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BIOLOGY

Unraveling trait relationships for the selection of drought-tolerant wheat genotypes

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ABSTRACT: The study of genotypic relationships between drought tolerance indices and agronomic traits of interest in wheat breeding is useful for designing selection strategies. The objective of this research was to investigate the cause-and-effect relationships between agronomic traits and drought tolerance indices through the analysis of canonical correlations. Two trials (control and stress) were conducted in winter 2020 in Viçosa, MG, Brazil. The traits evaluated were: (days for heading, plant height, mass and number of grains per spike, mass of one hundred grains, and grain yield). Grain yield data from the control and stress conditions were used to construct five drought tolerance indices. The data were subjected to mixed model analysis for estimation of genetic parameters and prediction of genotypic values (REML/BLUP), and then the genotypic values were used to calculate the correlation coefficients between the traits. Two groups of traits were established for the study of canonical correlations, the first group consisting of agronomic traits and the second by drought tolerance indices. There was a significant genotype effect for all evaluated traits. The canonical pairs were significant, which indicated the existence of dependence between the groups. Days to heading trait can be used in the indirect selection of wheat genotypes for drought tolerance.
Key words: *Triticum aestivum* L., mixed model, drought stress tolerance, tropical wheat breeding, genetics parameters.

Desvendando as relações de caracteres para a seleção de genótipos de trigo tolerantes a seca

RESUMO: O estudo das relações genotípicas entre índices de tolerância à seca e caracteres agronômicos de interesse no melhoramento de trigo é útil para traçar estratégias de seleção. Objetivou-se com este trabalho investigar as relações de causa e efeito entre características agronômicas e índices de tolerância à seca via análise de correlações canônicas. Dois ensaios (controle e estresse) foram conduzidos no inverno de 2020 em Viçosa, MG, Brasil. Foram avaliados os caracteres (dias para o espigamento, altura da planta, massa e número de grãos por espiga, massa de cem grãos e rendimento de grãos). Os dados de produtividade dos ensaios de controle e estresse foram utilizados para construir cinco índices de tolerância à seca. Os dados foram submetidos à análise de modelos mistos para estimação dos parâmetros genéticos e predição dos valores genotípicos (REML/BLUP), em seguida, os valores genotípicos foram utilizados para calcular os coeficientes de correlação entre os caracteres. Dois grupos de caracteres foram estabelecidos para o estudo das correlações canônicas, sendo o primeiro grupo constituído pelas variáveis agronômicas e o segundo pelos índices de tolerância à seca. Houve efeito significativo de genótipo para todas as características avaliadas. Os pares canônicos foram significativos, o que indicou a existência de dependência entre os grupos. O caráter dias para o espigamento pode ser utilizado na seleção indireta de genótipos de trigo para tolerância à seca.
Palavras-chave: *Triticum aestivum* L., modelos mistos, tolerância a estresse hídrico, melhoramento de trigo tropical, parâmetros genéticos.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is of great importance worldwide (ALI et al., 2022). It is widely cultivated, being considered the third most-produced cereal, and responsible for providing 20% of the calories consumed by the population (SINGH et al., 2021). World wheat production was 779.9 million tons (USDA, 2022). However, studies show that the need for wheat will increase about 60-70% by 2050 due to increasing world population (POUR-ABOUGHADAREH et al., 2019).

In Brazil, wheat production occurs mainly in the South region; however, a growing exploration of the Central region is expected, mainly in the Cerrado biome (PASINATO et al., 2018). This region is characterized by high temperatures and greater water restriction, under rainfed cultivation conditions (PASINATO et al., 2018; HOFMANN et al., 2021).

Water stress negatively affects crop productivity (POUR-ABOUGHADAREH et al., 2019) and the wheat crop, in turn, is considered sensitive to drought conditions (NARDINO et al., 2022), especially in the reproductive and grain-filling

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phases (DONG et al., 2017). With the interest of circumventing this problem, the selection of cultivars and lines tolerant to water stress is a promising alternative (POUR-ABOUGHADAREH et al., 2019; POUR-ABOUGHADAREH et al., 2020; ANWAAR et al., 2020; NARDINO et al., 2022).

One of the potential strategies to evaluate and select drought-tolerant wheat genotypes is using water stress tolerance indices. These indices are based on mathematical relationships between the grain yield performance of the genotypes evaluated under stress and control conditions. Among the various indices available, the following stand out: GMP, geometric mean productivity (SCHNEIDER et al., 1997); MP, mean productivity (ROSIELLE & HAMBLIN, 1981); HM, Harmonic mean (JAFARI et al., 2009); STI, stress tolerance index (FERNANDEZ, 1992); and YI, yield index (GAVUZZI et al., 1997).

In addition to drought tolerance indices, agronomic traits of interest in wheat breeding (cycle, height, yield components) should also be considered during the selection of stress-resilient genotypes. Thus, it is of fundamental importance to know the relationships between the evaluated traits to obtain desirable gains with the selection. The understanding of the relationships between distinct groups of traits through canonical correlations can be used for this purpose (CRUZ et al., 2012). In addition, it reasonable that the inferences regarding relationship among the traits rely on best linear unbiased predictors (BLUP).

A few studies aiming to understand the relationship among traits in tropical wheat applying canonical correlations were conducted. For instance, MEZZOMO et al. (2021) verified the existence of canonical correlation between physiological and agronomic traits. SILVA et al. (2023) used canonical correlations to assess the existence of linear and multivariate relationships between high and low heritability traits. Studies of canonical correlations between water stress tolerance indices and agronomic traits of wheat using a mixed-model approach have not been reported to date. In this sense, unveiling the relationships between agronomic traits and drought tolerance indices in wheat is relevant.

Given the above mentioned, this study investigated the cause-and-effect relationships between agronomic traits and drought tolerance indices via analysis of canonical correlations using the predicted genotypic values of tropical wheat genotypes.

MATERIAL AND METHODS

Field experiment and plant material

The present study was conducted in the experimental area Professor Diogo Alves de Mello (lat 20º 45’ 14” S, long 42º 52’ 55” W, alt 648 m) belonged to the Universidade Federal de Viçosa (UFV), Viçosa, Minas Gerais, between June and October 2020. A total of 36 tropical wheat genotypes were evaluated. Genotypes consisted in 31 lines in the cultivation and use value (VCU) developed by the UFV Wheat Breeding Program and five commercial cultivars: BRS 264 (Empresa Brasileira de Pesquisa Agropecuária – EMBRAPA®), CD 151 (Cooperativa Central de Pesquisa Agrícola – COODETEC®), ORS 1403 (OR Genética de Sementes®), TBIO Aton and TBIO Duque (Biotrigo Genética®). Experiments were conducted under randomized block design with three replications. Two experiments were conducted, one under control condition (regular water supply) and the other under stress condition (drought stress). The experimental plot consisted of five rows of 5 m each, spaced 0.20 m apart, with a final population density of 300 m^{-2} plants.

Management

The base and cover fertilizations were carried out according to the interpretation of the chemical analysis of the soil, to meet to the needs of the crop. In the seeding line, 300 kg ha^{-1} of formulation 8-28-16 (nitrogen-phosphorus-potassium) were distributed and, in the cover fertilization, 90 kg ha^{-1} of nitrogen was applied in two phases, 50% at the beginning of tillering and 50% at the beginning of rubberization, stages 21 and 45 (ZADOKS et al., 1974). Urea (45% N) was used as a nitrogen source, totaling 200 kg ha^{-1}.

Control and stress

The 36 wheat genotypes were subjected to the control condition (irrigation) and the drought stress condition. Thus, two experiments were installed and were conducted simultaneously. The control, conducted with the aid of sprinkler irrigation, according to the water needs of the crop and the stress, in which there was a restriction of irrigation from the phenological stage of spiking, stage 65 (ZADOKS et al., 1974), with 30 days of stress due to drought, until the harvest.

Initially, soil samples were collected at depths of 0.00 to 0.10 m and 0.10 to 0.20 m for each environment. The homogenized samples were sent to the laboratory to perform the chemical analysis and to obtain the soil water retention curve. To monitor soil moisture, soil samples were collected every two days at 10 points in each area with the aid of a "Dutch" auger, at depths of 0.00–0.10 and 0.10–0.20 m. Then, the soil samples were weighted on a precision scale (0.001 g) and placed in an oven with air circulation at 60 °C for 48 hours. Subsequently, the samples were weighted again, and soil moisture was estimated.

The soil physical analysis data for the soil water retention curve (CRA, kpa) were: -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 -1500 kpa = 0.234 kg kg^{-1}.

Traits evaluated and drought tolerance indices

The number of days for heading (DH, days) was evaluated when at least 50% of the plants in the plot had fully exposed spikes. Five representative plants from each plot were used to measure the average plant height (PH, cm) with the aid of a ruler graduated in centimeters. Five representative spikes were collected from each plot to obtain the average grain mass per spike (GMS, g), number of grains per spike (NGS, units), and 100-grain mass (HGM, g). Grain yield (GY) was determined in kg ha^{-1}, with adjustment for 13% humidity in all plots. Finally, grain yield from both control and drought stress trials were used to calculate five different drought tolerance indices for each of the 36 genotypes (Table 1).

Statistical analysis

Data were subjected to analysis of deviance for estimation of genetic parameters, predict genotypic values, and obtain confidence intervals via REML/BLUP methodology, as follows (RESENDE, 2016).

$$y = X_r + Z_g + e$$

where y is the data vector; r is the vector of replication effects (assumed to be fixed) added to the overall mean; g is the vector of genotypic effects (assumed to be random) [$g \sim NID(0, \sigma_g^2)$], where σ_g^2 is the genotypic variance; and e the vector of errors or (random) residuals [$e \sim NID(0, \sigma_e^2)$], where σ_e^2 is the of residual variance; and X and Z are incidence matrices for said effects.

For the analysis of canonical correlations, the traits were divided into four groups. The first and third groups were composed of stress tolerance indices (GMP, MP, HM, STI, and YI). The second and fourth groups gathered agronomic traits, such as cycle (DH), height (PH), grain mass per spike (GMS), number of grains per spike (NGS), e 100-grain mass (HGM) and grain yield (GY), under control conditions (c) and stress (s), respectively.

The traits were subjected to a multicollinearity diagnosis, with condition number and variance inflation factor used as indicators of multicollinearity severity levels (MONTGOMERY et al., 2012).

RESULTS

Table 2 shows the results of the mixed model analysis. The heritability estimates ranged from 0.52 (NGS) to 0.84 (DH), and from 0.66 (GY) to 0.90 (DH) for the control and stress conditions, respectively. The highest accuracy was observed for DH (0.92), followed by GMS, PH, HGM, GY, and the lowest for NGS, in the control condition. In the stress condition, the highest is observed in DH (0.95), followed by GMS, HGM, NGS, PH, and the lowest for GY. For the drought tolerance indices, the highest was 0.89 for GMP, MP, and HM, and the lowest 0.80 for YI.

Table 1 - Drought tolerance indexes applied in the evaluation wheat genotypes.

Drought tolerance index	Code	Equation	Reference
Stress tolerance index	STI	$\frac{Y_S * Y_C}{(Y_C)^2}$	FERNANDEZ (1992)
Yield index	YI	$\frac{Y_S}{\bar{Y}_S}$	GAVUZZI et al. (1997)
Geometric mean productivity	GMP	$\sqrt{Y_S * Y_C}$	KRISTIN et al. (1997)
Mean productivity	MP	$\frac{Y_S * Y_C}{2}$	ROSIELLE & HAMBLING (1981)
Harmonic mean	MH	$\frac{2(Y_C - Y_S)}{Y_C + Y_S}$	JAFARI et al. (2009)

Y_S and Y_C are drought-stress and nonstress grain yeld of a given genotype, respectively. $\bar{Y}_S$ and $\bar{Y}_C$ are average yield of an all genotypes under drought-stress and nonstress conditions, respectively.

Table 2 - Variance components and genetic parameters of wheat genotypes for days to heading (DH), plant height (PH), weight of grain per spike (GMS), number of grains per spike (NGS), weight of 100 grains (HGM) and grain yield (GY), in condition of control and stress. And five drought tolerance indexes, geometric mean productivity (GMP) Mean productivity (MP), Harmonic mean (HM); Stress tolerance index (STI), Yield index (YI).

Parameters	DH	PH	GMS	NGS	HGM	GY
Control						
$\hat{\sigma}_g^2$	4.20	13.54	0.05	8.36	0.12	193721.05
$\hat{\sigma}_e^2$	2.39	18.11	0.07	23.54	0.19	342299.86
$\hat{\sigma}_f^2$	6.59	31.65	0.12	31.89	0.31	536020.91
$\hat{h}^2$	0.84	0.69	0.70	0.52	0.66	0.63
$\hat{h}$	0.92	0.83	0.84	0.72	0.81	0.79
CV_g	3.53	4.13	9.16	4.81	8.99	10.55
CV_e	2.66	4.78	10.34	8.08	11.19	14.02
CV_g/CV_e	1.32	0.86	0.89	0.60	0.80	0.75
Stress						
$\hat{\sigma}_g^2$	3.75	12.10	0.07	16.80	0.23	131836.48
$\hat{\sigma}_e^2$	1.20	16.04	0.03	16.47	0.13	203932.51
$\hat{\sigma}_f^2$	4.95	28.14	0.10	33.27	0.35	335768.99
$\hat{h}^2$	0.90	0.69	0.87	0.75	0.84	0.66
$\hat{h}$	0.95	0.83	0.93	0.87	0.92	0.81
CV_g	3.41	4.14	12.03	7.75	12.60	9.90
CV_e	1.93	4.76	8.07	7.67	9.52	12.31
CV_g/CV_e	1.76	0.87	1.49	1.01	1.32	0.80

Indexes	GMP	MP	MH	STI	YI
$\hat{\sigma}_g^2$	169243.75	168353.96	170138.80	0.03	0.01
$\hat{\sigma}_e^2$	134501.52	137723.11	132421.26	0.03	0.02
$\hat{\sigma}_f^2$	303745.27	306077.07	302560.06	0.06	0.02
$\hat{h}^2$	0.79	0.79	0.79	0.78	0.65
$\hat{h}$	0.89	0.89	0.89	0.88	0.80
CV_g	10.56	10.47	10.65	20.57	9.68
CV_e	9.41	9.47	9.40	19.14	12.39
CV_g/CV_e	1.12	1.11	1.13	1.07	0.78

Note: $\hat{\sigma}_g^2$, component of genotypic variance; $\hat{\sigma}_e^2$, component of residual variation; $\hat{\sigma}_f^2$, component of phenotypic variation; $\hat{h}^2$, heritability; $\hat{h}$, accuracy; CV_g, coefficient of genotypic variation; CV_e, coefficient of experimental variation.

In the control condition, only the DH showed the greatest genotypic variation effect (CVg/CVe > 1.00), in explaining the total phenotypic variation. The stress condition presented for most traits, except for PH and GY, greater genotypic variation (Table 2). Drought tolerance indices; on the other hand, showed greater genotypic variation in most indices except for YI (Table 2).

Figure 1 shows the boxplot for the six traits evaluated, in the two conditions (control and stress) studied. For the traits associated with production (GY, NGS, and GMS), the wheat genotypes under control condition presented higher means than those of the stress condition. For plant height (PH) it is observed that in the control condition the plants were taller. The traits DH and HGM, in turn, showed a high amplitude of variation. The DH in the drought stress condition, the high amplitude of variation, can be explained by the existence of genotypes, which in this condition, presented reduced cycle (Figure 1).

In table 3, canonical correlation analysis shows that all canonical pairs were significant. This demonstrates that there is a dependence between the groups under study. The first pair indicates that the five drought tolerance indices studied (GMP, MP, HM, STI, and YI) are directly related to GY, both

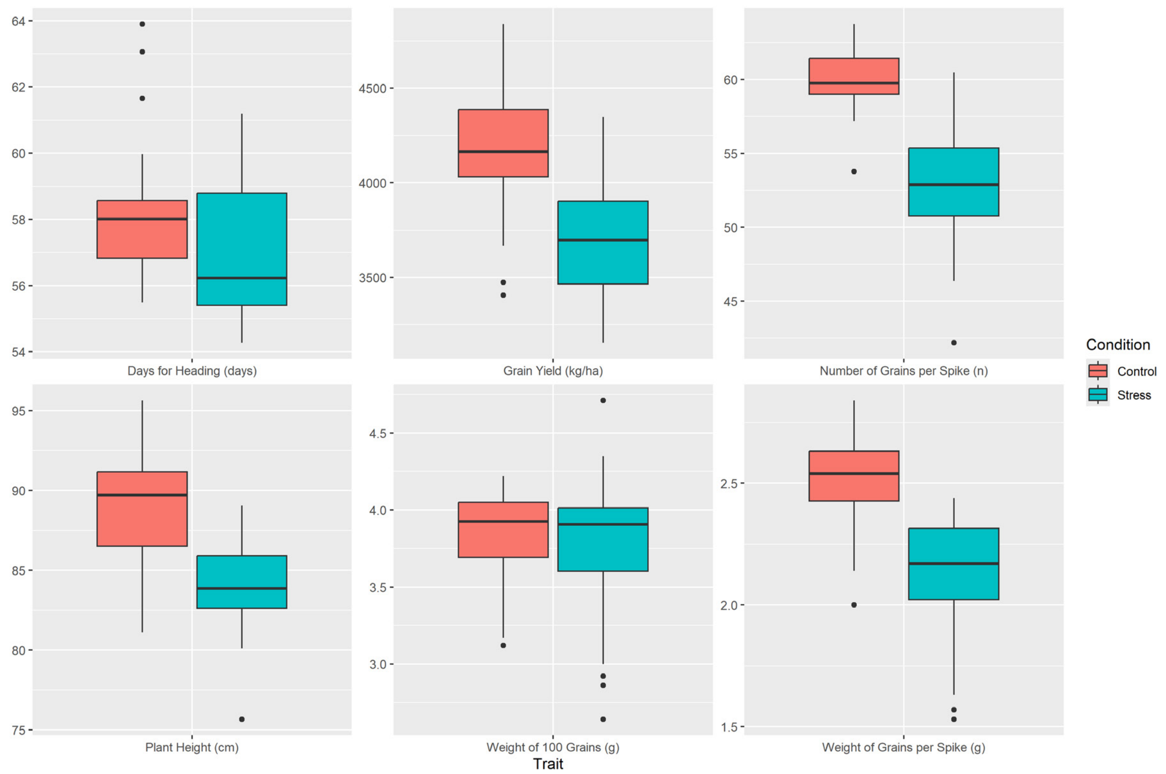

Figure 1 - Boxplot showing differences between two distinct field trial conditions (control and stress conditions) for nine agronomic traits.

for the control condition (GYc) and for the stress condition (GYs). Relationship of the indices with HGM can also be observed in both conditions. The second pair shows us positive relationships of the indices with the traits DHc and PHc; however, in the stress condition for the same traits the relationships are opposite (Table 3).

The correlation network between tolerance indices and agronomic traits under control and stress conditions is shown in figure 2. It is observed that there is cohesion between the drought tolerance indices and a positive correlation with the group of agronomic parameters (GYc and GYs). The indices are also shown to correlate positively with HGMc and HGMs, as can also be seen in table 3. Negative correlation is observed between the group of indices, with DHs and DHc, which also presents a negative correlation with GYc, GYs, HGMc and HGMs. This fact allows us to understand that shorter-cycle genotypes would be more productive. This corroborated the view in table 3, in which we can observe the opposite relationship of the cycle with the tolerance indices.

DISCUSSION

For the selective accuracy values, we observed very high values (> 0.90) and high values (> 0.70) for the variables studied, varying according to the condition studied. For stress indices, all are considered high (RESENDE & DUARTE, 2007). Very high and high accuracy for the variables indicate good experimental accuracy, which shows the reliability of the selection of wheat genotypes (RESENDE & DUARTE, 2007). The coefficient of genetic variation (CVg) explains the portion of genetic over the phenotypic variance. Greater contribution of CVg was observed in the drought stress condition, ranging from 3.41 to 12.60, and, in the drought tolerance indices (9.40 to 20.57), indicating therefore, the presence of genetic variation for the traits evaluated in this condition (RESENDE, 2002). The literature tells us that high values for CVg allow genetic gains in genotype selection (RESENDE, 2002).

Drought stress negatively affected GY and the other grain production components (except HGM), as well as PH, of the 36 genotypes

Table 3 - Estimates of canonical correlations pairs between indexes (Geometric mean productivity – GMP; Mean productivity – MP; Harmonic mean – HM; Stress tolerance index – STI and Yield index – YI) and agronomic traits (Days to heading – DH; plant height – PH; weight of grain per spike – GMS; number of grains per spike – NGS; weight of 100 grains – HGM and grain yield – GY) under control and stress in 36 tropical wheat genotypes.

Traits	Canonical pairs				
	1st	2nd	3rd	4th	5th
Group I					
GMP	0.93	0.00	-0.05	0.00	-0.03
MP	0.94	0.01	-0.05	0.00	-0.03
HM	0.92	-0.01	-0.05	0.01	-0.03
STI	0.92	0.01	-0.02	0.00	-0.03
YI	0.73	0.02	-0.12	0.03	-0.06
Group II					
DHc	-0.50	0.62	-0.05	-0.45	0.12
PHc	0.12	0.56	0.09	0.47	0.16
GMSc	0.30	-0.22	-0.58	0.65	-0.31
NGSc	-0.16	-0.28	-0.33	0.73	0.32
HGMc	0.40	-0.19	-0.74	-0.02	-0.29
GYc	0.99	0.01	0.02	0.03	0.03
r[1]	0.98	0.58	0.35	0.28	0.11
Group III					
GMP	0.90	0.16	-0.05	0.09	0.00
MP	0.89	0.17	-0.04	0.10	0.00
HM	0.91	0.15	-0.05	0.09	0.00
STI	0.90	0.15	-0.02	0.10	0.00
YI	1.00	0.00	0.00	0.00	0.00
Group IV					
DHs	-0.34	-0.67	0.24	-0.32	0.47
PHs	0.27	-0.71	-0.33	0.49	-0.27
GMSs	0.24	0.35	-0.81	0.20	0.08
NGSs	-0.18	-0.05	-0.69	-0.27	-0.31
HGMs	0.31	0.55	0.06	0.50	0.44
GYs	1.00	-0.01	-0.01	0.02	0.02
r[1]	0.99	0.60	0.46	0.30	0.20

[1]values greater than 0.10 are significant.

under study, compared to the control condition. Studies show that plant height and productivity are more sensitive to stress than the weight of 1000 grains (AHMAD et al., 2018; ALI, 2019). The lower plant height observed in the drought stress condition may be due to lower water availability, culminating in reduced cell elongation (HUSSAIN et al., 2021). In wheat studies, plant height reduction is documented by other authors (POUR-ABOUGHADAREH et al., 2020).

Lower NGS and GMS, in stress, can be explained by the sensitivity of drought anthesis (DONG et al., 2017). Lower water availability affects pollen grain viability, thus reducing the number of grains per ear (DONG et al., 2017; AHMAD et al., 2022; SHAMUYARIRA et al., 2022). Studies with water stress verified the reduction of NGS in durum wheat genotypes (POUR-ABOUGHADAREH et al., 2019) and bread wheat (DONG et al., 2017). We verified a large DH amplitude under stress conditions. DH reduction is configured as an adaptive strategy, in which the plant accelerates its cycle to escape an upcoming water shortage. In such a way it enables a reduction in exposure to water stress during the most drought-sensitive stages (SHAVRUKOV et al., 2017).

High cohesion between the tolerance indices can be explained because both lead to their common component formulas (GY). That also explains their positive correlation with GY. However, some indices may not positively correlate with GY

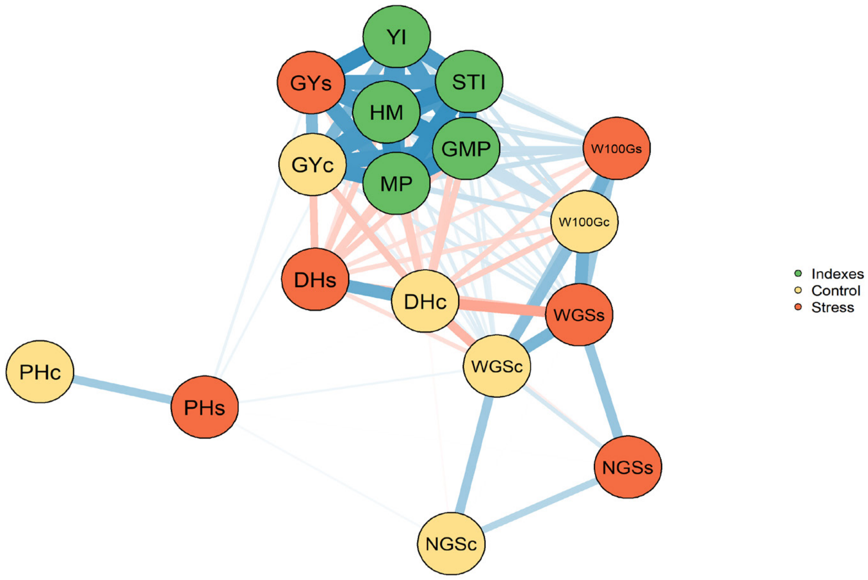

Figure 2 - Network correlations among the days to heading – DH; plant height – PH; weight of grain per spike – GMS; number of grains per spike – NGS; weight of 100 grains – HGM and grain yield – GY, in condition of control (c) and stress (s). Red and blue lines indicate negative and positive genotypic correlations. The line widths are proportional to the values of correlation. Cutoff: 0.2; Maximum correlation: 0.99.

(HUSSAIN et al., 2021), a fact that did not occur in our study significant and positive associations of stress tolerance indices, with GY under control and stress, indicate that the indices are promising for the evaluation of high-yield wheat genotypes under both conditions (ALHAG et al., 2021). A study with rice (HUSSAIN et al., 2021), identified four indices (GMP, STI, M_{PRO}, and M_{HAR}) that can be used to indicate stress tolerance in rice breeding programs. In barley (JAMSHIDI & JAVANMARD, 2018) they identified GMP and STI as indicative indices for salinity tolerance. Positive correlation of indices with productivity in both conditions (control and drought) is observed in wheat by different authors (POUR-ABOUGHADAREH et al., 2020; ALHAG et al., 2021). In other crops such as chickpeas (ARIF et al., 2021) and rice (HUSSAIN et al., 2021) this is also observed.

From the results obtained in this research, we observed that, with the correlations obtained between agronomic traits and drought tolerance indices, it was possible to identify traits, indirectly, that help in the identification of drought-tolerant genotypes. Therefore, we can use the cycle (DH) as a tool, selecting the earliest genotypes, which in turn will be the most productive in both conditions. Earlier genotypes will have a higher hectoliter weight and mass of one hundred grains (SILVA et al., 2023).

CONCLUSION

By correlations obtained between agronomic traits and drought tolerance indices was possible to identify traits that can help in the identification of drought-tolerant genotypes. The agronomic trait days to heading (DH) can be used for the indirect selection of drought-tolerant genotypes with high grain yield.

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DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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