



# Multivariate approach for identifying wheat *Triticum aestivum* L. tolerant to heat and drought stress

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**ABSTRACT.** Breeding wheat for production in the Brazilian Cerrado region should aim at developing productive genotypes adapted to heat and drought conditions; therefore, this study evaluated the tolerance of wheat cultivars to heat/thermal and drought stress. To meet this objective, 30 wheat cultivars were cultivated in 3 greenhouse environments, namely control, drought, and heat, applying stress from the booting stage to the end of anthesis. The following parameters were evaluated: cycle, plant height, spike number per plant, spike length, spike weight, spikelet number per spike, percentage of fertile spikelets, number of grains per spike, and grain weight per spike. Principal component analysis and cluster analysis were performed by the Ward and “K-means” methods. Principal component analysis showed that the correlations between the productivity traits and the principal components were more affected by drought than by the control and heat environments. The cluster analyses formed five clusters for the control and heat environments and seven clusters for the drought environment. Grain productivity was highlighted in the heat environment for ‘BRS 210’, ‘BR18 Terena’, ‘IPR Catuara TM’, ‘CD 154’, ‘BR 220’, ‘MGS Aliança’, ‘IAC 350’, ‘IAC Tucunaré’, ‘BR 24’, ‘IAC 5 Maringá’, ‘UFVT1 Pioneiro’, and ‘CD 151 4’ with a positive relationship in principal component analysis. Considering spikelet number per spike in the drought environment, cultivars ‘BRS 208’, ‘IAC 350’, ‘Supera’, ‘BRS 210’, ‘IPR 85’, ‘IPR Catuara TM’, ‘Anahuac 73’, ‘BR 24’, ‘IAC 5 Maringá’, and ‘UFVT1 Pioneiro’ were highlighted. In the drought environment, most of the productivity-related traits exhibited components with negative relationships among cultivars and the evaluated traits, confirming their response to the induced stress.

**Keywords:** *Triticum aestivum*; thermal stress; water stress; Cerrado; genotype × environment interactions; Python.

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## Introduction

Wheat (*Triticum aestivum* L.) is one of the most cultivated grass species in the world and is considered a worldwide staple food. In Brazil, the average wheat production is 4368.8 tons, ranging from 1524.3 to 10,554.4 tons, with more than 88.97% of the harvest coming from the central-south region of Brazil (Companhia Nacional de Abastecimento [CONAB], 2023). Due to weather conditions, the 2024 harvest is expected to fall by 2.4% compared to the last season (CONAB, 2023). However, Brazil imports 515,300 tons of wheat, 2.3% less than last month and 10.1% more than the same period last year (Companhia Nacional de Abastecimento [CONAB], 2022) because domestic production does not meet domestic demand. Therefore, to reduce external dependence on wheat imports, it is important to look for alternative production areas in Central Brazil, including in SP, MG, GO, MS, DF, and parts of BA, that constitute an excellent alternative for expanding the wheat cultivation area in Brazil for both rainfed and irrigated crops (CONAB, 2022).

Despite the plasticity of wheat for cultivation in different regions of the world, yield and economic feasibility can be strongly influenced by climate conditions. The very high temperature registered in the central region of Brazil during the wheat crop cycle is the main limitation for culture since it causes losses in all stages of plant growth, leading to reduced grain yield (Bigolin & Talamini, 2024).

Wheat is the most sensitive at the beginning of the reproductive phase during the differentiation of the apical gem of the stem at the beginning of the spike (Souza & Pimentel, 2013). The occurrence of stress in this phase reduces the number of fertile flowers per spikelet and the number of grains per spike (Farooq et al.,



2011). Dolferus et al. (2011) found that wheat was most sensitive to heat stress during the anthesis period and the five days preceding it.

According to Ribeiro Junior et al. (2006), another limiting factor is a short period of dry and hot sunny summer weather during the rainy season. Wheat productivity depends on the amount of water available in the soil, which is clearly evidenced by the fact that the average yield is three times higher for irrigated crops than for rainfed crops. The water deficiency level that reduces growth differs between and within species since the cultivars have different growth and developmental characteristics (Rodrigues et al., 1998).

Once genetic variability is verified, the main objective of breeding programs is to obtain cultivars adapted to cultivation under stress conditions. In this context, the objective was to characterize heat- and drought-tolerant wheat cultivars to providing data and information to subsidize wheat-breeding programs.

## Material and methods

The present study was conducted in partnership with the Advanced Research Center of Tropical Wheat, created by Embrapa Wheat, located in Uberaba, Minas Gerais State, Brazil. The wheat cultivars used in this study were provided by the Embrapa Wheat Germplasm Bank and have different indicated growing regions (Table 1).

**Table 1.** List of the 30 wheat cultivars evaluated in greenhouse experiments, their respective genealogies, and indications of cultivation areas.

Nº	Genotypes	Indications	Nº	Genotypes	Indications
1	Anahuac 75	MS/SP/PR	16	CD 151	4SP/4MS/GO/DF/MG/BA
2	BH 1146	4SP/4MS/GO/DF/MG/BA	17	CD 154	4SP/4MS/GO/DF/MG/BA
3	BR 18 Terena	4SP/4MS/GO/DF/MG/BA	18	Embrapa 21	4SP/4MS/GO/DF/MG/BA
4	BR 24	4SP/4MS/GO/DF/MG/BA	19	IAC 350	4SP/4MS/GO/DF/MG/BA
5	BRS 208	2SP/3MS	20	IAC 5 Maringá	SP/PR/MG
6	BRS 210	4SP/4MS/GO/DF/MG/BA	21	IAC 24 Tucuruí	4SP/4MS/GO/DF/MG/BA
7	BRS 220	2SP/3MS	22	IPR 144	23SP/3MS
8	BRS 229	3MS/PR	23	IPR 85	4SP/3MS
9	BRS 327	SP/MS	24	IPR Catuara TM	23SP/3MS
10	BRS 49	4SP/4MS/GO/DF/MG/BA	25	MGS Aliança	4SP/4MS/GO/DF/MG/BA
11	BRS Gralha Azul	3MS/3PR	26	Quartzo	PR
12	BRS Guabijú	3SP/3MS	27	Supera	4SP/4MS/GO/DF/MG/BA
13	BRS Guamirim	4SP/3MS	28	TBIO Sinuelo	23PR
14	CD 117	4SP/4MS/GO/DF/MG/BA	29	Topázio	1RS/1SC/1PR
15	CD 150	4SP/4MS/GO/DF/MG/BA	30	UFVT1 Pioneiro	4SP/4MS/GO/DF/MG/BA

The experiments were conducted in a greenhouse at 21°14'32.8" S and 48°17'56.6" W, and 595 m altitude. The cultivars were evaluated in three environments and arranged in a randomized block design with five replications. The limitations in obtaining larger seed quantities made it impossible to carry out a study with more repetitions. There were 30 genotypes, with 5 replications and 4 environments, totaling 20 plants of each genotype throughout the experiment. Seed germplasm banks provide few seeds. However, there were five plants per genotype per environment, and there was no experimental error. There were 472 degrees of freedom of the residual, representing 78.67% of the experiment's total. This substantial degrees of freedom value indicates a significant amount of information not explained by the statistical model, reinforcing the reliability of the results.

The substrate, fertilization, and light conditions were the same in all environments. Irrigation was performed by drip irrigation, respecting the soil capacity in each pot. The environments differed regarding temperature and irrigation conditions so that the genotypes were submitted to temperature and water stress, and in an optimal environment. The control environment was controlled with temperature and irrigation throughout the cycle; the temperature was maintained between 15 and 25°C, with irrigation until the physiological maturity of the seeds (8.9 Zadoks growth stage) (Zadoks et al., 1974). For the drought (water stress) environment, the temperature was maintained between 15 and 25°C until the physiological maturity of the seeds, and water stress consisted of suspending irrigation from the booting period until the end of anthesis (4.5 and 6.9 Zadoks growth stages, respectively). Water stress consisted of removing the drip irrigation hose from the pot as soon as 50% of the plants from the plot reached the booting growth stage, and it was replaced at the end of anthesis. For the heat stress environment (temperature stress), the temperature was maintained between 25 and 35°C from the booting period until the end of anthesis, and irrigation was maintained until the physiological maturity of the seeds. Heat stress was applied in two stages and separated



into two compartments of the greenhouse. First, the pots were placed in a compartment in which room temperature varied between 15 and 25°C, and when 50% of the plants reached the booting stage in each plot, the pot was transferred to a room in which the temperature varied from 25 to 35°C, returning to the lower temperature compartment at the end of the anthesis.

Four individual plants in each pot were evaluated to determine the following agronomic and productivity traits: cycle (CY) was determined as the number of days in which 50% of the plot plants presented hard or very hard grains (8/9 Zadoks growth stage); plant height (PH) was measured as the height of each plant in the plot at physiological maturation and calculated as the plot average (cm); for the spike number per plant (SNPP), the number of spikes present in each pot was counted at physiological maturation, and the average number of spikes per plant was estimated by dividing the obtained value by the total number of plants in the plot; spike length (SL) was measured after full maturation by cutting off the spikes of each plot with scissors and measuring their length with a ruler, and the summation was divided by the number of spikes in the plot (cm); spike weight (SW) was calculated as the summation of weight (g) of all spikes of the plot divided by the total number of spikes in that plot (g); spikelet number per spike (SNPS) was calculated as the summation of the number of spikelets on each spike in the plot divided by the number of spikes; to calculate the percentage of fertile spikelets (PFS), the spikes from each plot were evaluated to determine the number of full (fertile) and empty (infertile) spikelets; the number of grains per spike (NGPS) was calculated by counting the total number of grains in each pot and dividing it by the number of harvested spikes; the grain weight per spike (GWPS) was calculated by dividing the weight (g) of all grains harvested in the plot by the number of harvested spikes (g).

### Statistical analysis

Before performing multivariate analyses, the data were standardized so that the parameters had the same weight in the calculation of the similarity coefficient between the objects, resulting in a mean equal to zero and variance equal to the unit, according to recommendations from Ferraudo (2008). This step is crucial, especially in techniques such as principal component analysis (PCA), which is a set of statistical procedures used to analyze data that simultaneously observe and analyze more than one statistical variable in each research unit (Johnson & Wichern, 2007). PCA was performed for each environment, as well as cluster analysis using the Ward method. From the defined number of clusters, the clustering analysis was performed using the non-hierarchical “K-means” method. These analyses were performed using Python 3.11.3.

## Results and discussion

### Principal component analysis

The eigenvalues above the unit were considered because they generate components with significant amounts of original information (Kaiser, 1958) while highlighting the traits whose correlation values were above 0.6, regardless of the signal (Ferraudo, 2010).

In the control environment, the first 3 eigenvalues generated by the PCA of wheat cultivars were greater than 1, explaining 84.88% of the variance contained in the 9 original traits (Table 2).

**Table 2.** Correlations between the principal components (PC) and the agronomic traits evaluated in 30 wheat cultivars in control, heat (thermal stress), and drought (water stress) environments.

	Control			Heat			Drought		
Eigenvalue	5.14	4	1.1	5.25	3.32	1.35	3.99	2.87	2.45
Variance (%)	42.59	33.15	9.14	43.47	27.5	11.17	33	23.76	20.3
Accumulated V. (%)	42.59	75.74	84.88	43.47	70.97	82.13	33	56.76	77.07
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
CY	−0.4	0.84	−0.02	0.7	−0.6	−0.09	0.77	0.4	−0.26
PH	0.48	0.47	−0.17	0.67	−0.17	−0.09	0.31	0.56	0.23
SNPP	0.94	0.04	0.13	0.72	0.64	0.04	−0.24	0.87	0.01
SW	0.93	−0.05	−0.04	0.69	0.63	−0.13	−0.18	0.85	−0.28
GWPS	0.8	0.36	0.23	0.62	0.47	0.49	−0.24	0.14	0.87
SL	0.73	0.5	0.25	0.84	0.26	0.34	−0.07	0.25	0.89
SNPS	0.73	0.5	0.25	0.84	0.26	0.34	−0.07	0.25	0.89
PFS	0.55	0.12	−0.76	−0.09	0.37	−0.89	−0.52	0.55	−0.38
NGPS	0.85	0.31	−0.32	0.58	0.66	−0.32	−0.52	0.63	0.05

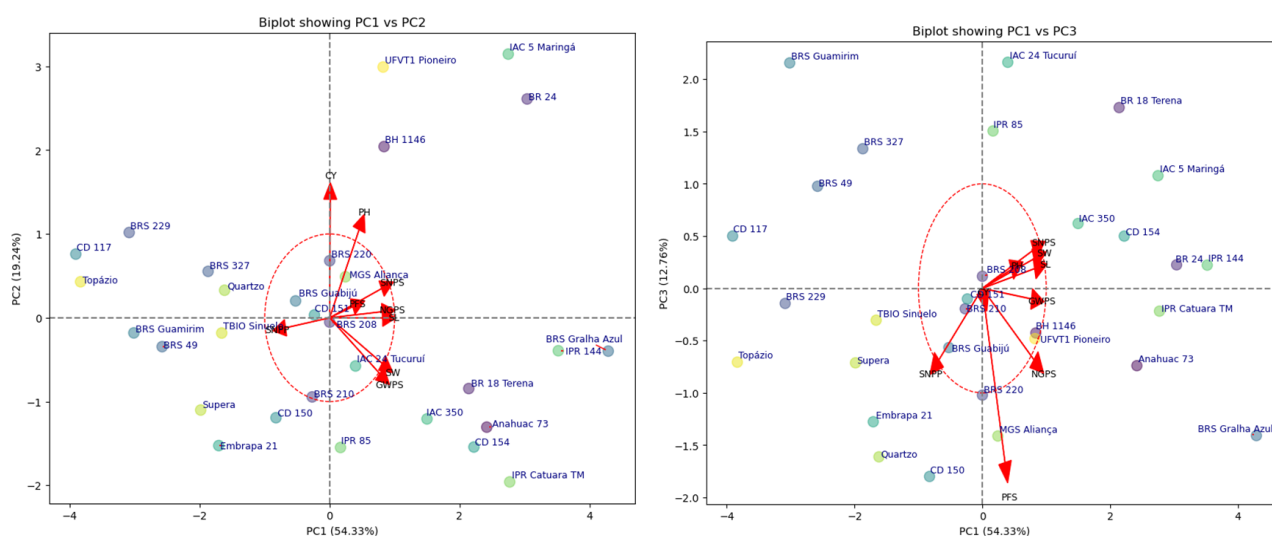
Cycle (CY), plant height (PH), spike number per plant (SNPP), spike weight (SW), grain weight per spike (GWPS), spike length (SL), spikelet number per spike (SNPS), number of grains per spike (NGPS), and percentage of fertile spikelets (PFS).



The first principal component (PC1) retained 42.59% of the original variance, which was explained by the spike number per plant, spike weight, grain weight per spike, spike length, spikelet number per spike, and number of grains per spike. The second principal component (PC2) retained 33.15% of the original variance (Table 2), which was explained by the cycle, percentage of fertile spikelets, and number of grains per spike.

Although  $PC1 \times PC2$  already captured over 60% of the variance, analysis was continued to capture additional aspects related to the behavior of the variables under environmental stress. Thus, in PC3, the PFS explained the variance retained in the third principal component, highlighting the importance of this specific variable in the data structure. The correlations between traits and principal components are shown in Table 2.

Figure 1 shows the distribution of each cultivar according to a coordinate factor plan considering its relationship with the variables. The two-dimensional plane formed by PC1 and PC2 retained 75.74% of the variance (Figure 1A). The positive and negative values of the principal component vector reflect how each cultivar responds to the traits when exposed to the environment in question. The biplot, as a visual representation, facilitates the understanding of the relationship between cultivars and the variables being studied.



**Figure 1.** Biplot showing the dispersion of wheat genotypes in the control environment as a function of the principal components  $PC1 \times PC2$  (a) and  $PC1 \times PC3$  (b) and projection of trait vectors: CY: cycle; PH: plant height; SNPP: spike number per plant; SNPS: spikelet number per spike; NGPS: number of grains per spike; SL: spike length; GWPS: grain weight per spike; SW: spike weight, and PFS: percentage of fertile spikelets.

The same reasoning allows us to conclude that the cultivars located outside the highlighted range are specific, differing significantly from the other for a certain trait: positive when located in the direction of the trait vector or negative when opposing the vector. However, this analysis must consider the set of projections of the trait relevant for the component, as well as the trait itself, since a higher average does not always imply that the cultivar is superior, as in the case of plant height and cycle.

Figure 1A shows that the ‘IAC 350’, ‘BR 18 Terena’, ‘Anahuac 73’, ‘CD 154’, ‘BRS Gralha Azul’, ‘IPR 144’, ‘IPR 85’, and ‘IPR Catuara TM’ cultivars stand out for traits related to productivity (SW, GWPS). The ‘BRS Gralha Azul’ and ‘IPR 144’ cultivars are highlighted for SW and GWPS, which correlates positively with the principal component; however, for SNPP, there was a negative correlation with this trait. Thus, for these environments, the projection of this trait occurred in the opposite direction to the productivity traits, so that the cultivars that surround SNPP had low SW, GWPS, NGPS, SL, and SNPS values and vice versa. In contrast, Camargo et al. (1998) found a highly significant and positive correlation between SNPP and SNPS in hybrid wheat populations from parents improved for these traits.

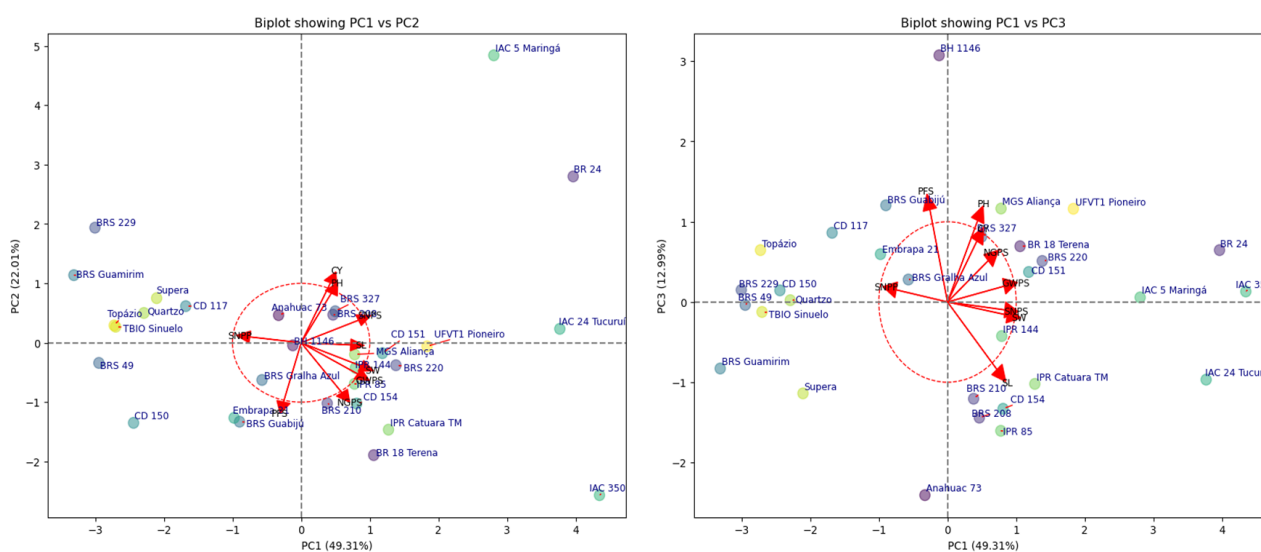
‘UFVT1 Pioneiro’, ‘BH 1146’, ‘BR 24’, and ‘IAC 5 Maringá’ stood out for SNPS but not SNPP; however, when analyzing PC2, they are also characterized by a long cycle. To select the shortest cycle cultivars in the control environment, it is necessary to pay attention to ‘IPR 85’, located opposite the CY vector (Figure 1A). In the  $PC1 \times PC3$  (Figure 1B) biplot, 12.76% of the variance was retained. The only agronomic trait retained in PC3 was PFS, allowing selection of cultivar MGS ‘Aliança’.

For heat stress, the first three eigenvalues explained 82.13% of the variance contained in the original variables in PCA (Table 2). PC1 retained 43.47% of the variance and was explained by the traits SNPP (0.72),



SW (0.69), GWPS (0.62), SL (0.84), SNPS (0.84), and NGPS (0.58). The cycle (−0.60) and plant height (−0.17) traits explained PC2, which retained 27.50% of the variance, while PFS (−0.89) explained PC3, which retained 11.17% (Table 2).

The PC1 × PC2 (Figure 2A) biplot allowed selection of the cultivars ‘MGS Aliança’, ‘BRS 220’, ‘CD 154’, ‘BR 210’, ‘IPR Catuara’, ‘BR 18 Terena’, and ‘IAC 350’, which presented positive vectors enabling selection for the SL, SW, GWPS, and NGPS traits. However, these cultivars were positive for PH and CY, which hinders selection, as cultivars with shorter heights and shorter cycles are priorities in breeding wheat. Souza et al. (2011) reported that a high temperature causes grain yield losses, wrinkled grain development, and reduced weight and commercial quality of wheat, especially due to damage during the grain-filling growth stage (Dias et al., 2008). Cunha et al. (2016) affirmed that grain yield could be hampered due to increased flower sterility and incomplete grain filling, especially from the booting growth stage onward. Cargnin et al. (2006) and Oliveira et al. (2011) reported losses of up to 50% in wheat production potential due to thermal stress.



**Figure 2.** Biplot showing the dispersion of wheat genotypes in the heat stress (thermal stress) environment as a function of the main components PC1 × PC2 (a) and PC1 × PC3 (b) and projection of trait vectors: CY: cycle; PH: plant height; SNPP: spike number per plant; SNPS: spikelet number per spike; NGPS: number of grains per spike; SL: spike length; GWPS: grain weight per spike; SW: spike weight, and PFS: percentage of fertile spikelets.

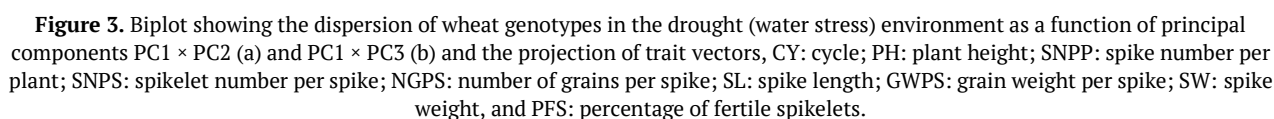
Cultivars ‘Embrapa 21’, ‘BRS Guabiju’, ‘CD 150’, and ‘BRS 49’ could be selected for height and cycle since they were in the opposite direction to these vectors. Tall plants are not desirable when the wheat crop is irrigated since areas irrigated by sprinkling require plants with a semi-dwarf genotype; in addition, tall cultivars are susceptible to lodging (Fornasieri Filho, 2008).

‘CD 117’, ‘Quartzo’, ‘TBIO Sinuelo’, ‘Topázio’, ‘Supera’, ‘BRS Guamirim’, and ‘BRS 229’ were located close to SNPP in the graph, orienting in the direction of the negative vector, and should not be selected for productivity. In contrast, ‘BR 24’, ‘IAC 5 Maringá’, and ‘IAC 24 Tucuruí’ were highlighted for their height and long cycle traits, which are both undesirable. Figure 2B highlights cultivar ‘BRS Guabiju’ for its PFS trait, which was retained in PC3.

For the drought environment, PCA retained 77.06% of the variance contained in the original traits. PC1 retained 33% of the variance and was explained by the SL, SNPS, PFS, and NGPS traits. PC2 retained 23.76%, being characterized by CY, SW, GWPS, and NGPS. Finally, 20.30% of the variance was retained in PC3, with the CY, PH, and GWPS traits. The correlation values between the traits and the components are shown in Table 2. Figure 3 shows the plots PC1 × PC2 (A) and PC1 × PC3 (B).

Figure 3A shows the plan characterized by productivity-related characteristics PFS, NGPS, and GWPS, discriminating cultivars ‘CD 117’, ‘Quartzo’, ‘BRS Gralha Azul’, ‘CD 117’, ‘Embrapa 21’, and ‘CD 154’. The relationship was negative with these variables, indicating a possible response to the stress caused by this environment in these cultivars. ‘IPR 144’, ‘IAC 24 Tucuruí’, ‘IAC 5 Maringá’, and ‘BR 24’ cultivars, located in quadrants opposite to the productivity component, were not indicated for studies investigating drought tolerance, as they had a higher CY and PH as a response to stress.





The higher variance component retained both the same variables in the control and heat environments, unlike the drought environment, in which SW and GWPS were retained only in the second component. This result may indicate that water stress influenced the grain and spike weight traits of the cultivars. However, under water stress, it is common to use secondary productivity traits to assist in the selection of genotypes capable of using water more productively or efficiently since selection for these traits results in higher yield gains compared to the weight of grains per se (Bänziger et al., 2000).

Likewise, the PFS trait was retained in the first PC in the drought environment, while a correlation with PC2 higher than 0.6 was observed in the control and only with PC3 in the heat environment, indicating that water stress during booting and anthesis affected spikelet fertility, significantly reducing PFS and increasing the variation and amplitude of the means. Bennani et al. (2016) evaluated the efficiency of selection criteria for productive and drought-tolerant wheat genotypes and reported a positive correlation between the number of fertile spikelets and grain yield, indicating that the first should be used in direct selection for high yields under drought conditions.

According to Spher (1967), the most critical phase for the occurrence of water deficit is immediately before the heading stage (panicle completely emerged from the stem), in the period between meiosis and the release of tetrads in the anthers. The second most sensitive phase is during anthesis and grain initiation, when stress causes loss of pollen fertility, spikelet death, or abortion of the newly formed seeds, due to less available water in the reproductive structures (Saini & Lalonde, 1998). Both phases were subjected to water deficit in this study.

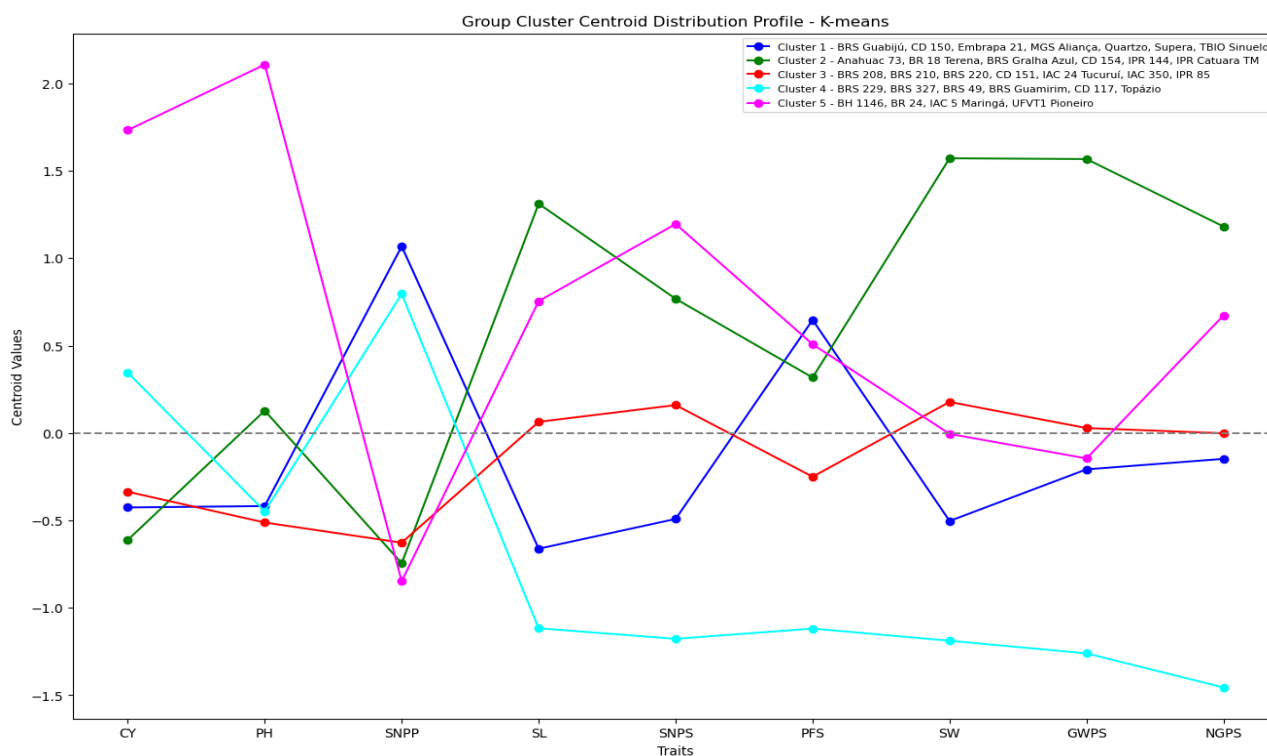
Cultivars 'BRS 210', 'BRS 220', 'CD 151', 'IPR 85', and 'MGS Aliança' did not present extremes in any of the evaluated environments. 'Topázio' is the only evaluated cultivar indicated for wheat-producing region 1 because it is adapted to lower temperatures, explaining the low productive performance of this cultivar in the conducted experiments.

When the environment changes, cultivars, such as ‘BRS 210’, ‘BRS 220’, ‘CD 151’, ‘IPR 85’, and ‘MGS Aliança’, tend to offer a stability advantage, maintaining consistent performance without extreme fluctuations, which is beneficial in variable conditions. In contrast, the ‘Topázio’ cultivar, adapted to colder climates, showed reduced performance under warmer conditions. Its yield tends to improve at lower temperatures, aligned with its genetic characteristics, while in warmer climates, its productivity is consistently lower.



### Clustering analysis

The Ward hierarchical clustering method formed five clusters for the control environment from the cutoff limit in which sharp changes were observed, as recommended by Cruz et al. (2014). From the number of clusters in the dendrogram obtained by the Ward method, the “K-means” analysis was performed to identify the best clustering solution. According to Ferraudo (2010), the ability to refine non-hierarchical methods allows more satisfactory results to be achieved than those obtained by a single method individually. The “K-means” graph for the control environment is shown in Figure 4.



**Figure 4.** Centroid distribution profile of the cluster analysis by “K-means” for the wheat cultivars in the control environment based on the following traits: CY: cycle; PH: plant height; SNPP: spike number per plant; SNPS: spikelet number per spike; NGPS: number of grains per spike; SL: spike length; GWPS: grain weight per spike; SW: spike weight; and PFS: percentage of fertile spikelets.

Cluster 1 (blue line) included ‘BRS Guabijú’, ‘CD 150’, ‘Embrapa 21’, ‘MGS Aliança’, ‘Quartzo’, ‘Supera’, and ‘TBIO Sinuelo’. This cluster excelled in SNPP and PFS, which are indicators of high productive potential and reproductive efficiency crucial for maximizing grain yield and ideal for stable, efficient production.

Cluster 2 (green line) comprised ‘Anahuac 73’, ‘BR 18 Terena’, ‘BRS Gralha Azul’, ‘CD 154’, ‘IPR 144’, and ‘IPR Catuara TM’, which were notable for ear size and productivity traits, including SL, SW, GWPS, and NGPS. These characteristics are essential for high agricultural yield and are especially beneficial in environments conducive to vigorous plant growth.

Cluster 5 (pink line) contained ‘BH 1146’, ‘BR 24’, ‘IAC 5 Maringá’, and ‘UFVT1 Pioneiro’ and stands out for its quick vegetative cycle (CY), tall PH, and high SNPS. These traits contribute to better light competition and potentially higher grain counts, making this cluster suitable for short growing seasons and challenging environmental conditions.

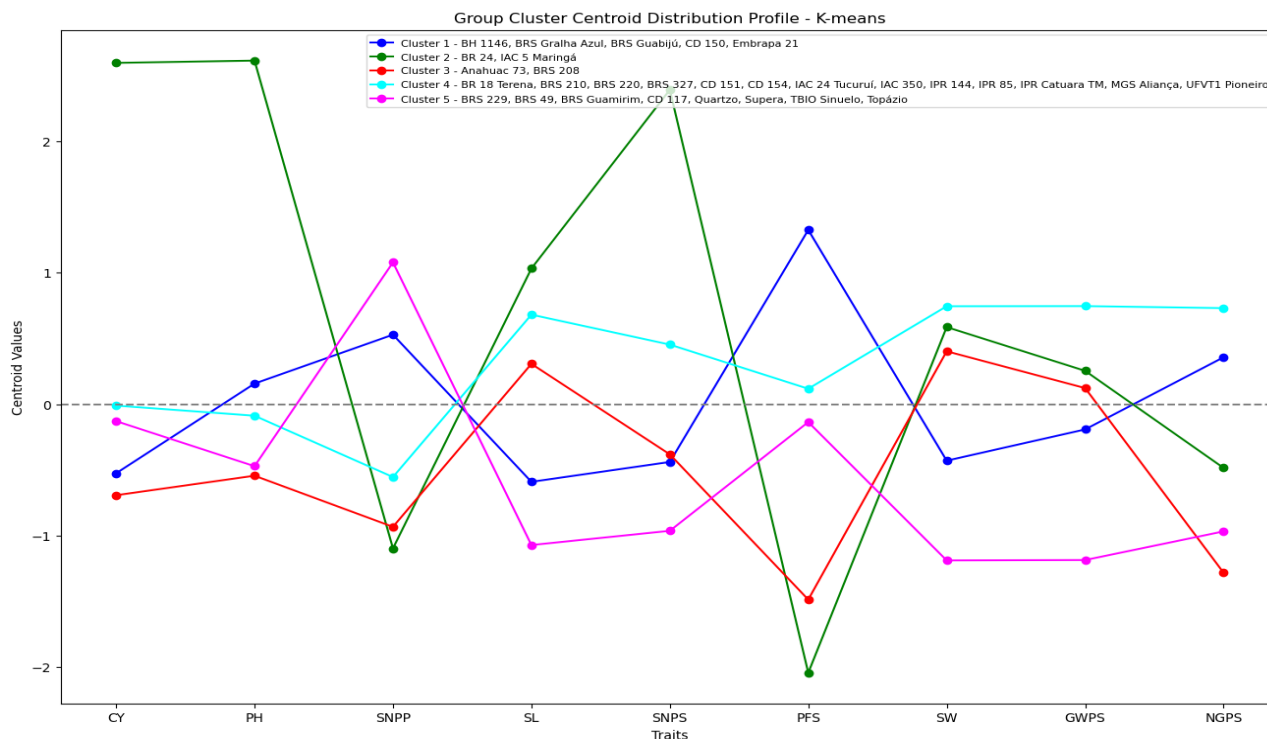
The Ward method also formed five clusters in the heat environment. Figure 5 shows that K-means Cluster 2 (green line) includes cultivars ‘Anahuac 73’, ‘BR 18 Terena’, ‘BRS Gralha Azul’, ‘CD 154’, ‘IPR 144’, and ‘IPR Catuara TM’. This cluster is distinguished by its long vegetative cycle (CY), high PH, and large, dense ears (SL and SNPS), features advantageous for regions with long growing seasons and densely planted environments that promote high grain productivity.

Cluster 5 (pink line) included ‘BH 1146’, ‘BR 24’, ‘IAC 5 Maringá’, and ‘UFVT1 Pioneiro’. It is characterized by a rapid vegetative cycle and high floral density, adapting well to short growing seasons and adverse conditions and maximizing ear production.



Cluster 1 (blue line) was composed of ‘BRS Guabijú’, ‘CD 150’, ‘Embrapa 21’, ‘MGS Aliança’, ‘Quartzo’, ‘Supera’, and ‘TBIO Sinuelo’. It stands out for its high reproductive efficiency (PFS), indicating a great capacity of the spikelets to produce grains, which is ideal for agricultural practices seeking stable and efficient production.

Cluster 4 (turquoise line) featured large and heavy ears, with a high grain production capacity (SW, GWPS, and NGPS), ideal for strategies aimed at maximizing grain yield in environments conducive to robust growth.



**Figure 5.** Centroid distribution profile of the cluster analysis by “K-means” for the wheat cultivars in the heat environment (thermal stress) based on the following traits: CY: cycle; PH: plant height; SNPP: spike number per plant; SNPS: spikelet number per spike; NGPS: number of grains per spike; SL: spike length; GWPS: grain weight per spike; SW: spike weight, and PFS: percentage of fertile spikelets.

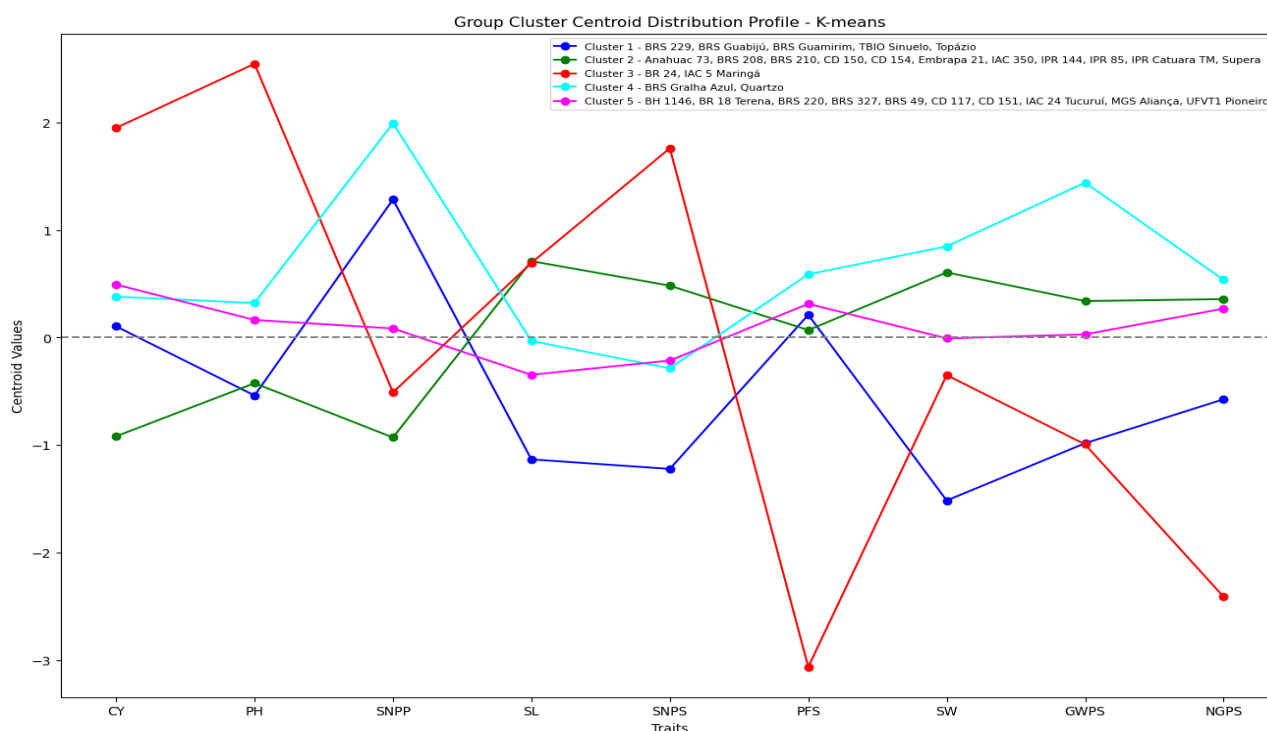
Clustering by the Ward hierarchical method separated the wheat cultivars into seven clusters in the drought environment. Figure 6 shows the non-hierarchical “K-means” method for the drought environment. Cluster 3 (green line) included ‘BR 24’ and ‘IAC 5 Maringá’, which excelled in vegetative cycle length (CY), PH, and SNPS. These cultivars are quick-growing, robust plants ideal in short-season regions or tough conditions.

Cluster 4 (turquoise line) contained ‘BRS Gralha Azul’ and ‘Quartzo’, which stood out for their ear productivity, with high values in SNPP, PFS, SW, and GWPS. These cultivars are especially effective in environments conducive to intense growth, focusing on maximizing grain yield (Figure 6).

Cluster 2 (green line) was comprised of ‘Anahuac 73’, ‘BRS 208’, ‘BRS 210’, ‘CD 150’, ‘CD 154’, ‘Embrapa 21’, ‘IAC 350’, ‘IPR 144’, ‘IPR 85’, ‘IPR Catuara TM’, and ‘Supera’. These cultivars showed a superior SL, indicating potential for higher grain capacity, and would be ideal for enhancing ear size and quality under extreme growth conditions (Figure 6).

Clustering analysis is widely used in plant breeding because it classifies individuals into clusters to maximize homogeneity within and heterogeneity between groups. Thus, this analysis is important for maintaining the genetic variability from crosses of genetically divergent clusters, representing an important strategy to obtain superior genotypes and, consequently, achieve greater gains with selection (Dallastra et al., 2014). PCA and “K-means” can be used as complementary tools for selecting superior genotypes in breeding programs. For a wheat breeding program focused on enhancing productivity across diverse environments, key variables such as NGPS, GWPS, SNPP, PFS, and CY are paramount. To achieve high productivity, it is essential that the selected plants not only demonstrate high yield potential but also possess shorter growth cycles. This adaptation is crucial in regions characterized by brief rainy seasons and limited periods of mild weather, necessitating varieties that can mature rapidly and effectively capitalize on shorter growing conditions.





**Figure 6.** Centroid distribution profile of the cluster analysis by “K-means” for the wheat cultivars in the drought environment (water stress) based on the following traits: CY: cycle; PH: plant height; SNPP: spike number per plant; SNPS: spikelet number per spike; NGPS: number of grains per spike; SL: spike length; GWPS: grain weight per spike; SW: spike weight, and PFS: percentage of fertile spikelets.

## Conclusion

The number of spikes per plant trait was negatively correlated with the other productivity traits evaluated in all environments. Based on PFS, spikelet fertility was more affected by water stress than by thermal stress. The cultivars highlighted in the heat environment (thermal stress) were ‘BRS 210’, ‘BR18 Terena’, ‘IPR Catuara TM’, ‘CD 154’, ‘BR 220’, ‘MGS Aliança’, ‘IAC 350’, ‘IAC Tucunaré’, ‘BR 24’, ‘IAC 5 Maringá’, ‘UFVT1 Pioneiro’, and ‘CD 151’ due to their grain productivity-related traits. Considering SNPS, cultivars ‘BRS 208’, ‘IAC 350’, ‘Supera’, ‘BRS 210’, ‘IPR 85’, ‘IPR Catuara TM’, ‘Anahuac 73’, ‘BR 24’, ‘IAC 5 Maringá’, and ‘UFVT1 Pioneiro’ were highlighted in the dry environment (water stress). In the drought environment, most of the productivity-related traits exhibited a negative relationship among cultivars and the evaluated traits, confirming their response to the induced stress.

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