

Identification of drought-tolerant spring wheat genotypes

Abstract – The objective of this work was to use drought-tolerance indices to classify spring wheat genotypes regarding their tolerance to drought environments, as well as to evaluate their genetic diversity for the selection of potential parents with this trait. Thirty-six wheat genotypes were evaluated in 2020, under two treatments in the field: one with irrigation (control) and the other with water stress. Grain yield was determined, and the obtained results were used to build five different drought-tolerance indices. The data were subjected to the mixed model analysis to estimate genetic parameters and predict genotypic values. The genotypic values of the studied variables were subjected to the genetic diversity analysis using the unweighted pair group method with arithmetic mean and to the principal component analysis. The random genotypic effect was significant for all studied traits. Genotypes VI 131313, VI 14001, VI 14055, VI 14118, VI 14197, VI 14214, VI 14239, VI 9004, and 'BRS 264' can be grown under drought-stress conditions. The used indices are useful to classify the genotypes in terms of drought-tolerance. Therefore, the proposed methodology allows of investigating genetic diversity and selecting genotypes based on their tolerance to drought.

Index terms: climate change, drought stress, multivariate analysis.

Identificação de genótipos de trigo de primavera tolerantes à seca

Resumo – O objetivo deste trabalho foi utilizar índices de tolerância à seca para classificar genótipos de trigo de primavera quanto à tolerância a ambientes secos, bem como avaliar a sua diversidade genética para a seleção de potenciais genitores com esta característica. Trinta e seis genótipos de trigo foram avaliados em 2020, em dois tratamentos em campo: um com irrigação (controle) e outro com estresse hídrico por seca. Foi determinada a produtividade de grãos, e os resultados obtidos foram utilizados para construir cinco diferentes índices de tolerância à seca. Os dados foram submetidos à análise de modelos mistos para estimação dos parâmetros genéticos e predição dos valores genotípicos. Os valores genotípicos das variáveis estudadas foram submetidos à análise de diversidade genética via o método de agrupamento de média aritmética não ponderada e à análise de componentes principais. O efeito aleatório de genótipos apresentou significância para todas as características. Os genótipos VI 131313, VI 14001, VI 14055, VI 14118, VI 14197, VI 14214, VI 14239, VI 9004 e 'BRS 264' podem ser cultivados sob condições de estresse hídrico por seca. Os índices utilizados são úteis para a classificação de genótipos quanto à tolerância à seca. Portanto, a metodologia proposta permite investigar a diversidade genética e selecionar genótipos com base na sua tolerância à seca.

Termos para indexação: mudanças climáticas, estresse por seca, análise multivariada.

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Introduction

Wheat (*Triticum aestivum* L.) is one of the most important crops worldwide for maintaining food security, standing out as the second most consumed cereal (Shahbandeh, 2022). However, climate change is gradually affecting agriculture and, consequently, the agronomic performance of major crop species (Liaqat et al., 2022). In the case of wheat, drought imposes great limitations, with potential to reduce grain yield in about 50–60% (Zhao et al., 2020), mainly when these events coincide with the reproductive period of the species (Nezhadahmadi et al., 2013).

In the Cerrado biome, the new agricultural frontier for wheat cultivation in Brazil, reductions of up to 50% in total rainfall and number of rainy days have been recorded during the dry season and the beginning of the wet season (Hofmann et al., 2023), which can hinder crop production. However, plants have developed several morphological, biochemical, and physiological features and molecular mechanisms to handle drought-stress (Nardino et al., 2022), which is an indicative that the challenge posed by water deficit can be overcome (Van Oosten et al., 2016).

The negative effects of drought could be minimized through the identification and use of drought-tolerant genotypes. According to Pereira et al. (2019), for the advancement of the wheat crop in the Cerrado, breeding researchers should focus on identifying phenotypic tolerance through factors related to drought.

Among the promising methodologies for the identification of drought-tolerant genotypes, drought-tolerance indices stand out, having been used previously in several crops, such as sunflower (*Helianthus annuus* L.) by Darvishzadeh et al. (2010), wheat by Ghasemi & Farshadfar (2015), maize (*Zea mays* L.) by Barutcular et al. (2016), and rice (*Oryza sativa* L.) by El-Hashash et al. (2018). To distinguish genotypes that present uniform superiority under stressed and unstressed conditions, indices that take into account genotype performance under both scenarios can be used, such as the stress tolerance index (Fernández, 1992), the yield index (Gavuzzi et al., 1997), geometric mean productivity (Kristin et al., 1997), mean productivity (Rosielle & Hamblin, 1981), and harmonic mean (Jafari et al., 2009). Darvishzadeh et al. (2010) highlighted that these indices should be used jointly, by means of the principal component analysis (PCA), in order to avoid biases due to their different statistical parameters.

The identification of drought-tolerant genotypes is not only necessary for the per se recommendation of the genotypes, but also to compose crossing-blocks in wheat breeding programs, aiming to obtain segregating populations for the selection of new genotypes. Casagrande et al. (2020), for example, when studying genetic diversity based on the best linear unbiased prediction (BLUP), observed the importance of identifying genotypes with superior and complementary traits to define parents that will compose groups in partial diallel schemes.

The objective of this work was to use drought-tolerance indices to classify spring wheat genotypes regarding their tolerance to drought environments, as well as to evaluate their genetic diversity for the selection of potential parents with this trait.

Materials and Methods

Two experiments were conducted from June to October 2020, using a total of 36 wheat genotypes. Of these, five are commercial tropical wheat cultivars adapted to the Cerrado, which were used as controls: BRS 264, from Empresa Brasileira de Pesquisa Agropecuária (Embrapa); CD 151, from Cooperativa Central de Pesquisa Agrícola (COODETEC); ORS 1403, from OR Melhoramento de Sementes Ltda; TBIO Aton, from Biotrigo Genética; and TBIO Duque, from Biotrigo Genética Ltda. The remaining 31 genotypes are wheat lines developed by the wheat breeding program of Universidade Federal de Viçosa.

The 36 wheat genotypes were subjected to two treatments in the field: control, with sprinkler irrigation, following the technical recommendations for wheat in Brazil (Reunião da Comissão Brasileira de Pesquisa de Trigo e Triticale, 2024); and water stress, with restricted irrigation for 30 days, from the spikelet phenological stage, representing stage 65 of the scale of Zadoks (Zadoks et al., 1974), until plant physiological maturity.

The experimental design was a randomized complete block, chosen based on the experience of the research team in conducting experiments in the study location. Parameters of heritability and accuracy, as well as the coefficient of variation, were used to monitor the precision and quality of the conduction of the experiment, assuring the correctness of the chosen experimental design. The plots were located in the

Professor Diogo Alves de Mello experimental area, belonging to Universidade Federal de Viçosa, in the municipality of Viçosa, in the state of Minas Gerais, Brazil (20°45'14"S, 42°52'55"W, at 648 m altitude). The experimental units consisted of five 5.0 m long rows, with 0.20 m between rows. The target population density was 350 plants per square meter.

Cultivation was carried out under the climatic conditions shown in Figure 1. Base fertilization was performed according to the interpretation of soil chemical analysis in order to meet the nutritional needs of the crop. In the seeding line, 300 kg ha⁻¹ of the N-P-K formula 08-28-16 were applied. Broadcast fertilization was carried out using 90 kg ha⁻¹ nitrogen in two phenological stages: 50% at the beginning of tillering

and 50% at the beginning of booting, representing stages 21 and 45 of the scale of Zadoks et al. (1974). Urea (45% nitrogen) was used as a nitrogen source, totaling 200 kg ha⁻¹.

Soil samples were collected at the depths of 0.00 to 0.10 and 0.10 to 0.20 m in each environment. The homogenized samples were sent to the laboratory to undergo the physicochemical analysis. The soil water retention curve (kpa) was obtained using data from the soil physical analysis, as follows: -10 kpa = 0.391 kg kg⁻¹, -30 kpa = 0.350 kg kg⁻¹, -50 kpa = 0.327 kg kg⁻¹, -100 kpa = 0.294 kg kg⁻¹, -300 kpa = 0.274 kg kg⁻¹, and -1,500 kpa = 0.234 kg kg⁻¹. To monitor soil moisture, soil samples were collected every two days in ten points in each area using a Dutch auger at the

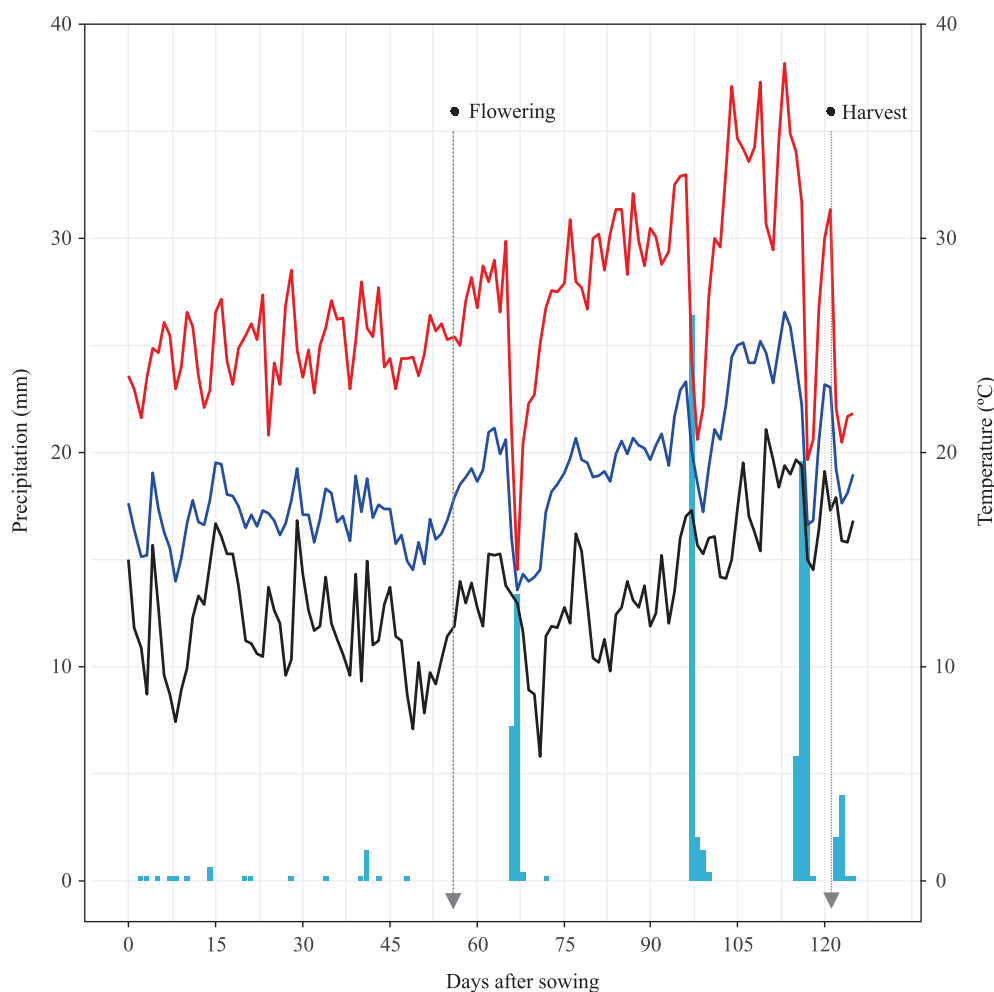


Figure 1. Meteorological data during the growing season of wheat (*Triticum aestivum*) collected from the station located at Universidade Federal de Viçosa, in the municipality of Viçosa, in the state of Minas Gerais, Brazil. Black line, minimum temperature; blue line, mean temperature; and red line, maximum temperature.

depths of 0.00–0.10 and 0.10–0.20 m. Afterwards, the soil samples were weighed and placed in an oven with air circulation, at 60°C, for 48 hours. Subsequently, the samples were weighed again, and the amount of water in the soil was estimated.

Grain yield (kg ha⁻¹) was determined for each genotype in each of the evaluated conditions, i.e., under irrigation (control) and water stress. From these results, five different drought-tolerance indices were calculated for each of the 36 genotypes (Table 1).

The data from each environment were subjected to the mixed model analysis in order to estimate genetic parameters and predict genotypic values using the following genetic-statistical model:

$$Y = X\beta + Z\mu + \varepsilon$$

where Y is the data vector; β is vector of repetition effects assumed to be fixed plus the general average; μ is the vector of the genotypic effects assumed to be random ($\mu \sim N(0, \sigma^2_\mu)$), where σ^2_μ is genotypic variance; ε is the vector of residual random effects ($\varepsilon \sim N(0, \sigma^2_\varepsilon)$), where σ^2_ε is the residual variance matrix; and X and Z are the incidence matrices for the fixed and random effects, respectively.

The significance of the genotype effect was verified through the likelihood ratio test (LRT) according to Wilks (1938), as follows:

$$LRT = -2 (\text{Log}L_F - \text{Log}L_R)$$

Table 1. Equations and references of the drought-tolerance indices used for the wheat (*Triticum aestivum*) crop.

Drought-tolerance index	Equation ⁽¹⁾	Reference
Stress tolerance index	$GYS \times GYC / (GYC)^2$	Fernández (1992)
Yield index	GYS / GYS	Gavuzzi et al. (1997)
Geometric mean productivity	$\sqrt{GYS \times GYC}$	Kristin et al. (1997)
Mean productivity	$(GYS \times GYS) / 2$	Rosielle & Hamblin (1981)
Harmonic mean	$\frac{2(GYC - GYS)}{GYC + GYS}$	Jafari et al. (2009)

⁽¹⁾GYS and GYC, grain yield of a given genotype under water stress and control (irrigation) conditions, respectively; and GYS, average yield of all genotypes under stress.

where $\text{Log}L_F$ is the logarithm of the restricted likelihood function of the full model, and $\text{Log}L_R$ is the logarithm of the restricted likelihood function of the reduced model. Significance was tested using the chi-square distribution, at 5 and 1% probability.

The variance estimates and the genetic value obtained by the restricted maximum likelihood approach and by the BLUP, respectively, were performed in the R, version 4.1.2, software (R Core Team, 2023) using the metan package (Olivoto & Lúcio, 2020). The PCA and genetic diversity were carried out using the factoextra (Kassambara & Mundt, 2020) and FactoMineR (Le et al., 2008) R packages. The unweighted pair group method with arithmetic mean (UPGMA) was also performed in the R software (R Core Team, 2023). The optimal number of groups of the UPGMA was determined by the k-means clustering method, based on the within sum of squares method, enabled by the fviz_nbclust() function of the NbClust package (Charrad et al., 2022). The assumptions of normality, homoscedasticity, and independence of errors were checked through the tests of Shapiro-Wilk, Breusch-Pagan, and Durbin-Watson, respectively. The assumption of multicollinearity was verified using the condition number from Montgomery et al. (2012) and the variance inflation value from O'Brien (2007).

Experimental precision was evaluated using the residual coefficient of variation (%), through the equation:

$$RCV = 100 \frac{\sigma_\varepsilon}{\bar{\mu}}$$

where RCV is the residual coefficient of variation, σ_ε is the residual covariance, and $\bar{\mu}$ is the overall mean.

The genotypic coefficient of variation (%) was calculated using the following equation:

$$GCV = 100 \frac{\sigma_\mu}{\bar{\mu}}$$

where GCV is the genotypic coefficient of variation, and σ_μ is the genotypic covariance.

Heritability was calculated using the equation:

$$h^2_m = \frac{\sigma^2_\mu}{\sigma^2_p}$$

where h_m^2 is broad-sense heritability, and σ_p^2 is the phenotypic variance component.

Selective accuracy was calculated through the equation:

$$h = \sqrt{h_m^2}$$

where h is selective accuracy.

The inputs to the analyses were the BLUP values from the genotypes. The Euclidean distance between each pair of genotypes was calculated for all studied genotypes using the predicted BLUP values, as follows

$$d_{ij} = \sqrt{E_j(Y_{ij} - Y_{i\cdot})^2}$$

where d_{ij} is the Euclidean distance based on the standardized data, and Y_{ij} is the i -th genotypic value of the j -th trait.

A $g \times g$ distance matrix was obtained, where $g=36$. Then, the UPGMA was applied. The association between the matrix that generated the graph by the UPGMA methodology and the Euclidean distance matrix was determined by the method of the cophenetic correlation coefficient, and significance was obtained by Mantel's test, with 10,000 permutations.

The genotypes tolerant and/or susceptible to drought-stress were identified for further grouping through the PCA, based on all applied drought-tolerance indices (Table 1) using the Naghavi et al. (2013) ranking method. The best-performing genotype in each of the indices received a ranking score equal to 1, while the worse-performing one received a score of 36 ($n = 36$ genotypes). Then, the genotypes received an average ranking score (r), which was used to discriminate them according to their drought-tolerance level (Zuffo et al., 2020). For this, the quartile values that divide the 36 ranks into four equal parts were considered. According to the used criteria, four classes were obtained: class 4, tolerant, $r \leq 9$; class 3, moderately tolerant, $9 < r \leq 18$; class 2, moderately susceptible, $18 < r \leq 27$; and class 1, susceptible, $r > 27$.

Results and Discussion

The LRT indicated significance of the genotypic random effect at 5% probability for all traits (Table 2). The estimates for broad-sense heritability ranged from 0.63 for grain yield under control conditions to 0.79 for geometric mean productivity, mean productivity, and harmonic mean, whereas those for selective accuracy varied from 0.79 to 0.89. In addition,

Table 2. Variance components, genetic parameters, and likelihood ratio test (LRT) results for the grain yield of 36 tropical wheat (*Triticum aestivum*) genotypes under control (irrigation) and water stress conditions and for the five used drought-tolerance indices⁽¹⁾.

Source of variation	Grain yield		Drought-tolerance index				
	GYC	GYS	STI	YI	GMP	MP	HM
Variance component							
Genetic variance	193,721.05*	131,836.49*	0.03**	0.01*	169,243.75*	168,353.96*	170,138.80*
Residual variance	342,299.86	203,932.50	0.03	0.02	134,501.52	137,723.11	132,421.26
Phenotypic variance	536,020.91	335,768.99	0.06	0.02	303,745.27	306,077.07	302,560.06
Genetic parameter							
Average heritability	0.63	0.66	0.78	0.65	0.79	0.79	0.79
Selective accuracy	0.79	0.81	0.88	0.80	0.89	0.89	0.89
GCV (%)	10.55	9.90	20.57	9.68	10.56	10.47	10.65
RCV (%)	14.02	12.31	19.14	12.39	9.41	9.47	9.40
GVC/RCV ratio	0.75	0.80	1.07	0.78	1.12	1.10	1.13
Likelihood ratio test							
Mean (kg ha ⁻¹)	4,173.56	3,669.18	0.89	1.00	3,895.75	3,918.93	3,872.89
LRT p-value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

⁽¹⁾GYC, grain yield under control (irrigation) conditions (kg ha⁻¹); GYS, grain yield under water stress (kg ha⁻¹); STI, stress tolerance index; YI, yield index; GMP, geometric mean productivity; MP, mean productivity; HM, harmonic mean; GCV, genotypic coefficient of variation; and RCV, residual coefficient of variation. *Significant at 5% probability.

genotypic variance was higher than residual variance for most drought-tolerance indices, since the genotypic coefficient of variation/residual coefficient of variation ratio was above unity (>1.00) in most cases. Furthermore, the heritability and accuracy estimates obtained for the evaluated traits were classified as high (Resende & Alves, 2020), which can be considered satisfactory. These results are an indicative that the studied indices can be used for the selection of drought-tolerant genotypes, the calculation of genetic gains with the selection of these genotypes, and experiment planning (Resende & Alves, 2020).

Grain yield showed a greater variation due to the environments. The presence of significant differences between the genotypes under control and drought-stress conditions indicates the existence of genetic variability, which can be a useful resource for the selection of genotypes tolerant to drought. Similar results were found by Moosavi et al. (2008) and Ali & El-Sadek (2016) when studying different drought-tolerance indices in wheat. Wheeler & Von Braun (2013) highlighted that identifying drought-tolerant genotypes is important in face of global climate change, which results in fluctuations in precipitation and in increases in temperature, mainly in inter-tropical regions. A consequence of drought is the reduction in wheat grain yield, whose average was 27.50% according to Zhang et al. (2018) when evaluating 60 database studies on the species; in the present work, this reduction was approximately 12%.

The performance of the genotypes against the indices was based on their yields under control and water stress conditions (Figure 2). Cultivar BRS 264 showed higher values in all indices and a greater grain yield both under control and stress conditions. The VI 131313, VI 14001, VI 14055, VI 14118, VI 14197, VI 14214, VI 14239, and VI 9004 lines also stood out. However, as a result of the limitation of water availability (stress), grain yield decreased from 4,173.56 kg ha⁻¹ in the control to 3,669.18 kg ha⁻¹ under stress.

The drought-tolerance indices already used in previous studies with sunflower (Darvishzadeh et al., 2010) and rice (El-Hashash et al., 2018) overlapped (Figure 3). Darvishzadeh et al. (2010) highlighted the difficulties faced when using several indices based on different parameters, observing biases in pretermittting a certain genotype based on its performance in a control or stress environment. In this line, Zuffo et al.

(2020) added that estimates of different indices may lead to a contradictory identification of tolerant or susceptible genotypes. An alternative strategy in this scenario is using rankings that consider these indices together, such as the one proposed by Naghavi et al. (2013).

Through rankings, it was possible to effectively identify four classes of genotypes, ranging from tolerant to susceptible (Figures 3 and 4). A strong cohesion between tolerance indices was assumed due to the common components in their formulas, such as grain yield (Ribeiro et al., 1981), although some indices may not correlate positively with this trait (Hussain et al., 2021).

The tolerant class was composed by lines VI 131313, VI 14001, VI 14055, VI 14118, VI 14197, VI 14214, VI 14239, and VI 9004, as well as by cultivar BRS 264. In a previous study, Nardino et al. (2022) also observed that the VI 14055 and VI 14001 lines showed the highest potential for commercial use under drought-stress conditions due to their positive agronomic, biochemical, and physiological performance. This information is extremely important for dryland wheat producers in the Cerrado, a region prone to drought (Pereira et al., 2019) and conditioned to climate risks of 20 to 40% (Pasinato et al., 2018).

In the tolerant class, the genotypes were clustered into the following two groups according to the UPGMA diversity analysis based on the Euclidean distance (Figure 4): lines developed by the wheat breeding program of Universidade Federal de Viçosa; and cultivar BRS 264. The genotypes in the moderately tolerant class also formed two groups: lines VI 14050 and VI 14980 clustered with the other tolerant lines in the susceptible group; and lines VI 9007, VI 14127, VI 14668, and VI 14774 plus cultivar TBIO Aton.

The identification of divergent genotypes is especially important to define parents that will compose crossing blocks in diallel schemes, since the alleles fixed for a particular locus in one group usually differ from those fixed for the same locus in another, which can leverage complementarity between gene loci and, in turn, increase the chance of identifying superior segregants (Casagrande et al., 2020). Therefore, combining the different groups indicated by UPGMA is a promising strategy to find complementarity between alleles favorable to drought-tolerance traits.

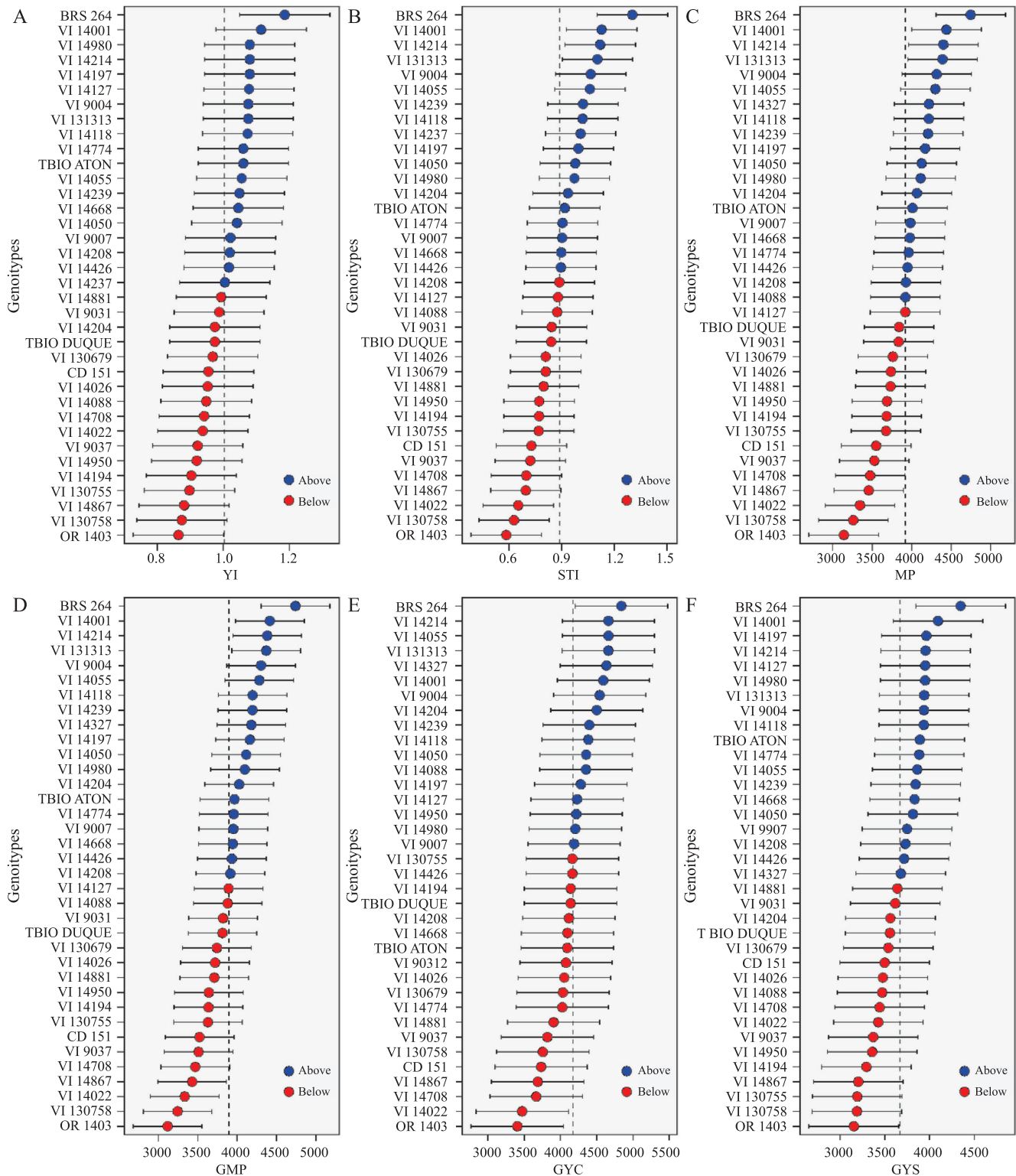


Figure 2. Best linear unbiased predictions for the evaluated wheat (*Triticum aestivum*) traits regarding: A, yield index (YI); B, stress tolerance index (STI); C, mean productivity (MP); D, geometric mean productivity (GMP); E, grain yield under control (irrigation) conditions (GYC) in kg ha⁻¹; and F, grain yield under water stress (GYS) in kg ha⁻¹. The blue and red dots represent genotypic values above or below the general average. The bars are the confidence intervals of the genotypic value.

The clustering of the genotypes by the UPGMA method allowed of their stratification into six different groups with clustering consistency, given the cophenetic correlation estimate of 0.67 between the original matrix and the distance plot (Figure 4). Groups 1 and 2 were composed by only one commercial cultivar each, i.e., BRS 264 and ORS 1403, respectively. Groups 4, 3, 5, and 6 consisted of lines from the wheat breeding program of Universidade Federal de Viçosa; however, the three latter groups also included, at least, one of the control cultivars.

The results of the PCA for the indices of stress tolerance and grain yield under control and stress conditions revealed that the first two components explained 93.44% of total variation (Figure 3). The stress tolerance index, geometric mean productivity, mean productivity, and harmonic mean were associated with the class of tolerant genotypes. However, grain yield was isolated for the control, but grouped with the

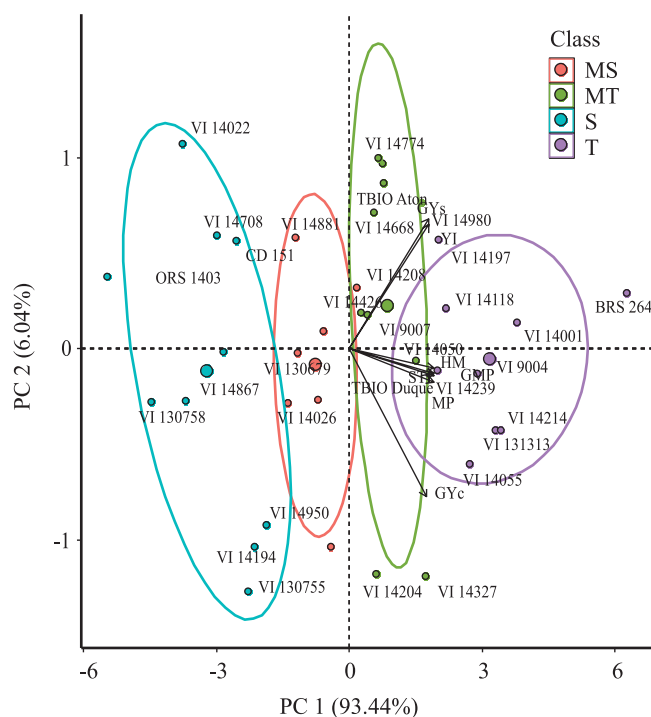


Figure 3. Principal component analysis of 36 tropical wheat (*Triticum aestivum*) genotypes based on five drought-tolerance indices and grain yield under two water regimes (control and stress). Classes: T, tolerant; MT, moderately tolerant; MS, moderately susceptible; and S, susceptible.

yield index under stress conditions, being associated with the class of moderately tolerant genotypes.

El-Hashash et al. (2018) found that the stress tolerance index, mean productivity, geometric mean productivity, and harmonic mean are suitable for the selection of genotypes with a stable and high yield under stress conditions, but also recommended the PCA technique. Moreover, it is also necessary to explore genomic selection for drought-tolerance as already done for other traits, particularly for diseases in wheat (Gupta et al., 2017). In a genome-wide study on 502 wheat genotypes, associating four different drought-tolerance indices (stress susceptibility index, stress tolerance index, tolerance index, and yield stability index) and single nucleotide polymorphisms (SNP), Ballesta et al. (2020) identified SNPs linked to quantitative trait loci (QTL), with up to 6% of phenotypic variation explained by these markers. These authors also detected QTL-rich regions on chromosome 4A,

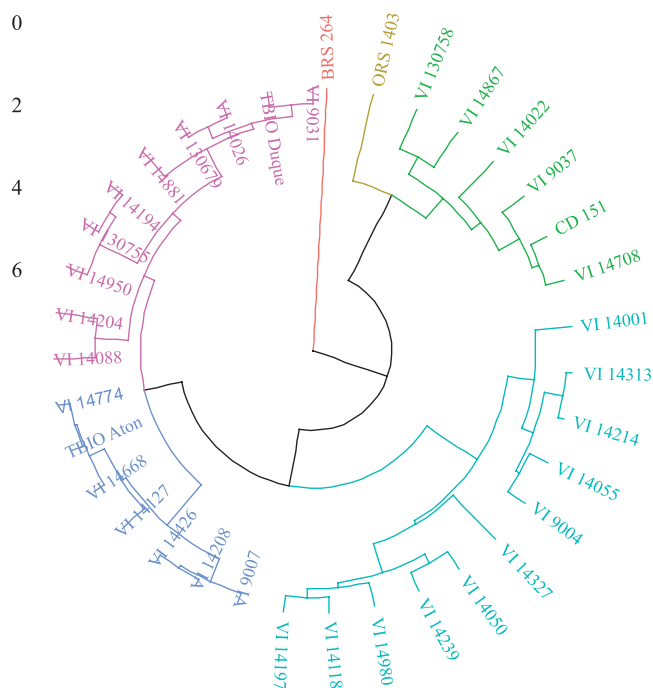


Figure 4. Dendrogram of the genetic diversity among 36 tropical wheat (*Triticum aestivum*) genotypes calculated by the unweighted pair group method with arithmetic mean based on the standardized average Euclidean distance of the genotypic values (each color represents a group). Cophenetic correlation: 0.67, significant by Mantel's test, at 1% probability, with 10,000 simulations.

supporting the hypothesis that it has a key role to play in drought tolerance, together with the drought tolerance-related B genome (Poersch-Bortolon et al., 2016). In short, the different indices should be used as a first selection of promising genotypes, with the confirmation of allele identification through the QTL analysis (Darvishzadeh et al., 2010).

In addition to cultivar BRS 264, eight lines developed by the wheat breeding program of Universidade Federal de Viçosa stood out as drought-tolerant according to the ranking proposed by Naghavi et al. (2013). These genotypes may contribute to the expansion of wheat in the drought-prone Cerrado region or to form combination panels within the breeding program. Even though drought is probably the main abiotic stress affecting wheat yield (Lobell et al., 2011), with likely worsening rainfall instability in future scenarios (Hofmann et al., 2023), the obtained results are an indicative that the challenge generated by water deficit can be overcome and that the negative effects of drought can be coped with through the development, selection, and use of drought-tolerant genotypes (Van Oosten et al., 2016).

Conclusions

1. All drought-tolerance indices allow of the classification of wheat (*Triticum aestivum*) genotypes into tolerant, moderately tolerant, moderately susceptible, and susceptible.

2. The methodology proposed allows of the investigation of genetic diversity and selection of genotypes based on their drought-tolerance response.

3. The drought-tolerant genotypes are lines VI 131313, VI 14001, VI 14055, VI 14118, VI 14197, VI 14214, VI 14239, and VI 9004, as well as cultivar BRS 264, which can be parents in wheat crossbreeding blocks in order to obtain drought-tolerant progenies.

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