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MULTIVARIATE ANALYSIS FOR WHEAT GENOTYPES CULTIVATED IN BRAZILIAN SAVANNA (CERRADO)

SUMMARY

The Cerrado is an important agricultural region for the production of food, feed and (bio)fuel, with great potential for the cultivation of tropical wheat (*Triticum aestivum* L.). However, it is necessary to understand how some genotypes of that crop would fit into this biome, as well as to identify possible physiological and chemical markers that may contribute to the selection of the best plant materials. The objective of this research was to evaluate the metabolic, physiological, biometric and productive characteristics of six wheat cultivars, commonly cultivated in the Cerrado of Minas Gerais, through multivariate analysis (Principal Components Analysis: PCA), in order to characterize the performance of these cultivars in the Cerrado conditions, in two years (2016 and 2017). The PCA showed that monosaccharides content (glucose and fructose) and net CO_2 assimilation rate were highly correlated in both years. Significant differences in rainfall between the two years resulted in different responses of the cultivars and their respective metabolic, physiological, biometric and productive behaviour. Furthermore, it was demonstrated that the CD 151 and Bio Sintonia cultivars grew better when exposed to favourable rainfall conditions, whereas BRS 264 and BRS 394 were the cultivars most suited to lower rainfall.

Keywords: *Triticum aestivum* L., physiology, sugars, dryland cultivation

INTRODUCTION

The Cerrrado, a Brazilian neotropical Savanna, covers 204.7 million hectares in the centre of the country (Sano *et al*., 2010), and it can be considered a region with great potential for the production of tropical wheat (*Triticum aestivum* L.) (Pasinato *et al*., 2018). Specifically, in the state of Minas Gerais, the land used by agriculture covers 2,122,452 hectares and the wheat cultivation, in 2018, occupied approximately 84,000 hectares (CONAB, 2018).

The Cerrado biome is characterized by a dry period from May to September (Wolf, 1977), which hampers agricultural production (Assad *et al*., 1993). For the last 40 years there has been a search for better wheat cultivars for the Cerrrado and, currently, agronomists specialising in the tropical wheat crop

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seek genetic materials adapted to dryland or irrigated conditions (Caierão *et al.*, 2014). The successful cultivation of tropical wheat in the dry climate of the Cerrado is explained by the early sowing (between February and March) of cultivars with a short growth-cycle on to water retentive soils (Pasinato *et al.*, 2018). Furthermore, this crop stands out because it is harvested at a different time compared to the wheat grown in the main cereal producing regions in Brazil (Ribeiro *et al.*, 2012). Approximately 20% of the total proteins and calories consumed by the world's population are supplied by the wheat crop and, because of this global significance, it is vital that cultivars are developed that are adapted to environmental changes, such as: increasing temperature, carbon dioxide, drought and salinity (Shiferaw *et al.*, 2013; Curtis and Halford, 2014). In order to combat such environmental stresses, plants are likely to have developed the use of some sugars as 'signalling molecules', which control the plants growth and development (Sheen *et al.*, 1999; Miller *et al.*, 2010; Keunen *et al.*, 2013) and protect the plants tissues under inhospitable conditions (Couée *et al.*, 2006). These latter four papers suggest that many carbohydrates function as 'adjustment markers' in plants under adverse conditions, with important signalling in their growth and reproductive stages.

The identification of those biological processes that act as markers in tropical wheat is essential for its cultivation across new agricultural frontiers, the promotion of new farming practices, and the attainment of higher yields. The aim of this research is to determine the physiological, metabolic and production characteristics of wheat cultivars grown under dryland conditions in the Cerrado.

MATERIAL AND METHODS

Two experiments were conducted in 2016 and 2017 at the Cooperativa Agropecuária do Alto Paranaíba (COOPADAP), Rio Paranaíba, MG, Brazil, whose geographic coordinates are 19º 12' 26" S and 46º 10' 05" W, with 1,140 m of altitude. The Köppen classification of the climate is Aw, with tropical and dry winter, as seen in the course of the experiments (Table 1).

Table 1. Means of air relative humidity (%), precipitation (mm) and air temperature (°C) in experimental area (COOPADAP), Rio Paranaíba (MG), during two years of analysis.

	2016			**2017**		
	RH (%)	Precipitation (mm)	Temperature (°C)	RH (%)	Precipitation (mm)	Temperature (°C)
April	68.60	19.50	21.90	81.75	34.20	23.11
May	74.60	9.39	19.50	75.86	95.20	20.23
June	73.40	28.46	17.40	70.28	12.40	19.10
July	61.50	0.00	18.60	65.26	0.20	16.84
August	58.30	13.22	19.90	54.00	0.00	20.80
Mean	67.28	14.11	19.46	69.43	28.4	20.01

The seeds for the first experiment were planted on 04.24.2016, and for the second on 04.17.2017. Five wheat cultivars were studied in both experiments: Coodetec CD 151 and Coodetec 1104 (Coodetec), together with Biotrigo Sintonia (Biotrigo), BRS 264 and BRS 394 (Embrapa).

Both experiments consisted of four replicates of each cultivar, with each replicate consisting of 5 rows, 5 m in length and spaced at 17 cm, using 400 seeds m^{-2}. Measurements were taken solely from the three central rows of each replicate. Following chemical analysis of the soil in the experimental area, 300 Kg ha^{-1} of Mono-ammonium-phosphate fertilizer (MAP) was applied at planting, and 250 kg ha^{-1} of formulated 21-00-21 (N-P_2O_5-K_2O) during the vegetative stages. Weeds, pests and diseases were controlled, according to the needs of the crop, by products registered for use on wheat crops in Brazil.

The gas exchange analyses were done by a portable infrared gas analyser (IRGA), model LI-6400XT (LI-COR, Lincoln, Nebraska, USA), during the 'boot' stage (Feekes 10.0 phenological stage) (Large, 1954), at 53 days after plant emergence (APE) in the first experiment, and 51 days APE in the second experiment. In both experiments were measured: photosynthetic rate (*A* - µmol m^{-2} s^{-1}); stomatal conductance (*gs* - mol m^{-2} s^{-1}); transpiration rate (*E* - mol H_2O m^{-2} s^{-1}); and intracellular CO_2 concentration (*Ci* - Pa). These measurements were taken between 8:30 am and 11:30 am, under photosynthetic active radiation of 1,500 µmol photons m^{-2} s^{-1}.

A ruler was used to measure plant height (PH) and source-sink distance (SD): i.e. the sum of flag leaf length and distance between the upper culm node and spike insertion (Fioreze and Rodrigues, 2014). Also measured were the numbers of total tillers per plant (TP) and of fertile tillers per plant (FT). The productive components were measured, at 105 days APE in 2016 and at 103 days APE in 2017, by counting: the number of spikelets per plant (NSP); the number of grains per spikelet (NGS); and the total number of grains per plant (NG).

Flag leaves were collected for glucose and fructose analyses between the phenological stages: Feekes 9.0 (ligule of flag leaf visible) and Feekes 10.0 (boot stage), according to the Feekes-Large scale (Large, 1954). After collection, the flag leaves were put into 50 mL Falcon® tubes, which were then placed in Styrofoam boxes containing ice and taken to the laboratory, where approximately 150 mg of leaves were grounded in liquid nitrogen to obtain a fine powder and extracted with 1.5 ml of an ethanol/water mixture (80/20; v/v) during 1 h, then the samples were centrifuged at 3000×*g*, at 4 °C, during 10 min. The supernatant was recovered and evaporated at reduced pressure. The extract was finally dissolved in 1ml water, filtered at 0.45 m and analyzed (Guignard *et al.*, 2005). The concentrations of sugars were measured by High Performance Liquid Chromatography (HPLC), using the model LC-10 of Shimadzu Co., with a refractive index detector (Shimadzu Co. model RID 6A) and column (Shimadzu Co. model CLC-NH2 (M)), 15 cm x 6.0 mm, with amine groups chemically bonded to silica. The mobile phase consisted of a mixture of acetonitrile and ultrapure water at a ratio of 80:20 v/v, with a flow of 2.0 mL min-1, at oven temperature used was 40 °C. The sample was filtered with Millipore disposable filter (Hidrophilic PVDF) with membranes 0.45 µm of pore, and stored in vial of 1.5 ml, being the injected volume equal to 20 µL.

Multivariate principal component analysis (PCA) was applied to identify possible clusters between all dependent variables evaluated in both experimental years. The data were transformed for standardization (a procedure necessary to homogenize the scales), and the calculation of the Eigen vectors values was adopted to determine the importance of each variable (McGarigal *et al.*, 2000). The data were analysed using the software PAST - Paleontological Statistics (Hammer *et al.*, 2001).

RESULTS AND DISCUSSION

In our research we observed that water scarcity inhibited the genetic potential of crop growth and production, this behaviour is expected in plants under drought stress, according (Fotouhi et al., 2017) this is one of the several environmental factors that greatly limiting crop production and plant establishment, and we adopted the multivariate analysis for understand different mechanisms in wheat plant, under different water conditions by two consecutive years.

By recognizing patterns, rather than classifying data, PCA reveals the existence (or not) of relations or groupings between observed samples (Lyra *et al.*, 2010). In this research PCA was used to assess the relation between six wheat cultivars and their physiological and biochemical properties, during two years of cultivation under dryland conditions. According (Bro and Smilde, 2014), the components of PCA explain the variation in the whole dataset in a certain sense, and the technique is able to identify some phenomena. The results of this research showed how the components of PCA could explain 59.98% of total variances obtained in 2016 and 49.85% in 2017 (Table 2). Where it was observed that, in the first year of evaluation, principal component 1 (PC1) explained 37.38 % of variance and principal component 2 (PC2) explained 22.60% of variance, while in the second year PC1 explained 30.48 % of variance and PC2 explained 19.37% of variance (Table 2).

For the 2016 crop, the biplot graph (Figure 1) showed the existence of an independent gradient, characterized by a positive grouping among glucose, fructose, number of grains per spikelet (NGS) and photosynthetic rate (*A*) in the right lower quadrant. The parameters more directly involved with the regulation of water status, i.e. transpiration (*E*), stomatal conductance (*gs*) and source-sink distance (SD) of the wheat plants, are also located in the right upper quadrant (Figure 1). With regard to cultivars in 2016, it was observed that BRS 264 and BRS 394 showed a close relationship to *gs*, *E* and SD. For PC2 another independent gradient was seen, characterized by the grouping of productive parameters: number of spikelets per plant (NSP), fertile tillers (FT) and number of grains per plant (NG), all positively correlated (Figure 1).

Table 2: Eigen values and variances for each principal component in the 2016 and 2017 crop seasons.

PC	2016		2017	
	Eigenvalue	**% Variance**	**Eigenvalue**	**% Variance**
1	4.850	37.388	3.963	30.483
2	2.930	22.600	2.518	19.371
3	1.482	11.407	2.226	17.120
4	0.962	7.403	1.501	11.547
5	0.891	6.857	1.114	8.570
6	0.693	5.338	0.740	5.694
7	0.466	3.591	0.457	3.519
8	0.353	2.719	0.249	1.916
9	0.226	1.743	0.113	0.869
10	0.076	0.585	0.102	0.784
11	0.033	0.256	0.009	0.073
12	0.011	0.091	0.004	0.034
13	0.002	0.017	0.003	0.022

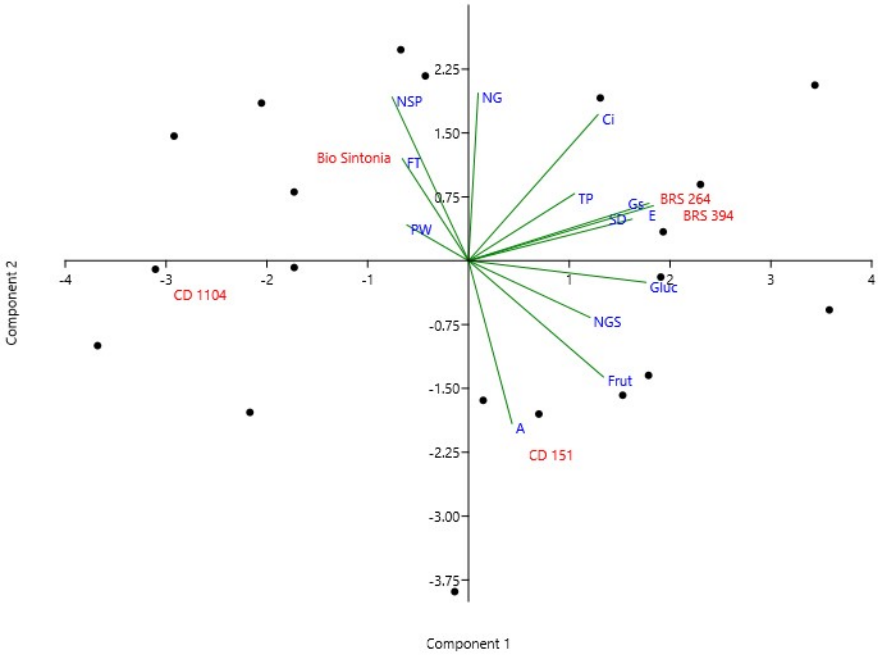

Figure 1: Biplot graph for cultivars in relation to physiologic, biometric and production parameters in 2016 crop session.

It is believed that this plant response was due to major divergent environmental conditions between the two years of analysis: specifically, the low precipitation of 2016. This result is similar to the model proposed by (Zhou *et al.*, 2013), which asserts that water levels are strong limiters of stomatal conductance, but this is not the only restricting effect of water loss, and the plant finds different strategies to combat water deficit in order to maintain its stable metabolism.

In 2017 there was an inversion in the phenomena observed, in relation to physiology, biochemistry and productive parameters, where the non-structural carbohydrates (glucose and fructose) and photosynthetic rate (*A*) remained grouped, but located in the left on the PC1, and the productive parameters (NSP, FT and NG) are grouped on the right of PC1 (Figure 2). This observation represents a positive grouping of these components with water status in the environment, because in 2017 the rainfall was double that of 2016 (Table 1). For PC2 there was a positive grouping of cultivars BRS 264 and BRS 394 for the productive parameter NG.

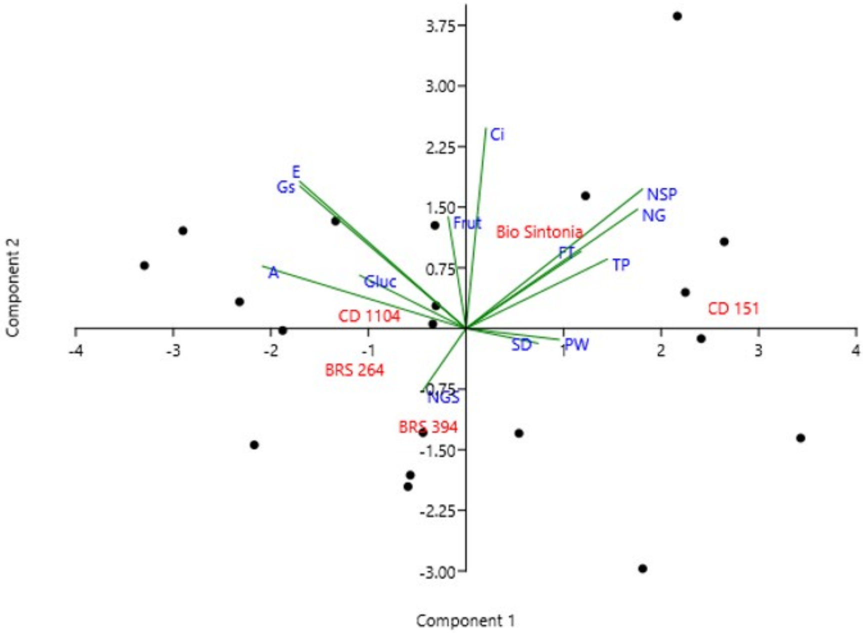

Figure 2: Biplot graph for cultivars in relation to physiologic, biometric and production parameters in 2017 crop session.

There were distinct differences in the growth of the crop in 2017 compared to 2016, possibly due to the amount of water available to the crop. In

2017 the cultivars BRS 264 and BRS 394 are in the negative quadrant of the biplot graph, and the Bio Sintonia and CD 151 genotypes are grouped in the same quadrant, and highly correlated to the productive parameters, because CD 1104 was highly correlated with the non-structural carbohydrates (Figure 2).

It was verified that, in the years 2016 and 2017, the concentration of both carbohydrates evaluated (glucose and fructose) were always found in the same quadrant as photosynthetic rate, and there was a positive grouping of these carbohydrates and photosynthetic rate (*A*) with the cultivars CD 151 and CD 1104, respectively, in 2016 and 2017 (Figures 1 and 2). The cultivar BioTrigo stood out by being influenced mainly by the NSP and FT parameters in both years, with very similar responses, inferring that these productive characteristics are very important for this cultivar (Figures 1 and 2).

Different genotypes of wheat present distinct photosynthetic and source-sink responses, so that some cultivars are better in reallocating their carbohydrates in more expressive sinks (Zhang *et al*., 2014), Moreover, the leaves are not the only carbohydrate source of wheat plants: the ears and stalks can contribute to the synthesis of photoassimilates (Sanchez-Bragado *et al*., 2014; Zhang *et al*., 2014), thus justifying the variation observed in the number of grains per plant and per spikelet in 2016, despite low photosynthesis. Also, these results show that glucose and fructose have a key role in the regulation in wheat: this phenomenon was confirmed by the research of (Kameli and Lösel, 1993) into the physiological adjustment in wheat plants under water stress.

In 2016 PC1showed that the biometric and productive indices: plant height (PH), fertile tiller (FT) and number of spikelets per plant (NSP) occupied a negative position in the biplot graph, whereas for 2017, the majority of the biometric and productive parameters were found on the positive side of the biplot graph, demonstrating the sensitivity of the wheat crop to changes in the Cerrado environment. This research did not find a positive link between the concentrations of glucose and fructose and grain index per plant (Figures 1 and 2). According to (Tack *et al*., 2015), a favourable environment, with stable rainfall rates during the crop’s growth cycle, will not only stimulate the growth of the crop, but will also aid in the reduction of stress caused by an elevated temperature, which is otherwise likely to result in major damage to wheat production.

In this study we verify distinct responses between wheat cultivars, under two cycle life and contrasting environments, according (Rakašćan *et al,* 2019) this fact occurs mainly by impact of different agro-ecological conditions they were submitted. So the recognition of morphological and physiological characters and traits that affecting the drought tolerance (the ability of a plant to survive under on water restriction) are important for improvement of wheat productivity under inhospitable conditions, where the severity, frequency, and duration of drought stress are essential factors for choosing the place of cultivation and selection of genotypes (Mohammadi and Abdulahi, 2016).

CONCLUSIONS

The performance of the wheat cultivars was distinct in both years. Physiological indicators enabled the differences between the genotypes to be understood, within the imposed environmental conditions. There was a strong, positive correlation between photosynthetic rate and sugar content. The cultivars of EMBRAPA adjusted their concentrations of glucose and fructose to cope with low rainfall. The increased rainfall in 2017 seemingly caused the productive parameters to overlap with the physiological and metabolic parameters, reversing the analyses compared to 2016.

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