaúba - MG, 39440-000
E-mail: arley.portugal@embrapa.br
https://orcid.org/0000-0001-6056-3233

Paulo César Magalhães
Embrapa Milho e Sorgo, Rod MG 424 Km 45, Zona Rural, Sete Lagoas - MG, 35701-970.
E-mail: paulo.magalhaes@embrapa.br
https://orcid.org/0000-0001-7842-4506

Karla Jorge da Silva
Embrapa Milho e Sorgo, Rod MG 424 Km 45, Zona Rural, Sete Lagoas - MG, 35701-970.
E-mail: karla.js@hotmail.com
https://orcid.org/0000-0002-6069-0619

Crislene Vieira Santos
Embrapa Milho e Sorgo, Rod MG 424 Km 45, Zona Rural, Sete Lagoas - MG, 35701-970.
E-mail: cris-vieira15@hotmail.com
https://orcid.org/0000-0003-2204-8673

Alexon Fernandes Campos
Embrapa Milho e Sorgo, Rod MG 424 Km 45, Zona Rural, Sete Lagoas - MG, 35701-970.
E-mail: alexoncampos614@gmail.com
https://orcid.org/0000-0003-3945-4889

Simonica Maria de Oliveira
Universidade Estadual de Montes Claros – Campus Janaúba, Av. Reinaldo Viana, 2630, Janaúba - MG, 39440-000.
E-mail: simonicaagronomia@gmail.com
https://orcid.org/0000-0002-3087-7386

Ignacio Aspiazú
Universidade Estadual de Montes Claros – Campus Janaúba, Av. Reinaldo Viana, 2630, Janaúba - MG, 39440-000.
E-mail: ignacio.aspiazu@unimontes.br
https://orcid.org/0000-0002-0042-3324

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ABSTRACT - Selection for drought tolerance is one of the main challenges of sorghum breeding programs. Early phenotyping in the seedling phase can increase selection gains for this trait. This work evaluated characteristics related to root morphology as indicators for the early selection of sorghum genotypes tolerant to water restriction. Twenty-six hybrids were assessed, of which twenty-three were experimental and three were commercial (BRS310, DKB540, and 1G282). The hybrids were grown in tube containers in a greenhouse with non-stress-stressed water. The evaluated characteristics were Root length (RL), root surface area (SA), root diameter (RD), root volume (RV), the surface area of the thin roots (from 0-1 mm in diameter) (SA1), as well as the root/shoot dry mass ratio (R/S). In water restriction conditions, the hybrids with the best performances for most of the evaluated characteristics were BRS310, 1516057, 1719034, 1719026, 1716041, 1G282, 1516059, 1720052, 1716049, 1718036, and 1716045. The RV, SA, SA1, and R/S characteristics most contributed to selecting these hybrids.

Keywords: *Sorghum bicolor*; drought tolerance; early selection.

SELEÇÃO DE MUDAS DE SORGO PARA TOLERÂNCIA À SECA BASEADA NA MORFOLOGIA DE RAIZ

RESUMO - A seleção para tolerância à seca é um dos principais desafios dos programas de melhoramento de sorgo, e a fenotipagem precoce, na fase de plântulas, pode aumentar os ganhos de seleção para essas características. Este trabalho avaliou características relacionadas à morfologia radicular como indicadores para a seleção precoce de genótipos tolerantes à restrição hídrica. Foram avaliados 26 híbridos, sendo 23 experimentais e três comerciais (BRS310, DKB540 e 1G282). Os híbridos foram cultivados em tubetes em casa de vegetação com e sem restrição hídrica. As características avalaidas foram: Comprimento da raiz (RL), área de superfície da raiz (SA), diâmetro da raiz (RD), volume da raiz (RV), área de superfície de raízes muito finas (de 0-1 mm de diâmetro) (SA1), razão da raiz pela massa seca da parte aérea (R/S). Sob restrição hídrica, os híbridos com os melhores desempenhos para a maioria das características avaliadas foram BRS310, 1516057, 1719034, 1719026, 1716041, 1G282, 1516059, 1720052, 1716049, 1718036 e 1716045. As características RV, SA, SA1 and R/S apresentaram a maior contribuição na seleção desses híbridos.

Palavras-chave: *Sorghum bicolor*; tolerância à seca; seleção precoce.

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The use of crops tolerant to drought is alternative farmers adopt for cultivation in regions or times subject to drought, mainly during the second crop planting in Brazil. Grain sorghum (Sorghum bicolor (L.) Moench) emerges as a potential option for cultivation under these conditions once it presents morphophysiological mechanisms of tolerance and escape, making it more efficient in the use of water than other cereals, such as corn and wheat (Magalhães et al., 2000; Tardin et al., 2013).

Despite its better performance compared to other crops under these conditions, the final yield of sorghum can also be affected when subjected to prolonged and severe periods of drought (Santana et al., 2017). Thus, selecting genotypes that are more adapted to water stress conditions can enable cultivation in places with water scarcity throughout the year, such as in regions with an arid or semiarid climate or the Midwest and Southeast of the country during the off-season (Menezes et al., 2015).

The search for sorghum genotypes more tolerant to drought and for phenotypic characteristics that facilitate the identification of these materials has been the objective of several studies (Sabadin et al., 2012; Tardin et al., 2013; Menezes et al., 2015; Batista et al., 2017; Souza et al., 2020). However, uncontrollable conditions in the field, such as soil heterogeneity, climate change, and other abiotic stresses that can lead to genotypes x environments interaction, hinder the selection process of the genotypes best adapted to the desired conditions and limit the number of genotypes that can be evaluated (Bibi et al., 2012).

Early selection, at the seedling stage, can be used to select genotypes with better performance under water restriction conditions to reduce the amount and variation between materials that will be evaluated in the field. Rauf et al. (2008) reports that among the benefits of this methodology, there is a reduction in costs, ease of handling, and the possibility of discarding susceptible genotypes in advance. Haussmann et al. (1998) understand that indirect selection characteristics should be easy, cheap, and quick to measure, thus allowing high-intensity selection.

The selection of materials based on seedling characteristics is efficient for crops such as rice (Gómez-Luciano et al., 2012), corn (Meeks et al., 2013), sunflower (Rauf et al., 2008), alfalfa (Hanson et al., 2015) and sorghum, in polyethylene glycol solution (Bibi et al., 2012; Chaniago et al., 2017).

Ali et al. (2009) correlated the results of seedling traits with field data. They suggested that root length, dry root mass/shoot dry mass ratio, coleoptile length, and grain yield can be improved simultaneously due to the existence of high and positive correlation among them, pointing them out as reliable selection criteria for drought tolerance.

Therefore, this work aimed to evaluate characteristics related to root morphology as indicators for the early selection of grain sorghum hybrids for drought tolerance.

Material and Methods

The trials were carried out in a greenhouse at Embrapa Milho e Sorgo, located at 19º 27’ 57” S and 44º 14’ 49” W. Tube-shaped containers measuring 6.3 cm x 19 cm were used filled with a typical dystrophic Red Latosol, fertilized with NPK (08-28-16), in the ratio of 50 g de N, 50

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g de P2O5, 35 g de K2O per m3 of soil. Ten days after sowing, irrigation with 5% urea was performed. The tubes were placed in trays of 6 x 9 cells, totaling 54 tubes per tray. Three seeds were sown per tube, leaving only one plant in each tube after germination. Each experimental unit consisted of three tubes. The design was in randomized blocks with four replications. Twenty-six hybrids were evaluated, 23 experimental and three commercial (BRS310, DKB540, and 1G282).

The plants were kept in a greenhouse at 32°C during the day and 25°C at night, irrigated daily with 50 mL of water per tube, with the aid of a graduated cylinder in millimeters. Eleven days after sowing, irrigation stopped only in the trial with water restriction. Nine days after the water restriction, when the seedlings began to show symptoms of water deficit, such as yellowing and curling of the leaves, the shoot was cut at the height of the seedling root collar and separated from the root system. Then, the root system of the seedlings was washed with a jet of water until it was free of soil and impurities. Washed roots were stored in flasks containing 70% ethanol to avoid dehydration.

Root length (RL, cm), the surface area of roots (SA, cm2), root diameter (RD, mm), root volume (RV, cm3), the surface area of fine roots (0-1 mm in diameter) (SA1, cm²) were evaluated, in addition to the root dry mass/ shoot dry mass ratio (R/S). The Winrhizo Pro 2007a system (Regent Instr. Inc.) was used, coupled with a professional Epson XL 10000 scanner equipped with an additional light unit (TPU) with a definition of 400 dpi to evaluate the characteristics related to root morphology (Magalhães et al., 2011). For scanning, the roots were placed in an acrylic vat 20 cm wide by 30 cm long, containing approximately 1 cm of water. This accessory allows for obtaining images in three dimensions, also avoiding the overlapping of roots; readings were carried out on three plants per plot. The material was stored in paper bags and dried in an oven with forced air circulation at 72 °C until a constant mass to determine the dry mass of shoots and roots. After drying, the plant material was weighed.

The data of the assays were subjected to joint analysis of variance according to the following statistical model:

$$Y_{ijk} = m + G_i + B/A_{jk} + A_j + GA_{ij} + \varepsilon_{ijk}$$

In which observation is done in the plot of the i-th treatment in the j-th block; m is the general mean; is effect of the i-th genotype. A_j is the effect of the j-th environment; is the effect of the interaction of the i-th genotype with the j-th environment; is the effect of the k-th block within the j-th environment; is the effect of the uncontrolled factors in the plot given by the i-th genotype in the j-th environment within the k-th block.

In case of significant effects, the hybrids were grouped by the Scott-Knott test, and the F test compared the environments. The analyses were performed using the Genes program (Cruz, 2013). First, the magnitude of the correlations between the evaluated characteristics was studied through the Pearson correlation performed for each environment, with and without water restriction. In addition, principal component analysis was performed, which was processed with the covariance matrix of the original variables, obtaining from it the

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eigenvalues that built the eigenvectors. These are linear combinations of the original variables and are called principal components. Finally, biplots of principal component analyses were plotted separately for the environments with and without water restriction, implemented in the R software (R Development Core Team, 2016).

Results and Discussion

There was a significant effect for the interaction genotypes x environments (G x E) in all the characteristics measured, except for root length, indicating that the hybrids showed different responses in the evaluated environments (Table 1). This result was expected, as these are two very different environmental conditions significantly influencing the root system. In addition, the effect of hybrids was significant for all traits except for root length (Table 1), indicating genetic variability among genotypes, allowing for the selection of genotypes based on these traits.

For most traits, there was more significant variability of the genotypes in the environment without water restriction than in the environment with water restriction. This result is related to the constraint of plant growth in the water-restricted environment, which, for growing less, also reduced the amplitude of growth differences between the hybrids evaluated (Table 1; Figures 1 and 2).

Root length (RL) was not influenced by the factors of hybrids and environments, with a mean of 277.8 cm (Table 1). However, this behavior may be associated with the size of the tube-shaped containers in which the plants were

Table 1 - Summary of joint analysis of variance for morphological characteristics of seedlings of grain sorghum hybrids in environments with and without water restriction. Sete Lagoas – MG, 2019.

SF	DF	Quadrado médio					
		RL	**SA**	**RD**	**RV**	**SA1**	**R/S**
Hybrids (H)	25	442.3^{ns}	840.4^{**}	0.001^{**}	0.04^{**}	491.7^{**}	0.0006^{**}
Environment (E)	1	757.8^{ns}	30386.5^{**}	0.02^{ns}	0.35^{**}	17319.1^{**}	0.0032^{ns}
H x A	25	532.5^{ns}	446.0^{**}	0.0004^{*}	0.04^{**}	245.9^{**}	0.0004^{**}
Resíduo	150	415.9	210.6	0.00	0.01	124.3	0.0002
Média		277.78	76.77	0.27	0.57	60.36	0.0450
CV%		7.34	18.91	5.93	17.54	18.47	27.99

Source of variation (SV), degree of freedom (DF); RL: Root length (cm); AS: Total area of the root surface (cm^2); RD: Root diameter (mm); RV: Total root volume (cm^3); AS1: Surface area of very fine roots between 0 and 1 mm in diameter (cm^2) and R/S: Root dry mass (g)/shoot dry mass (g) ratio. Coefficient of variation; (CV%), ^{ns} – not significant, $^{**, *}$= significant at 1% and 5% by F Test respectively.

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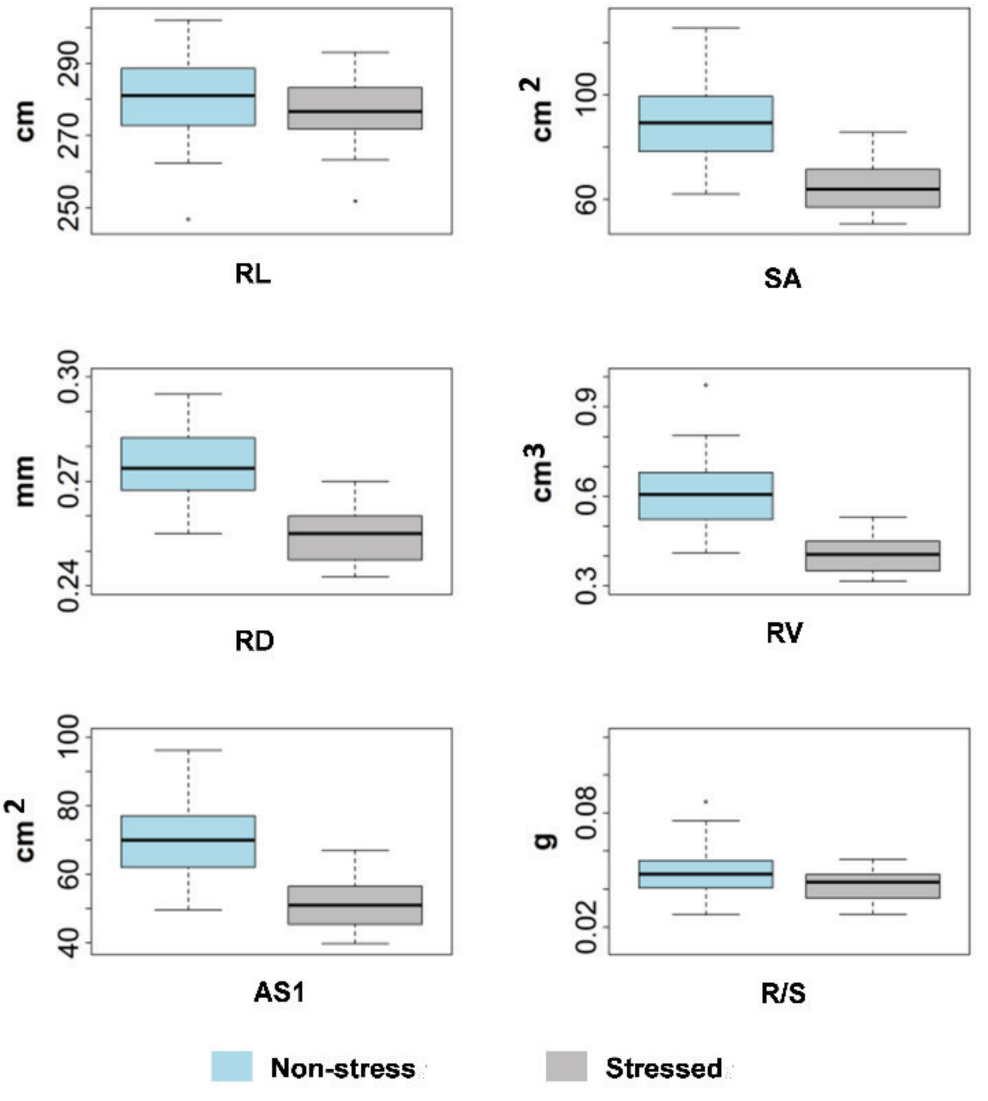


Figure 1. Means of root length (RL; cm); total area of the root surface (SA; cm^2); root diameter (RD; mm); total root volume (RV; cm^3); surface area of very fine roots between 0 and 1 mm in diameter (SA1; cm^2); Root dry mass/shoot dry mass ratio (R/S) of grain sorghum hybrids carried out with and without water stress. Sete Lagoas, MG, 2019.

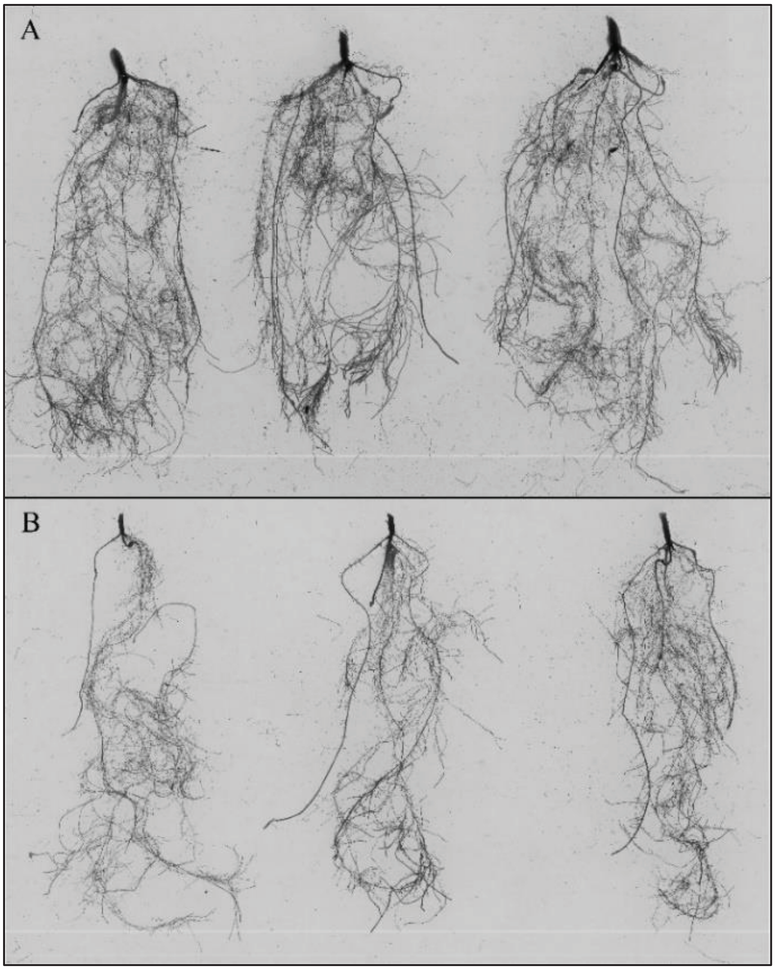
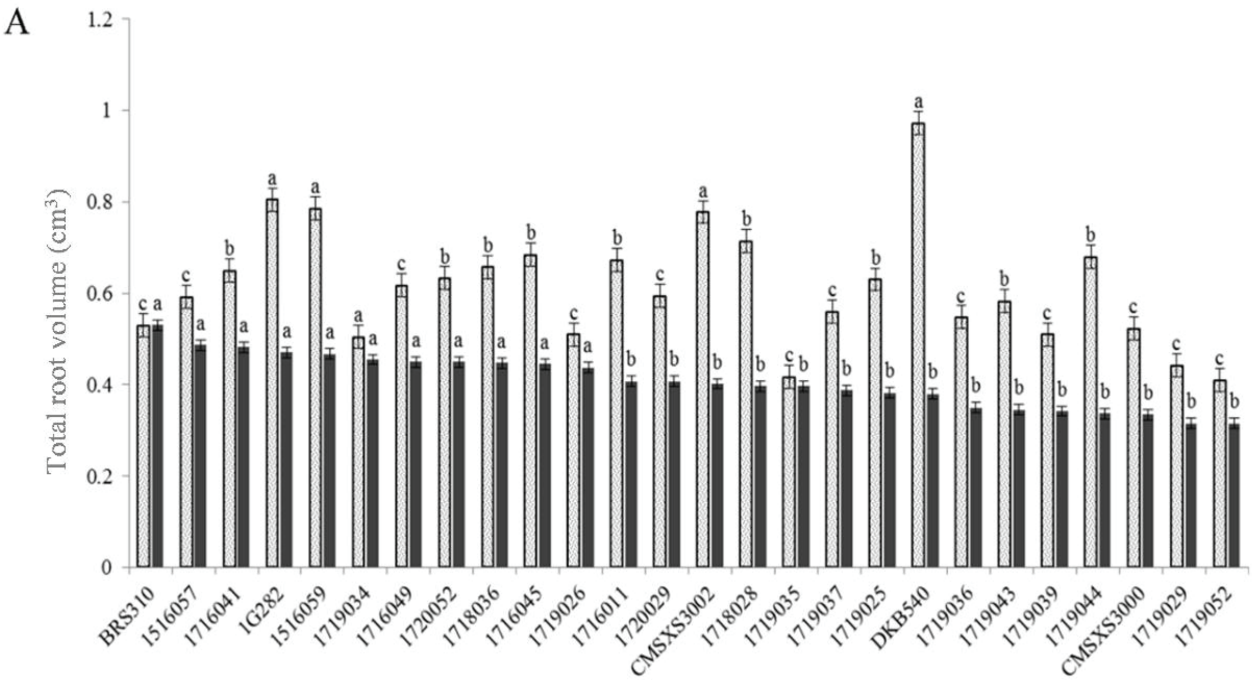


Figure 2 - Contrast among roots of a same sorghum genotype submitted to an environments without (A) and with water restriction (B). Sete Lagoas, 2019.

grown (19 cm) since the root system of all the genotypes had already reached the lower limit of the container when imposed the stress. However, the evaluation of this characteristic in breeding programs should be considered, given that changes in the morphology of the root system, especially length, and volume, have been related to correlate with grain yield (Bibi et al., 2012; Ali et al., 2017; Avila, 2018). Furthermore, according to Negri et al. (2014), the sorghum root length presents a positive correction with the total surface area and the surface area of fine roots, which allows the indirect selection of this characteristic in future evaluations.

For root volume, 19 out of the 26 hybrids showed significant differences compared to the two environments, with higher means in the environment without restriction (Table 1; Figure 3A). In the stressed environment, the group with the best means comprised the hybrids BRS310, 1516057, 1719034, 1719026, 1716041, 1G282, 1516059, 1720052, 1716049, 1718036, and 1716045, ranging from 0.530 to 0.437 cm^3. The hybrids BRS310, 1516057, 1719034, and



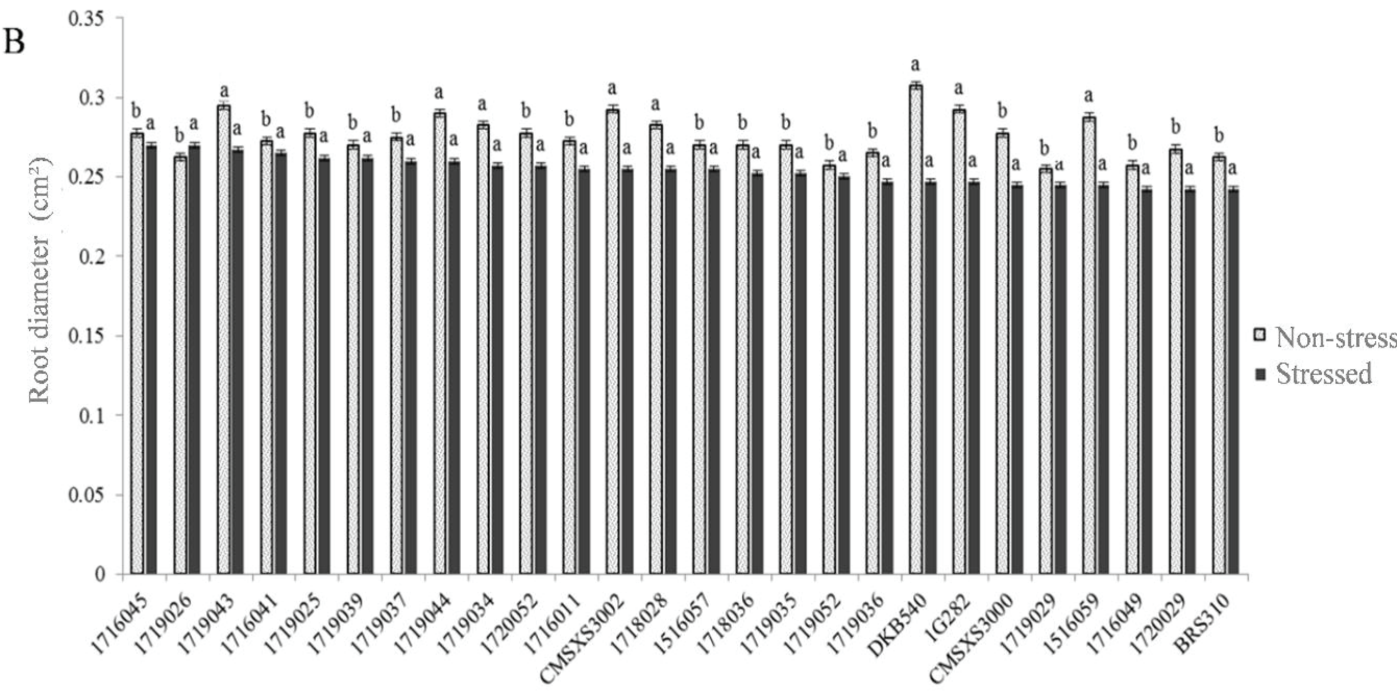


Figure 3 - Total root volume (RV; cm³) – A and mean root diameter (RD; cm²) – B of grain sorghum hybrid carried out in environments under water stress and non-stressed. Sete Lagoas, MG, 2019. Means followed by the same letter in the same environment do not differ statistically by the Scott-Knott test at 5% significance level. Bars represent the standard error of the mean.

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1719026 did not significantly reduce root volume when subjected to stress, indicating possible tolerance to environments with water restriction. On the other hand, when fully irrigated, only the hybrids DKB540, 1G282, 1516059, and CMSXS3002 were statistically superior to the others (Figure 3A).

The hybrids did not differ for root diameter (DR) under water stress, ranging from 0.270 to 0.242 mm, which, in practice, would not allow the selection of superior materials for this characteristic in this environment (Table 1; Figure 3B). In contrast, in the non-stressed environment, eight out of the 26 genotypes presented higher means than the others. Furthermore, these eight genotypes showed greater diameter under non-stressed environments. The reduction in root diameter when in a harsh environment is understood as an adaptive capacity of some species because, according to Bhom (1979), thinner roots play a fundamental role in the absorption of water and nutrients, while thicker roots are less efficient in this function, are more related to support.

The total root surface area (SA) and the surface area of fine roots (AS1) were also higher in most genotypes in the water-non-stressed environment compared to the stressed environment (Table 1; Figure 4A, B). The similarity in the results of these two characteristics is explained by the dominance of the surface of fine roots in the total area of the root surface, which, on average, represented 78 and 79% in environments without and with restriction, respectively, which was already expected, given that evaluations were performed on seedlings in the first 20 days of development. Except for 1720029, in the water-restricted environment, the hybrids that obtained the highest values of SA (85.7 to 65.7 cm²) and SA1 (67.0 to 52.1 cm²) also stood out concerning root volume.

Magalhães et al. (2014) observed that maize plants considered drought tolerant presented similar total root volume in an environment with and without water restriction but increased the length and volume of fine roots and the total surface area and volume of fine roots when subjected to water restriction.

Concerning the R/S ratio, only the hybrids DKB540, 1G282, and CMSXS3002 presented the highest means in the environment without water stress. However, in the stressed environment, 15 hybrids were grouped with the highest means, among them the three commercial hybrids, BRS310, 1G282, and DKB540, in addition to the hybrids: 1516059, 1720052, 1719034, 1716045, 1516057, 1718036, 1716041, 1716049, CMSXS3002, 1720029, 1719035 and 1716011 (Table 1; Figure 4C). Higher values presented by these hybrids concerning the others suggest an increase in the allocation of resources for root development. Turner and Begg (1981) report that, despite the influence of water restriction on the distribution of photoassimilates being dependent on the growth stage and sensitivity of each organ, in general, plants with greater tolerance to drought can reduce the development of the shoot under water-stressed conditions and increase the contribution of resources in roots.

Bibi et al. (2012), working with sorghum selection under stress conditions simulated by polyethylene glycol, observed that plants with higher root dry mass were less affected by water restriction. Similar results were also observed for the alfalfa crop evaluated in the seedling stage, showing that some varieties increased the root/

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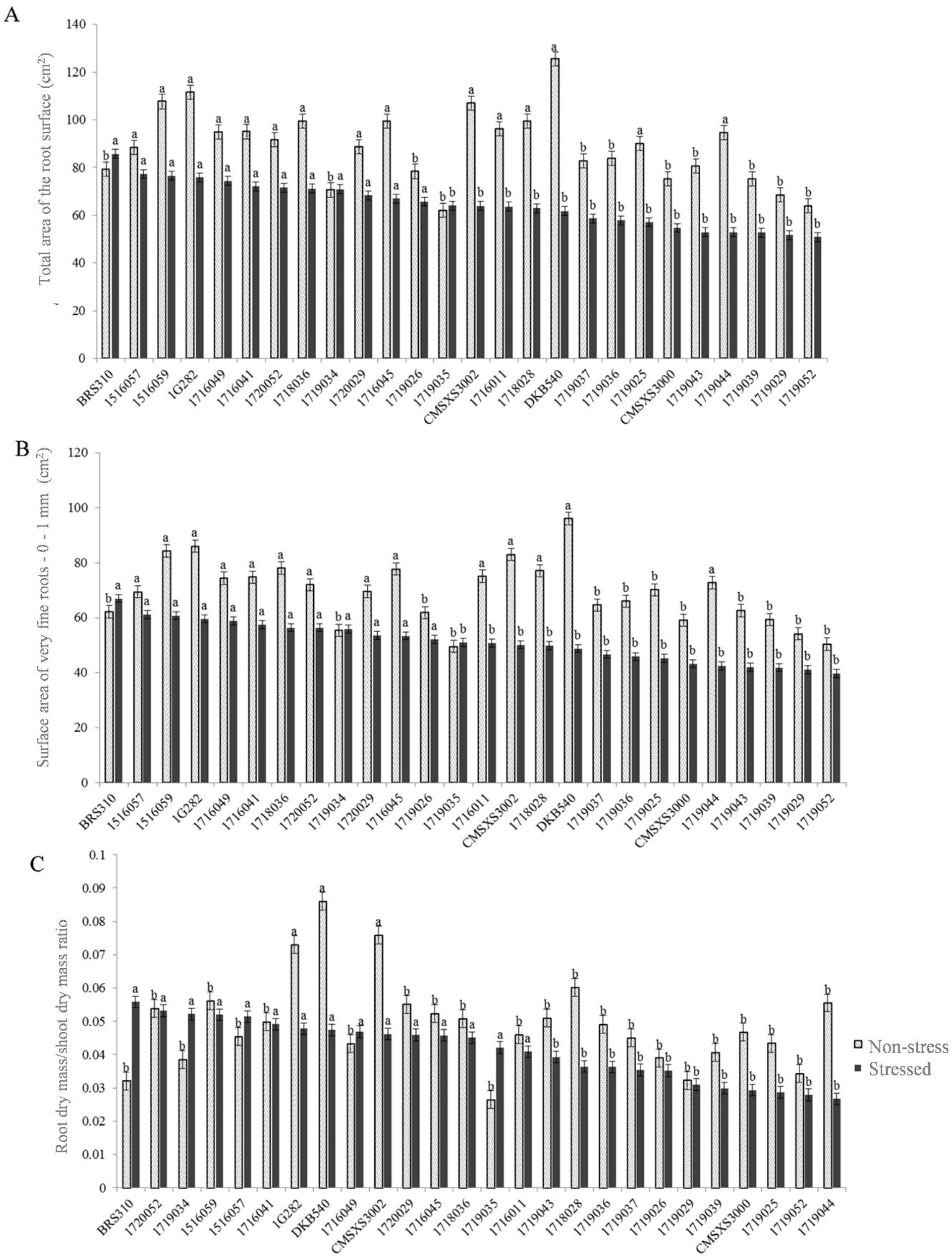

Figure 4. Total area of the root surface (SA, cm²) - A; surface area of very fine roots between 0 and 1 mm in diameter (SA1, cm²) - B; Root dry mass/shoot dry mass ratio (R/S) – C of grain sorghum hybrid carried out in environments with and without water stress. Sete Lagoas, MG, 2019. Means followed by the same letter in the same environment do not differ statistically by the Scott-Knott test at a 5% significance level. Bars represent standard error of the mean.

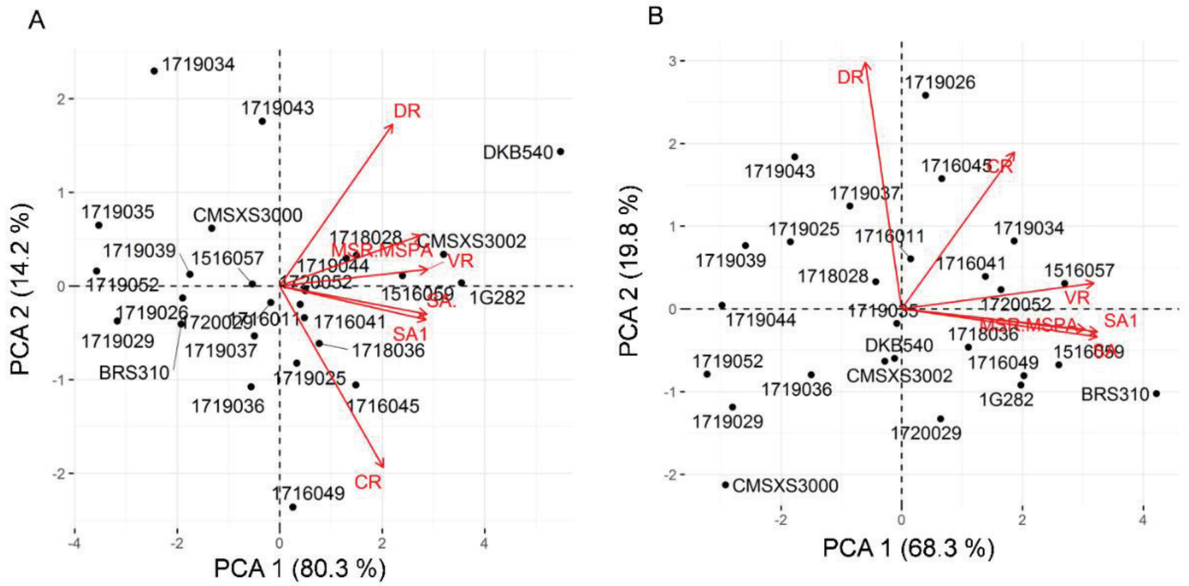

Figure 5. Principal Component Analysis - PCA Biplot for root length (RL; cm); total area of the root surface (SA; cm^2); root diameter (RD; mm); total root volume (RV; cm^3); surface area of very fine roots between 0 and 1 mm in diameter (SA1; cm^2); root/shoot dry mass ratio (R/S) of seedlings of grain sorghum hybrids conducted in environments without (A) and with (B) water restriction. The first and second principal components are on the x and y axes, respectively. Sete Lagoas, MG, 2019.

shoot ratio under severe drought, indicating that it is a trait with potential for evaluation in breeding programs for cultivars with drought tolerance (Hanson et al., 2015). Akinwale et al. (2017) observed that the root/shoot ratio is an essential indicator of drought tolerance for corn crops.

The correlations among the traits are in Figure 5. VR, SA, SA1, and R/S correlated positively, while RD correlated negatively to RL.

In the principal component analysis, each axis explains a percentage of the variation between the hybrids. For example, the PCA1 and PCA2 explained 94.5 and 88.1% of the total variation in the data for the environments without and with water restriction, respectively (Figure 6A, B), demonstrating the effectiveness of the analysis in the selection of these characteristics. According to Hongyu et al. (2016), at least 70% of the total variation of the data must be explained by the first and second main components and allow the discard of redundant characteristics or that have a low correlation with the essential characteristics in the main components.

In both environments, it is possible to observe that the characteristics VR, SA, SA1, and the R/S ratio significantly influenced the selection of hybrids, with practically matching contributions, allowing them to be considered together in an evaluation (Figure 5A, B).

In general, in the environment without water restriction, the commercial hybrids DKB540 and 1G282 were the ones that presented the best results for all characteristics (Figure 5A). However, in the water-restricted environment,

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the position of the DKB540 hybrid reveals that it was not among the highest potential hybrids (Figure 5B). Batista et al. (2017) observed that, under field conditions, the hybrid DKB540 showed high instability but still proved highly yielding. The less expressive response of this hybrid to the water-restricted environment in this work may be associated with the characteristics evaluated since, in addition to strategies in root morphology, tolerant genotypes may also present physiological mechanisms at the shoot level to minimize loss of water and increase its yield (Magalhães et al., 2014).

In the stressed environment, the hybrid with the best performance among the evaluated traits was BRS310 (Figure 5B). This hybrid performed well under water restrictions in field conditions (Tardin et al., 2013). The hybrid 1516059 also stands out, potentially expressing the characteristics of both environments. In addition, it is worth noting the hybrids 1516057, 1719034, 1719026, 1716041, 1G282, 1720052, 1716049, 1718036, and 1716045, which also showed good performance for characteristics when under water restriction conditions.

Conclusions

The seedling characteristics of root volume, root surface area, the surface area of fine roots, and the root dry mass/shoot dry mass ratio allow the discrimination of sorghum hybrids under drought stress.

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