Morphologically accessing wheat genotypes in response to drought on early stages of development

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ABSTRACT

As an alternative to decrease the internal deficit of wheat production in Brazil is the expansion of the cultivation area to the Cerrado, in which the occurrence of drought periods is common, causing damage when it occurs during the crop establishment and during the reproductive stage, thus develop genotypes tolerant to dry environments is essential. Development of drought-tolerant genotypes depends on the presence of genetic variability, thus the identify the variability within the accessions cultivated in Brazil allows the selection of genotypes with tolerance levels for use in crossing blocks. Thus, the objective of this study was to identify wheat genotypes displaying drought tolerance in the early stages of development through morphological characterization. In this sense, here we investigate 104 wheat genotypes cultivated in Brazil in response to drought at early stage of development. The seeds were deposited on germination paper with water and with PEG6000 at -0.5 MPa solution, simulating the control and drought stress conditions, respectively, and were kept in a germination chamber for eight days. The number of roots, root and shoot length, and root and shoot dry mass were measured, and a relative performance was calculated. The results were subjected to analysis of variance, Scoot Knot test and a Pearson correlation. The genetic distance was calculated based on the Euclidean distance and for the grouping the UPGMA method was applied. The principal component analysis was also performed. Based on the shoot and root phenotyping, it was possible to verify that the evaluated wheat panel displayed genetic variability associated with drought and present potential genotypes to drought tolerance on early stages of development, as FPS Nitron, which is a target genotype as a genitor to be used in crossing blocks aiming drought tolerance.

Highlighted Conclusions

1. The wheat genotypes growing in Brazil present genetic diversity in response to drought stress at early developmental stages.
2. FPS Nitron was less affected in drought stress condition which suggest its inclusion in crossing blocks.

INTRODUCTION

Wheat (Triticum aestivum L.) is a basic food since, at least, 8,000 years and remains one of the most important food crops in terms of harvested area, commercial value, source of energy and nutrition (Dudziak et al. 2019, Yadav et al. 2019). Brazil is one of the largest wheat importers in the world, importing ca. 6 million tons per year, which represents a significant impact on the trade balance. This impact could be reduced by expanding the area of cultivation and yield to different regions in the Country (Pereira et al. 2019).

In Brazil, due to the different climatic and geographical properties, four regions of wheat cultivation were outlined (Scheeren et al. 2008). Rio Grande do Sul, Santa Catarina and Paraná, represents more than 90% of wheat production, and belong mainly to region 1 (wet and cold) and region 2 (wet and moderately cold). The other two cultivation regions occur in the Cerrado biome, with hot and moderately dry (region 3) or hot and dry (region 4) climatic conditions, and in these regions, wheat can be grown under irrigated or rainfed conditions. Considering that irrigation leads to an increase in production costs and depends on the availability and use of water, the cultivation of rainfed wheat is the most promising alternative to increase grain production in the Cerrado (Pereira et al. 2019).
al. 2019). However, in this region there is an irregular distribution of rain and heat, with periods of drought, which can negatively impact the crop (Scheeren et al. 2008).

Drought is one of the most important environmental stresses and can reduce wheat yield by 50-90% (Wang et al. 2019). It is known that the yield of any crop depends on the performance of the plants during the cycle, with germination and seedling establishment being the critical stages (Tian et al. 2014). It has been shown, in most crops, that drought delays germination and can induce significant changes in seedling physiology and biochemistry (Bateman et al. 2016). Similarly, wheat seed germination is also negatively affected by drought (Yang et al. 2016). In the reproductive stage, drought also impacts yield due to reductions in number of grains (Senapati et al. 2019).

The identification and/or development of genotypes tolerant to dry environments is essential to enable the expansion of new cultivation areas in Brazil. The selection of a potential germplasm is the first step of a breeding program. Then, it must be decided whether the germplasm will be evaluated at a specific stage or in multiple growth stages, based on the climate and the objective of the study. After identifying/selecting a group of tolerant genotypes, the breeding program initiate directed crosses using the selected tolerant genotypes as donor parents (Sallam et al. 2019). Researchers have been applying different methods for screening genotypes (Mwadzingeni et al. 2016). However, drought conditions are not easily controlled in the field and the evaluation of the response of plants in the initial stage is commonly performed using polyethylene glycol (PEG), a compound able to alter the water potential of the medium (Mickky and Aldesuquy 2017).

Thus, this study aimed to characterize the response of different wheat genotypes under drought conditions induced with PEG during the germination and seedling establishment phase aiming at the identification of tolerant accessions to this condition for inclusion in crossing blocks.

**MATERIAL AND METHODS**

**Plant material and drought stress conditions.** A panel of 104 wheat genotypes (Table 1) was analyzed in this study. In order to characterize the response of these genotypes under drought conditions during germination and in the initial stage of development, an experiment using polyethylene glycol (PEG) 6000 was conducted. The seeds of each genotype were disinfected with sodium hypochlorite solution (2.0%) and then washed with sterile water. The seeds were deposited on germination paper (Germitest®) soaked with water and with PEG6000 at - 0.5 MPa solution (2.5x the weight of the paper), simulating the control and drought stress conditions, respectively. The rolls were kept in a germination chamber at 20 °C with a photoperiod of 12 hours of light for eight days. The experiment was carried out in a randomized block design, with three replicates of 50 seedlings.

**Morphological traits.** To characterize the wheat genotypes related to drought responses, the number of roots (RN), shoot length (SL), root length (RL), shoot dry mass (SDW) and root dry mass (RDW) were measured. SL and RL were evaluated using a pachymeter (Starrett, ± 0,05mm accuracy). After evaluating RN, SL and RL, the shoot and root were separated and both were packed in paper bags and dried in a forced air oven at 80 °C for 96 h, and subsequently, the samples were weighed in analytical balance (0.001 g accuracy).

Taking into account the intrinsic traits of each wheat genotype, a relative performance (RP) was calculated following the equation: RP variable = (variable at drought / variable at control) * 100.

**Statistical analysis.** The RP results were subjected to analysis of variance (data not shown) and the Scoot Knott test (P <0.05) for grouping means. A Pearson correlation analysis (P <0.001, P <0.05) between the variables was also conducted. Afterwards, the genetic distance matrix was calculated based on the Euclidean distance, and the results were converted into a heat map to facilitate the visualization of the distance between the accessions. For the grouping, the distance matrix was used and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method was applied. To delimit the number of groups, the cutoff point was calculated following Mojena (1977), in which the cutoff point = mean + k (1.25) * SD. A principal component analysis (PCA) was also performed and the number of components was determined by the proportion of the variation explained and to determine the number of groups, the K-means algorithm and the Elbow criterion were used. All analyzes were conducted using the Orange software. 3.18 (Demsar et al. 2013) and Genes (Cruz 2013).

**RESULTS AND DISCUSSION**

**Root traits and simulated drought.** In Brazil, new areas have been explored for wheat cultivation, as observed in Cerrado, which has frequent episodes of drought, requiring sustainable strategies to enable cultivation in this region (Pereira et al. 2019). Here, we characterize a panel of wheat genotypes used in Brazil in terms of tolerance to drought at germination and seedling stages, as well as the presence of genetic variability for this trait. For that, root and shoot characters were evaluated since are key traits associated with wheat drought tolerance (Mwadzingeni et al. 2016).
Table 1. Panel of wheat accessions evaluated for morphological traits under drought stress on early stages of development.

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Root characters affect the amount of water and nutrient absorption, and are important for maintaining crop yields under water stress conditions. Despite the importance of root traits in drought tolerance, few breeding programs take these traits into account when developing drought-tolerant wheat varieties. Information regarding the wheat genetic variability of the root traits is limited, and the exploitation of this variability can assist wheat breeding programs in the development of varieties with desired root traits for drought tolerance (Narayanan et al. 2014).

Considering the root number (RN), the wheat genotypes subjected to drought stress were grouped into three classes. The group of genotypes that had branches less affected by drought had a RP ranging from 83-109%; the group of genotypes with branches moderately affected by drought showed a RP of 72-82% and in the group most impacted by drought, the RP varied from 59-70% (Figure 1). It is well known that changes in the architecture of the root system, especially the proliferation of higher order roots, help in the short term to adapt to the water deficit (Mahmoudi et al. 2019). The capture of water occurs at the tip of the roots, thus, the production of new tips through the proliferation of roots may be more important for the capture of water and nutrients than the length and surface area of the root (Comas et al. 2013).
Figure 1. Grouping of averages of the relative performance of different variables in 104 wheat genotypes subjected to osmotic stress (PEG6000 -0.5MPa) using the Scoot Knott test (P <0.05). The values were converted into a heat map to facilitate visualization. Root number (RN), shoot length (SL), root length (RL), shoot dry weight (SDW) and root dry weight (RDW).
Relating root length (RL), the averages were also grouped into three classes. Some genotypes have better RP in drought than in control condition (112-150%), others were less affected or were not affected in this condition (80-100%). However, one group of genotypes was more affected showing a RP of 40-77% (Figure 1). Previous reports analyzing a 105 wheat genotypes panel showed that all the investigated genotypes were affected by drought stress reducing their RL (Ahmed et al. 2019). In this sense, Brazilian genotypes (Marlim, Mirante, Valente, FPS Nitron, CD 124, ORS Madre Pérola e Rubi) which do not were affected by drought stress and were able to maintain root growth are promising to be used in breeding programs aiming drought tolerance.

In the wheat panel studied, some genotypes showed promising root character behavior under simulated drought, such as FPS Nitron, Valente, Marlim, ORS Madre Pérola, Mirante, CD104 and CD107. These genotypes present potential to be used in regions with short drought episodes during the seedling stage, or can be exploited for use in breeding programs for the root system aiming at drought tolerance.

Shoot traits and simulated drought. Plant growth is drastically affected by drought, with a direct impact on shoot and root, the main components of adaptation to this condition. Plants generally limit the number and area of leaves in response to drought stress, to reduce water in the cost of lost income. On the other hand, root growth, density, proliferation and size are the main responses of plants to drought (Farooq et al. 2009). In fact, when shoot characters are considered, a negative impact of drought on RP in all evaluated genotypes was observed (Figure 1), different from the observed in the roots.

For shoot length (SL), the mean RP of the genotypes were grouped into five classes. The genotypes less affected by drought presented RP varying from 37-44%, followed by groups with RP from 31.6-36.6%; 24.4-31.4% and 16.1-24%. The genotypes that had SL most drastically affected by drought showed RP ranging from 6.1-14.8%. Ahmed et al. (2019) also identified, analyzing 105 wheat genotypes in response to drought, reduction in SL. Our results can be explained based on the findings of Schuppler et al. (1998), which found that in wheat seedlings under drought the leaf elongation rate was reduced by half and the mitotic activity of the mesophyll cells was reduced to 42%. The authors also verified a reduction in the length of the cell division zone of the mesophyll and that the period of division continued longer in the stressed leaves than in the control leaves, and the final number of cells in the stressed leaves reached 85% compared to control. Drought reduces cell expansion rates in roots and leaves, and probably the reduction occurs in the cell growth phase of the cell cycle. A longer period of mitotic activity in stressed leaves impact the final number of cells produced which was not different from that found in non-stressed leaves. However, the time required to reach the final cell number indicates that the cell growth of the stressed plant was lower compared to the control (Schuppler et al. 1998).

Shoot dry weight (SDW) was less affected by the stress then SL, however, a reduction in RP in all evaluated genotypes was also observed (Figure 1). The less impacted genotypes had RP ranging from 62-83%. Other genotypes showed RP of 46-58% and 107-128%. The genotypes that had the most impacted SDW, showed RP values ranging from 7.1 to30.1%. It can be an effect caused by reduction in SL observed in stressed plants. Previous studies also reported in wheat that, depending on the growth stage, genotype and nutritional status, more than 50% of the daily accumulated photoassimilates were transported to the root, and about 60% of that fraction was applied to respiratory system. Wheat genotypes sensitive to water deficit commonly spend higher amounts of glucose for water absorption during drought stress (Farooq et al. 2009). In addition, drought also affects chlorophyll pigments in wheat which impair the photosynthesis system, a key process which regulates in water culture medium under low concentration (Ahmed et al. 2019).

Among the genotypes that had the shoot less impacted by drought, Tec Frontale, CD119, CD1705, BRS Parrudo, BRS220, Fundacep Campo Real, Topázio, TBO Alpaca, Inova, FPS Amplitude, and FPS Nitron can be
mentioned. For the development of tolerant plants, the genotypes which displayed a better shoot performance profile can be used in crosses, together with the genotypes that showed a better root performance.

The impact of drought on root and shoot traits observed in this study can be associated with the increase in abscisic acid (ABA) synthesis during this condition. ABA alters the relative growth rates of various parts of the plant, such as increasing the ratio between the root dry weight of the root and shoot dry weight, inhibiting the development of the leaf area and producing prolific and deep roots. This phytohormone leads to a complex series of events that result in stomatal closure, a key response during water deficit (Farooq et al. 2009). ** Shoot growth maintenance directly affect root growth under drought stress.** Pearson's correlation analysis between the characters (RN, SL, RL, SDW and RDW) in wheat seedlings subjected to drought revealed significant correlations (P <0.001, P <0.05). The correlation coefficient varied from 0.210 (between SL and RN) to 0.785 (between SL and SDW). A significant weak positive correlation was observed between SL and RN (r = 0.210), SL and RDW (r = 0.250), RDW and SDW (r = 0.278) (Figure 2).

![Pearson Correlation of the characters root number (RN), shoot length (SL), root length (RL), shoot dry weight (SDW) and root dry weight (RDW) in 104 wheat genotypes subjected to osmotic stress (PEG6000 -0.5MPa).](image)

Positive correlation between shoot characters and root characters have been also observed in previous studies. That correlation indicates that the greater capacity for resource search and obtaining due the increase in root mass may contribute to the increase in shoot dry weight (Narayanan et al. 2014). Strong positive correlation was observed between SL and SDW (r = 0.785) and RL and RDW (r = 0.700) (Figure 2). This result suggests that under drought conditions, wheat seedlings that maintain shoot growth, also maintain photoassimilates accumulation; and the maintenance and/or induction of root growth is accompanied by the translocation of photoassimilates from the shoot to the root.

**Brazilian wheat genotypes present genetic variability for drought-related traits.** Genetic variability in plant species is an essential element for agricultural production. Accessing the genetic variability is essential to identify genes that control biological functions and that can be rationally used to develop new varieties (Onda and Mochida 2016). In wheat, variability can be obtained from modern cultivars, landraces, elite germplasm and wild species such as *Aegilops tauschii* and *Triticum dicoccoides*. However, cultivated varieties, landraces and elite germplasm are interesting candidates for improvement due the compatibility of crosses, adaptation and presence of desired traits (Mwadzingeni et al. 2017).

Genetic variability can be measured through morphological, physiological, chemical and molecular characterization. In this study, the genetic variability in response to drought at early stages of development of panel of wheat genotypes was evaluated through the morphological characterization of shoot and root traits. To verify the genetic variability a hierarchical cluster analysis based on genetic distance and principal component analysis were performed.

Considering the genetic distance, represented by the heat map, it was possible to observe genotypes very close to each other, while others are more distant (Figure 3). The hierarchical grouping method showed nine groups, three groups have only one genotype in each, two groups were composed of two genotypes each and one group presented three genotypes. The other groups housed a greater number of genotypes, one group with 11 genotypes, a group with 19 genotypes, and the largest group with 64 genotypes (Figure 3). This clustering profile showed that although a large number of genotypes belong to the same group, there is still genetic variability in response to drought, and this germplasm can be exploited for improvement for drought tolerance in the early stages of development.

The presence of genetic variability for response to drought can be explained by the genetic basis of wheat accessions used in Brazil. In a study developed by Scherlosky et al. (2018) analyzing 211 wheat varieties, being
185 developed in Brazil, were genotyped with 10,049 SNPs. The authors found that the wheat genotypes used in Brazil present genetic variability, which has been maintained for the past four decades. According to the authors, this variability is consequence of the strategy used in the country, which commonly opt for introduce germplasm and use commercial varieties from other companies in breeding programs. This strategy avoids narrowing the variability in Brazilian wheat.

Figure 3: Heat map demonstrating the Euclidean genetic distance and hierarchical grouping using the Unweighted Pair Group Method with Arithmetic Mean of 104 wheat genotypes subjected to osmotic stress (PEG6000 - 0.5MPa), considering the characters, root number (RN), shoot length (SL), root length (RL), shoot dry weight (SDW) and root dry weight (RDW). Cutoff point = mean + k (1.25)*SD.
Analyzing the origin of the genotypes (Table 1), it demonstrates that, the grouping is not dependent on breeding programs. The genotypes FPS Nitron, Valente and Rubi constitute individual groups, not grouping with the other genotypes developed by the obtaining companies. The CD120 and Supera genotypes were present in a same group, showing similarity in response to drought, however they belong to different breeding programs. Likewise, the largest group harbored genotypes from different breeding programs, which can be explained by the “exchange” of germplasm between breeding programs, as previously discussed (Scherlosky et al. 2018).

Our study demonstrates that there is still genetic variability in wheat elite germplasm which can be used in breeding programs aiming improvement for drought tolerance, and can be used as primary source of genetic variability. These results can help breeders to developing new genotypes to be cultivated at Brazilian Cerrado.

To complement the results obtained by the hierarchical method, a principal component analysis (PCA) was performed. Three components were needed to explain 90% of the variation. Based on the non-hierarchical grouping method (k-means) used, it was not possible to form groups (Figure 4). However, it is possible to observe a dispersion of genotypes, suggesting the presence of variability for drought tolerance in the early stages of development.

Different methods can be applied to grouping genotypes; however, the methods commonly used present different principle of analysis and can generate different forms of interpretation. While the UPGMA is a hierarchical approach to grouping data that uses the mean similarity across all cluster data points, the k-means try to optimize groups by minimizing relative distances based on a chosen index (Sokal and Michener 1958, Hartigan and Wong 1979). In this study, the UPGMA was the most appropriate method for separating genotypes into groups. In fact, UPGMA is one of the most popular methods for grouping biological data.

Figure 4. Principal component analysis from root number (RT), shoot length (SL), root length (RL), shoot dry weight (SDW) and root dry weight (RDW) characters in 104 wheat genotypes subjected to osmotic stress (PEG6000 -0.5MPa). The number of components necessary to explain a reasonable amount of variation is shown next to the figure. The green line indicates the accumulated variation, while the red line indicates the proportion of variation explained by each component. The K-means graph was also presented, which did not show any drop, not allowing the formation of groups.

The evaluated wheat panel present potential genotypes to drought tolerance on early stages of development. FPS Nitron is a potential genotype as a genitor to be used in crossing blocks aiming drought tolerance considering
that was less affected under this condition. Also, there is genetic variability associated with drought responses in the wheat genotypes used in Brazil.

The seedlings morphological profile in different wheat genotypes drought-treated can help the plant breeders to select genitors aiming at the drought tolerance improvement. In addition, this characterization allows the selection of genotypes to be used in localities experiencing drought episodes during field establishment.

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