Impact of drought and salinity stress on early growth of tropical wheat cultivars

Impacto do estresse hídrico e salino no crescimento inicial de cultivares de trigo tropical

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ABSTRACT

The use of wheat cultivars adapted to adverse environmental conditions is essential to ensure the stability of agricultural production in the face of climate change. This study evaluated the effects of drought and salinity stresses on the germination and early growth of 11 tropical wheat cultivars under controlled iso-osmotic conditions induced by polyethylene glycol (PEG) and sodium chloride (NaCl). Wheat plants were grown for 12 days at 25 °C under non-stressful conditions (control) and under simulated drought and salinity stress conditions using iso-osmotic solutions of –0.30 MPa prepared with polyethylene glycol (151.40 g L^{-1} PEG 6000) or sodium chloride (3.536 g L^{-1} NaCl), respectively. Germination, seedling growth, and dry matter accumulation were measured and used to calculate stress tolerance indices. The results revealed significant differences among cultivars in their responses to the applied stresses. The cultivars TBIO Sintonia and TBIO Calibre showed greater tolerance to drought stress, whereas TBIO Convicto demonstrated greater resilience under saline stress. In contrast, Biotrigo Valente and ORS Feroz showed greater sensitivity to the evaluated stresses. The integration of stress tolerance indices with multivariate statistical approaches allowed a consistent discrimination between tolerant and susceptible cultivars. These results highlight the variability of tropical wheat germplasm and indicate that the identification of tolerant cultivars may contribute to breeding strategies and to improving wheat adaptation to environments subject to drought and salinity.

Keywords: abiotic stress, germination, *Triticum aestivum* L.

RESUMO

O uso de cultivares de trigo adaptadas às condições ambientais adversas é essencial para garantir a estabilidade da produção agrícola diante das mudanças climáticas. Este estudo avaliou os efeitos dos estresses hídrico e salino na germinação e no crescimento inicial de 11 cultivares tropicais de trigo sob condições iso-osmóticas controladas induzidas por polietilenoglicol (PEG) e cloreto de sódio (NaCl). Plantas de trigo foram cultivadas por 12 dias a 25 °C sob condições não estressantes (controle) e sob condições simuladas de estresse hídrico e salino com soluções iso-osmóticas de –0,30 MPa preparadas com polietilenoglicol (151,40 g L^{-1} PEG 6000) ou cloreto de sódio (3,536 g L^{-1} NaCl), respectivamente. A germinação, o crescimento das plântulas e o acúmulo de matéria seca foram mensurados e utilizados para o cálculo de índices de tolerância ao estresse. Os resultados revelaram diferenças significativas entre as cultivares quanto às respostas aos estresses aplicados. As cultivares TBIO Sintonia e TBIO Calibre apresentaram maior tolerância ao estresse hídrico, enquanto TBIO Convicto demonstrou maior resiliência sob estresse salino. Em contraste, Biotrigo Valente e ORS FEROZ mostraram maior sensibilidade aos estresses avaliados. A integração de índices de tolerância ao estresse com abordagens estatísticas multivariadas permitiu uma discriminação consistente entre cultivares tolerantes e suscetíveis. Esses resultados evidenciam a variabilidade do germoplasma de trigo tropical e indicam que a identificação de cultivares tolerantes pode contribuir para estratégias de melhoramento e para a adaptação do trigo a ambientes sujeitos à seca e à salinidade.

Palavras-chave: estresse abiótico, germinação, *Triticum aestivum* L.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the three main cereal crops grown in the world. Wheat plays a key role in the food and nutritional security of the global population, and is widely used in the production of bread, dough, pasta, cookies and many other products. In Brazil, wheat cultivation has gained greater relevance in recent years, following high investments in crop genetic improvement programs, which aim to select genetic materials adapted to the high-temperature tropical conditions of the Brazilian Cerrado (Lima *et al*., 2022; Casagrande *et al*., 2024; Ribeiro *et al*., 2024). This selection of cultivars adapted to high temperatures has allowed the expansion of the wheat cultivation area in the new agricultural frontiers of the Cerrado region, which has increased national wheat production (Oliveira *et al*., 2022; Teixeira *et al*., 2023).

However, wheat production in the tropics faces challenges caused by climate change, including extreme drought events and heat waves that have contributed to soil salinization due to intense crop evapotranspiration, which can negatively impact germination and early plant growth (Guo *et al*., 2017; Thabet *et al*., 2018). Low water availability and excess salts in the soil limit the seed imbibition process, compromising germination and initial plant development in the field (Al-Tabbal & Al-Zboon, 2021). Furthermore, these abiotic stresses cause significant changes in the enzymatic, oxidative, hormonal and physiological metabolism of plants, which is a critical factor for plant development, especially in the leaf area and roots (Zuffo *et al*., 2020; Steiner *et al*., 2021; Argentel-Martínez *et al*., 2024a, b). Therefore, the identification of genotypes that are tolerant and adapted to adverse environmental conditions of drought and salinity is a key factor for the sustainability of wheat production in the tropics (Pereira *et al*., 2015; Aguilera *et al*., 2016, 2019; Rüdell *et al*., 2021; Ribeiro *et al*., 2024).

The evaluations conducted during the initial phase of seedling growth are essential for selecting genotypes adapted to these stresses, allowing optimization of time and conservation of resources (Oliveira *et al*., 2023; Monteiro *et al*., 2023). Studies demonstrate that techniques such as multivariate indices (Aguilera *et al*., 2019; Zuffo *et al*., 2020; Steiner *et al*., 2021; Argentel-Martínez *et al*., 2024b), physiological analyses (Rüdell *et al*., 2021; Severo *et al*., 2022), and machine learning (Oliveira *et al*., 2022, 2023) enable the identification of materials with greater tolerance to water deficit and salinity (Zuffo *et al*., 2020; Oliveira *et al*., 2023). These strategies aid in the development of new, more resilient cultivars, contributing to the maintenance of productivity under adverse scenarios (Steiner *et al*., 2021; Ribeiro *et al*., 2024).

Recent studies have investigated the response of tropical wheat cultivars to abiotic stresses using different methodological approaches. Multivariate adaptability and stability analyses have been employed to identify genotypes with superior performance under drought and salinity conditions, highlighting the genetic variability among cultivars adapted to the Brazilian Cerrado. In this context, Zuffo *et al*. (2025a) evaluated the multivariate adaptability of tropical wheat cultivars subjected to drought and salinity stresses during early plant development, demonstrating that different genotypes exhibit contrasting morphological responses under adverse environmental conditions. Similarly, approaches based on image analysis and RGB spectral information have been proposed as rapid and nondestructive tools for the selection of genotypes tolerant to abiotic stresses. In this regard, Zuffo *et al*. (2025b) demonstrated the potential of RGB spectral information for identifying wheat genotypes adapted to drought and salinity conditions during the early growth stage.

However, the comparative response of tropical wheat cultivars under iso-osmotic conditions induced by polyethylene glycol (PEG) and sodium chloride (NaCl) is still poorly understood. While PEG predominantly simulates osmotic stress, NaCl imposes osmotic stress associated with ionic stress. In this context, the integration of stress tolerance indices with multivariate statistical approaches, such as ranking and cluster analysis, can increase the accuracy in discriminating tolerant genotypes, providing a more robust basis for understanding tolerance mechanisms and supporting breeding programs aimed at improving wheat adaptation to environments subject to drought and salinity.

Given this context, the present study aimed to evaluate the resilience of tropical wheat cultivars to drought and salinity during germination and early seedling growth under iso-osmotic conditions, integrating stress tolerance indices with multivariate statistical approaches, such as ranking and hierarchical clustering analysis, in order to identify promising genotypes adapted to challenging edaphoclimatic conditions in Brazil.

MATERIAL AND METHODS

Plant material and stress treatments

Seeds from 11 tropical wheat cultivars (*Triticum aestivum* L.) commonly grown in the Brazilian Cerrado region were used in this study. Before starting the experiment, the water content, thousand seed weight, seed germination and plant emergence rate were determined following the Brazilian Official Rules for Seed Analysis (Brasil, 2009). The main characteristics of wheat seeds and cultivars are shown in Table 1.

To compare the impact of drought and salinity stresses on germination and plant growth rate, seeds of each wheat cultivar were exposed to –0.30 MPa osmotic solutions prepared with polyethylene glycol (PEG-6000) and sodium chloride (NaCl), respectively. This osmotic potential was selected because it represents a moderate stress level commonly used in seedling screening experiments, allowing adequate germination while enabling discrimination among genotypes in terms of stress tolerance. A similar approach has been adopted in studies evaluating the response of wheat genotypes to abiotic stresses during germination and early seedling development (Ribeiro *et al*., 2024). The amount of PEG-6000 added to obtain the solution with osmotic potential of –0.30 MPa was determined by the equation of Michel & Kaufmann (1973): $\Psi_S = [-(1.18 \times 10^{-2}) \times C - (1.18 \times 10^{-4}) \times C^2 + (2.67 \times 10^{-4}) \times C \times T + (8.39 \times 10^{-7}) \times C^2 \times T]/10$, where Ψ_S is the osmotic potential (MPa); C is the concentration (g L^{-1} of PEG-6000); and T is the temperature (°C). The amount of NaCl added to obtain the osmotic potential of –0.30 MPa was calculated by the van't Hoff equation (Hillel, 1971): $\Psi_S = -R \times T \times C \times i$, where R is the universal constant of noble gas (0.008314 MPa mol^{-1} K^{-1}), T is the absolute temperature (273.15 + °C), C is the molar concentration of the solute (mol L^{-1}) and i is the van't Hoff factor, that is the number of ions released when the solute is dissolved in water [i.e., for NaCl this value is 2.0 (Na^+ and Cl^-). Distilled water with an osmotic potential of 0.00 MPa was used as control treatment.

Germination and growth conditions

Four 25-seeds replicates from each wheat cultivar were evenly distributed between two sheets of paper towels, previously moistened with distilled water (control), PEG-6000 solution (drought stress) or NaCl solution (salinity), in an amount equivalent to three times the dry mass of the Germitest® paper. The paper towel sheets were then turned

Table 1 - Agronomic characteristics, water content, thousand seed mass, and germination rate of 11 Brazilian wheat cultivars used in this study

Cultivar	Maturation cycle	Water content (%)	1,000-seed weight (g)	Germination rate (%)	Emergency rate (%)
TBIO ATON	Medium	12.7	37.88 b	95 a	100 a
TBIO SINTONIA	Early	12.5	32.55 e	86 a	82 d
BRS 404	Early/Medium	12.8	38.73 b	89 a	92 b
BRS 264	Super early	12.7	36.13 d	92 a	100 a
TBIO CALIBRE	Super early	12.5	35.05 d	88 a	100 a
Biotrigo Valente	Medium/Early	12.6	37.08 c	83 b	100 a
TBIO DUQUE	Early	12.9	34.68 d	91 a	100 a
BIO 190038	Early	12.7	37.20 c	79 b	100 a
TBIO SOSSEGO	Medium	12.6	34.90 d	88 a	100 a
ORS FEROZ	Super early	12.5	40.10 a	94 a	100 a
TBIO CONVICTO	Medium/Late	12.8	40.33 a	82 b	84 c

Mean followed by distinct letters in the column for the wheat cultivars show significant differences by the Scott-Knott test at the 0.05 level of confidence.

into rolls, which were packaged into plastic bags to prevent evaporation and maintain the relative humidity close to 100%. Germination was conducted in a growth chamber under 12/12 h photoperiod (light/darkness), light intensity of 120 μmol m^{-2} s^{-1} and constant temperature of 25 °C for 12 days. Vitavax–Thiram® was added to the solutions at a concentration of 0.2% (v/v) to control the fungi infection as used by Zuffo *et al*. (2020). Plants remained exposed to abiotic stress for 12 days. In a previous trial, a period of 12 days was appropriate to identify differences in tolerance between soya bean genotypes exposed to osmotic stress conditions, especially since this period is ideal for the radicle and epicotyl elongation, two processes that are delayed when the germination environment has low osmotic potential.

Measurement of germination, plant growth, and tolerance indices

After 12 days of exposure to abiotic stress, the seed germination rate was recorded. Seeds were considered germinated when the radicle was longer than 10.0 mm. Subsequently, five plants per replicate were randomly chosen and the shoot length (SL), root length (RL), total length (TL), shoot dry matter (SDM), root dry matter (RDM) and total dry mass (TDM) were determined. The SL, RL and TL were measured using a millimeter ruler. The SDM, RDM and TDM were recorded on an analytical balance after oven drying at 85 °C for 48 h.

For seedling growth measurements, five normal seedlings were randomly selected per replicate from the germinated seedlings. This sampling procedure was adopted because the evaluated traits involved destructive measurements and is consistent with standard seedling growth bioassays conducted under controlled conditions. Moreover, the relatively low coefficients of variation observed for the measured traits indicated adequate experimental precision for detecting treatment differences.

The original data of germination, growth and dry matter accumulation of wheat plants were used to calculate stress tolerance indices (STI) according to Equation 1 used by Vilas-Boas *et al*. (2025):

$$\Delta STI = [(\Delta \text{ under stressful conditions} / \Delta \text{ under control conditions}) \times 100] \quad [Eq.\ 1]$$

where Δ are the dependent variables measured in wheat plants [i.e., G, SL, RL, TL, SDM, RDM, and TDM] under stressful conditions (drought or salinity) and non-stressful conditions (control).

Experimental design and Statistical analysis

The experimental trial was arranged in a completely randomized design (CRD) in a 3 × 11 factorial scheme: three stress treatments [control, drought and salinity) and 11 tropical wheat cultivars, with four replicates of 25 seeds.

Prior to the analysis of variance (ANOVA), the assumptions of normality of residuals and homogeneity of variances were verified. The normality of residuals was assessed using the Shapiro–Wilk test, while the homogeneity of variances was evaluated using Levene's test. After confirming these assumptions, the data were subjected to ANOVA, and the means of the stress tolerance indices for the wheat cultivars were grouped using the Scott–Knott test at the 5% significance level. All statistical analyses were performed using Rbio® software, version 140 for Windows (Bhering, 2017).

The identification of tolerant or susceptible cultivars to abiotic stress was performed based on all stress tolerance indices (STI), using two multivariate analysis methods [ranking method and hierarchical clustering analysis (HCA)].

The ranking method was used as proposed by Zuffo *et al.* (2020) and Cabral *et al.* (2022). In this method, a cultivar with the highest value for each STI of plant dependent variables received a ranking score equal to 1, while the cultivar with the lowest value for each STI received a ranking score equal to 11. The mean rank ($\overline{R}$) and standard deviation of ranks (SD_R) of all stress tolerance criteria were then calculated. The discrimination of wheat cultivars regarding their tolerance degree to drought and salinity stress was performed based on the mean rank score of each cultivar, considering the values of the quartiles that divide the 11 possible positions (i.e., 11 wheat cultivars) into four equal parts. A cultivar with a mean ranking ($\overline{R}$) lower than the value of the first quartile (<3.5 points) is classified as tolerant (T); a cultivar with an between the first and second quartiles (3.6 to 6.0

points) is classified as moderately tolerant (MT); a cultivar with an $\bar{R}$ between the value of the second and third quartiles (6.1 to 8.5 points) is classified as moderately susceptible (MS); and the group of genotypes susceptible (S) to drought and salinity stress is represented by cultivars with an higher than the value of the third quartile (>8.6 points).

Hierarchical clustering analysis (HCA) of 11 wheat cultivars based on the Euclidean distance method and unweighted pair group method with arithmetic mean (UPGMA) was performed using the Rbio® software version 140 for Windows (Bhering, 2017). The optimal number of tolerance groups to drought and salinity stress formed in the dendrogram was defined by Mojena's criterion (Mojena, 1977). This criterion is based on computing the highest amplitude between clusters through the following formula: $\alpha_j > \check{\alpha} + \omega S_{\alpha}$, where j = (1, 2, …, n) is the number of clusters; α_j is the correspondence joint point to n − j + 1 clusters; $\check{\alpha}$ and S_{α} are the mean and the standard deviation of α'_j s, respectively, and ω is a constant equal to 1.25, as suggested by Milligan & Cooper (1985).

A correlation network was constructed by employing the Pearson correlation values obtained by correlating the values of the seven proposed indices, the ranking classification, and the grouping. For these latter two methods, the classifications S, MS, MT, and T were converted into numerical values (1, 2, 3, and 4, respectively). Analysis was performed using the Rbio® software version 140 for Windows (Bhering, 2017).

RESULTS

Evaluation of wheat performance through different indices

The result of the GSTI of 11 wheat cultivars is shown in Table 2. The indices under water deficit and salinity conditions show that no significant differences were observed between the two stress conditions for the GSTI index within most cultivars. When comparing the indices within each cultivar, it is shown that only three cultivars (Biotrigo Valente, ORS FEROZ, and TBIO Duque) exhibit a differential effect when comparing the two applied stresses. The cultivars Biotrigo Valente and TBIO Duque showed a decrease of 12% and 10% in performance when subjected to saline stress compared to water stress, respectively. Among the cultivars that differed between the applied stresses, ORS FEROZ demonstrated better performance under saline stress (85%) compared to water stress (75%) (Table 2).

Table 2 - Germination stress tolerance index (GSTI) of 11 wheat (*Triticum aestivum* L.) cultivars exposed to drought and salinity stress

Wheat cultivar	GSTI (%)	
	Drought	Salinity
BIO 190038	88 a	90 a
Biotrigo Valente	87 a	78 a*
BRS 264	80 a	85 a
BRS 404	85 a	88 a
ORS FEROZ	75 a	85 a*
TBIO Aton	87 a	90 a
TBIO Calibre	88 a	93 a
TBIO Convicto	83 a	89 a
TBIO Duque	89 a	81 a*
TBIO Sintonia	90 a	90 a
TBIO Sossego	88 a	84 a
Mean	85	87
CV (%)	8.71	

Mean followed by distinct letters on the column for the wheat cultivars show significant differences by the Scott-Knott test at the 0.05 level of confidence. CV: coefficient of variation. Asterisks show that there was a significant difference (*F* test; $p < 0.05$) between drought and salinity stress for the wheat cultivar.

The indices obtained for stem and root length are shown in Table 3. In the SLSTI index, only under water stress did the 11 cultivars differentiate, with TBIO Aton, TBIO Calibre, TBIO Sintonia, and TBIO Sossego standing out, showing values of 78%, 78%, 87%, and 76%, respectively. When compared within each cultivar, the following cultivars showed differences with better performance under salinity: BIO 190038, Biotrigo Valente, ORS FEROZ, TBIO Aton, TBIO Convicto, TBIO Duque, and TBIO Sossego (Table 3). The root-related index (RLSTI) highlighted differences in the performance of the 11 wheat cultivars under both applied stresses. Under water stress for RLSTI, most cultivars performed well without statistical differences among them, except for Biotrigo Valente. Under saline stress for RLSTI, the best performances were for cultivars BRS 264, TBIO Aton, TBIO Convicto, and TBIO Sintonia, with performances of 81%, 87%, 92%, and 95%, respectively (Table 3).

Table 3 - Shoot length (SLSTI), root length (RLSTI), and total seedling length stress tolerance index (TLSTI) of 11 wheat (*Triticum aestivum* L.) cultivars exposed to drought and salinity stress

Wheat cultivar	SLSTI (%)		RLSTI (%)		TLSTI (%)	
	Drought	Salinity	Drought	Salinity	Drought	Salinity
BIO 190038	69 b	90 a*	90 a	72 b*	75 b	84 a
Biotrigo Valente	73 b	84 a*	82 b	55 c*	75 b	75 b
BRS 264	71 b	80 a	96 a	81 a*	78 a	80 a
BRS 404	72 b	78 a	89 a	75 b*	76 b	77 b
ORS FEROZ	69 b	84 a*	96 a	64 c*	78 a	77 b
TBIO Aton	78 a	91 a*	92 a	87 a	82 a	89 a
TBIO Calibre	78 a	85 a	97 a	78 b*	85 a	82 a
TBIO Convicto	64 b	89 a*	96 a	92 a	73 b	90 a*
TBIO Duque	72 b	88 a*	97 a	74 b*	79 a	84 a
TBIO Sintonia	87 a	89 a	93 a	95 a	88 a	90 a
TBIO Sossego	76 a	88 a*	94 a	70 b*	82 a	81 a
Mean	74	86	93	77	79	83
CV (%)	11.76		11.87		9.20	

Mean followed by distinct letters on the column for the wheat cultivars show significant differences by the Scott-Knott test at the 0.05 level of confidence. CV: coefficient of variation. Asterisks show that there was a significant difference (*F* test; $p < 0.05$) between drought and salinity stress for the wheat cultivar.

When comparing the percentage values within the RLSTI index, the comparison within each cultivar shows that eight out of the 11 cultivars differed between both stresses and exhibited better performance under water stress (Table 3). When considering the TLSTI index, variation is observed in the response of cultivars when subjected to water and salt stress. Among water stress, the cultivars that showed the best performance were BRS 264, ORS FEROZ, TBIO Aton, TBIO Calibre, TBIO Duque, TBIO Sintonia, and TBIO Sossego, with values ranging from 78% to 88% (Table 2). Among salt stress for TLSTI, the cultivars that demonstrated the best performance were BIO 190038, BRS 264, TBIO Aton, TBIO Calibre, TBIO Convicto, TBIO Duque, TBIO Sintonia, and TBIO Sossego, with values from 80% to 90%. When evaluating the greatest variation between the two applied stresses, only one cultivar, TBIO Convicto, showed differences, with the greater effect being water stress at 73% (Table 3).

Table 4 - Shoot dry matter (SMSTI), root dry matter (RMSTI), and total dry matter stress tolerance index (TMSTI) of 11 wheat (*Triticum aestivum* L.) cultivars exposed to drought and salinity stress

Wheat cultivar	SMSTI (%)		RMSTI (%)		TMSTI (%)	
	Drought	Salinity	Drought	Salinity	Drought	Salinity
BIO 190038	83	80	89	84	89	81
Biotrigo Valente	84	83	94	90	94	84
BRS 264	96	98	94	92	94	97
BRS 404	90	86	82	90	82	87
ORS FEROZ	87	83	91	96	91	86
TBIO Aton	83	87	78	77	78	84
TBIO Calibre	90	91	94	94	94	92
TBIO Convicto	88	92	94	94	94	93
TBIO Duque	67	71	86	85	86	74
TBIO Sintonia	97	85	89	89	89	86
TBIO Sossego	91	90	94	93	94	91
Mean	87	86	90	89	87	87
CV (%)	10.03		9.05		9.28	

Mean followed by distinct letters on the column for the wheat cultivars show significant differences by the Scott-Knott test at the 0.05 level of confidence. CV: coefficient of variation. Asterisks show significant differences (*F* test; $p < 0.05$) between drought and salinity stress for the wheat cultivar.

The indices related to dry matter accumulation (SMSTI, RMSTI, and TMSTI) did not show significant differences among cultivars (Table 4). However, differences among genotypes were clearly detected for growth-related indices (SLSTI, RLSTI, and TLSTI), which contributed to the discrimination of tolerant and susceptible cultivars (Table 3).

Performance evaluation through ranking using indices

The ranking of cultivars using the seven evaluated variables under water stress shows that there is variation in the performance of cultivars, a variation that is corrected when calculating the average ranking with maximum values of 8.0 in BIO 190038 and a minimum of 3.0 in TBIO Sintonia (Table 5). The values obtained from the average ranking allowed the classification of cultivars into tolerant (TBIO Calibre and TBIO Sintonia), moderately tolerant (BRS 264 and TBIO Sossego), moderately susceptible (BIO 190038, Biotrigo Valente, BRS 404, ORS FEROZ, TBIO Aton, TBIO Convicto, and TBIO Duque), and no susceptible cultivars were obtained (Table 5).

The ranking of cultivars considering salt stress shows that there is variation in the performance of

Table 5 - Rank, rank mean ($\bar{R}$), and standard deviation of ranks (SDR) of drought tolerance indices of 11 wheat (*Triticum aestivum* L.) cultivars

Wheat cultivar	Stress tolerance index							± SDR	Tolerance level†
	G	SL	RL	TL	SDM	RDM	TDM		
BIO 190038	3	10	9	9	9	7	9	8.0±1.7	MS
Biotrigo Valente	7	5	11	10	8	4	8	7.6±1.9	MS
BRS 264	10	8	4	7	2	1	1	4.7±3.1	MT
BRS 404	8	7	10	8	4	10	6	7.6±1.6	MS
ORS FEROZ	11	9	3	6	7	6	7	7.0±1.7	MS
TBIO Aton	6	3	8	3	10	11	10	7.3±2.8	MS
TBIO Calibre	5	2	1	2	5	5	4	3.4±1.5	T
TBIO Convicto	9	11	5	11	6	3	5	7.1±2.7	MS
TBIO Duque	2	6	2	5	11	9	11	6.6±3.2	MS
TBIO Sintonia	1	1	7	1	1	8	2	3.0±2.6	T
TBIO Sossego	4	4	6	4	3	2	3	3.7±0.9	MT

†T, refers to a drought-tolerant cultivar, having mean rank ($\bar{R}$) score of 1 to 3.5; MT, moderately tolerant cultivar with mean rank score of 3.6 to 6.0; MS, moderately sensitive cultivar with mean rank score of 6.1 to 8.5; and S, drought-sensitive cultivar with mean rank score of 8.6 to 11. Abbreviations: G: germination; SL: shoot length; RL: root length; TL, total seedling length; SDM: shoot dry matter; RDM: root dry matter; TDM: total seedling dry matter.

Table 6 - Rank, rank mean ($\bar{R}$), and standard deviation of ranks (SDR) of salinity tolerance indices of 11 wheat (*Triticum aestivum* L.) cultivars

Wheat cultivar	Stress tolerance index							± SDR	Tolerance level†
	G	SL	RL	TL	SDM	RDM	TDM		
BIO 190038	3	2	8	5	10	10	10	6.9±3.0	MS
Biotrigo Valente	11	8	11	11	9	7	8	9.3±1.5	S
BRS 264	7	10	4	8	1	5	1	5.1±2.7	MT
BRS 404	6	11	6	10	6	6	5	7.1±1.9	MS
ORS FEROZ	8	9	10	9	8	1	7	7.4±2.0	MS
TBIO Aton	2	1	3	3	5	11	9	4.9±3.0	MT
TBIO Calibre	1	7	5	6	3	3	3	4.0±1.7	MT
TBIO Convicto	5	3	2	2	2	2	2	2.6±0.8	T
TBIO Duque	10	5	7	4	11	9	11	8.1±2.4	MS
TBIO Sintonia	4	4	1	1	7	8	6	4.4±2.2	MT
TBIO Sossego	9	6	9	7	4	4	4	6.1±1.9	MS

†T, refers to a salinity-tolerant cultivar, having a mean rank ($\bar{R}$) score of 1 to 3.5; MT, moderately tolerant cultivar with a mean rank score of 3.6 to 6.0; MS, moderately sensitive cultivar with a mean rank score of 6.1 to 8.5; and S, salinity-sensitive cultivar with a mean rank score of 8.6 to 11. Abbreviations: G: germination; SL: shoot length; RL: root length; TL, total seedling length; SDM: shoot dry matter; RDM: root dry matter; TDM: total seedling dry matter.

the cultivars. The variation is corrected by calculating the average ranking with maximum values of 9.3 in Biotrigo Valente and a minimum of 2.6 in TBIO Convicto (Table 6). The values obtained from the average ranking allowed the classification of the cultivars into tolerant (TBIO Convicto), moderately tolerant (BRS 264, TBIO Aton, TBIO Calibre, and TBIO Sintonia), moderately susceptible (BIO 190038, BRS 404, ORS FEROZ, TBIO Duque, and TBIO Sossego), and susceptible only the cultivar Biotrigo Valente (Table 6).

Performance evaluation through UPGMA clustering

By using seven indices obtained from evaluating 11 wheat cultivars, two dendrograms were constructed, one for performance under water stress (Figure 1) and another for performance under salt stress (Figure 2).

When considering the grouping related to the performance of wheat cultivars under water stress, it is observed that with a dissimilarity degree of 3.8%, used as the cutoff point, four groups are formed (Figure 1). The formed groups separate the tolerant cultivars (TBIO Sintonia, TBIO Calibre, and TBIO Sossego), moderately tolerant only TBIO Duque, moderately susceptible (TBIO Convicto, BRS 264, and ORS Feroz), and susceptible (BIO 190038, Biotrigo Valente, BRS 404, and TBIO Aton) (Figure 1).

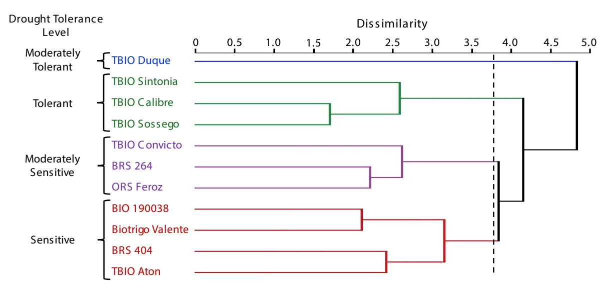

Figure 1 - Dendrogram of the hierarchical cluster analysis of 11 wheat [*Triticum aestivum* L.] cultivars using the Euclidean distance and the unweighted pair group method with arithmetic mean (UPGMA) based on the drought tolerance indices (GSTI, SLSI, RLSI, TLSI, SMSI, RMSI, and TMSI).

When considering the grouping related to the performance of wheat cultivars under salt stress, it is observed that with a dissimilarity degree of 3.7%, used as the cutoff point, four groups are formed (Figure 2). The groups formed in Figure 2 for salt stress have a different clustering compared to that observed for water stress (Figure 1), indicating that the cultivars exhibit distinct behaviors in response to the applied stress. The formed groups separate the tolerant cultivars (TBIO Convicto, TBIO Sintonia, TBIO Calibre, and TBIO Sossego), moderately tolerant (BIO 190038 and TBIO Aton), moderately susceptible (BRS 264, Biotrigo Valente, BRS 404, and ORS Feroz), and susceptible only TBIO Duque (Figure 2).

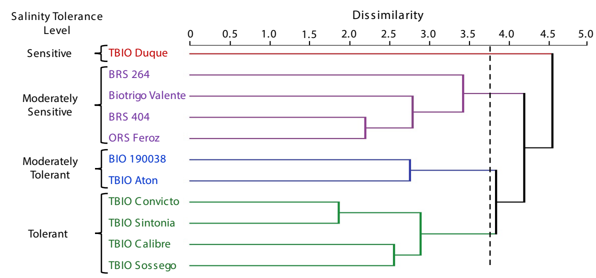

Figure 2 - Dendrogram of the hierarchical cluster analysis of 11 wheat [*Triticum aestivum* L.] cultivars using the Euclidean distance and the unweighted pair group method with arithmetic mean (UPGMA) based on the salinity tolerance indices (GSTI, SLSI, RLSI, TLSI, SMSI, RMSI, and TMSI).

Evaluation of the correlation between the assessed methods

From the results obtained with the seven indices, the ranking process and the UPGMA clustering method, correlations between the different methods were obtained for each applied stress [water stress (Figure 3A) and saline stress (Figure 3B)] to verify the best relationship between the results obtained from the different methods.

Figure 3A is related to water stress and shows the direct relationship between the ranking method and cluster analysis with a high correlation of 0.80 and significant ($p<0.01$), while both methods correlate with the TLSTI index with 0.82 and 0.74, both significant ($p<0.01$), respectively. The ranking method showed a high correlation (0.72, $p<0.05$)

with the SLSTI index, and the latter in turn has a high correlation with TLSTI (0.92, p<0.001), indicating that when assessing water stress, these two indices, due to their direct relationship, can be used interchangeably.

Figure 3B is related to salt stress and shows that under these conditions, the result obtained between the ranking method and cluster analysis demonstrates a moderate correlation of 0.55 and is significant (p<0.05). The ranking method shows a high correlation (0.89, p<0.001) with the RLSTI index (associated with root length), a moderate correlation with the TLSTI (0.75, p<0.01) and GSTI (0.68, p<0.05) indices. These correlations indicate that the ranking result is directly related to the ability to produce roots, the overall plant development, and the capacity to achieve good progress during the germination process. When observing the clustering method, it is noted that the correlation results with most indices are of positive magnitude and below 0.65 when combined with GSTI (p<0.05).

that there is variability in the responses for SLSTI, RLSTI, and TLSTI (Table 3). The index related to germination (GSTI) did not show a significant G×E interaction nor was it affected between the applied stresses (Table 2). Seed germination is directly associated with its physiological quality (Zuffo *et al.*, 2020). For wheat, the minimum germination percentage for commercialization is 80% (Brasil, 2013). Thus, the seeds used in our study exhibited excellent quality under different stresses, as they achieved values above 80%, with the exception of Biotrigo Valente with 78% under saline stress and ORS FEROZ with 75% under water stress (Table 2).

The seven indices described were used to establish two selection methods [ranking (Table 4 and Table 5) and grouping (Figure 1 and Figure 2)], while the relationship was verified through Pearson correlations (Figure 3) between the indices and the grouping methods, for each applied stress [water (Figure 3A) and saline (Figure 3B)].

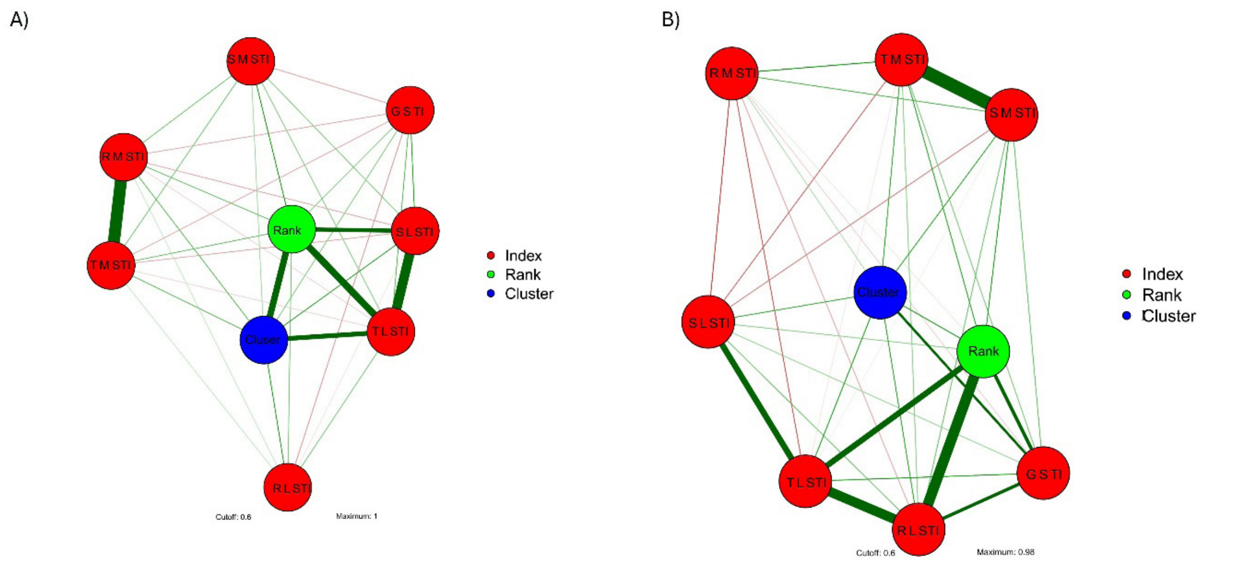

Figure 3 - Correlation network obtained by comparing 11 wheat cultivars (*Triticum aestivum* L.) based on indices (GSTI, SLSI, RLSI, TLSI, SMSI, RMSI, and TMSI) of tolerance to water stress (A) and saline stress (B), in combination with the ranking and dendrogram of the hierarchical cluster analysis.

DISCUSSION

The resilience of 11 wheat cultivars to drought and saline stresses during germination and early growth was evaluated by determining seven indices associated with plant growth, showing

In the literature, numerous selection strategies are carried out to make better recommendations of genotypes resistant to abiotic stresses, among them water deficit (Oliveira *et al.*, 2022, 2023; Argentel-Martinez *et al.*, 2024a; Ratke *et al.*, 2024) and salinity (Zuffo *et al.*, 2020). All these methods aim to select, in a more efficient way, the genotypes best adapted to stress conditions, where the main problem often lies in the recommendation of the best genotype, largely due to the variability in the response of genotypes across various environments or stress conditions, which complicates the recommendation.

Phenotyping for abiotic stress (drought and salinity) during developmental stages constitutes an excellent selection strategy and is applied in various crops such as wheat (Aguilera *et al*., 2019; Argentel-Martinez *et al*., 2024b, Ribeiro *et al*., 2024), tomato (Argentel-Martinez *et al*., 2024a, c), corn (Argentel-Martinez *et al*., 2024b), soybean (Steiner *et al*., 2021; Zuffo *et al*., 2023; Oliveira *et al*., 2022, 2023), cover crops (Zuffo *et al*., 2022), among other crops.

The results obtained show that there is variability in the responses of genotypes to each applied stress. In water stress, there is a direct relationship between the ranking method and cluster analysis with a high correlation of 0.80 ($p<0.01$), while both methods correlate with the TLSTI index at 0.82 and 0.74, respectively. Plant development was affected, with the drought stress imposed by the PEG 6000 solution reported to have a negative effect on seedling development (Thabet *et al*., 2018). Induced water stress causes oxidative damage, including increased H_2O_2 and O^- (ROS), generation of malondialdehyde (MDA), and higher levels of proline and soluble sugar (Guo *et al*., 2017; Argentel-Martinez *et al*., 2024b), which hinders seedling development (Steiner *et al*., 2021).

The high correlation obtained between both methods indicates that they can select with a high degree of accuracy, thus both methods recommend TBIO Sintonia and TBIO Calibre, however TBIO Sossego is recommended as tolerant by clustering (Figure 1) and moderately tolerant by ranking (Table 5). TBIO Sintonia is recommended as superior under soil flooding conditions for the number of spikelets, number of seeds, and for the seed mass attribute (Monteiro *et al*., 2023), as well as being indicated for favorable environments that simulate higher productivity (Rüdell *et al*., 2021). Recently, TBIO Sintonia was recommended for water and salt stress when evaluated for multiple stresses (Severo *et al*., 2022; Ribeiro *et al*., 2024), a result that aligns with that obtained in the present work.

The variability observed among wheat cultivars under drought and saline stress conditions may be associated with physiological mechanisms involved in stress tolerance. Under osmotic stress conditions, plants may maintain cell turgor through osmotic adjustment mediated by the accumulation of compatible solutes such as proline, soluble sugars, and glycine betaine (Farooq *et al*., 2009; Ghosh *et al*., 2021; Yang *et al*., 2021). These compounds help maintain cellular hydration and metabolic activity during water deficit conditions. Additionally, tolerance to salinity is often related to the capacity of plants to regulate ion homeostasis, particularly through mechanisms of Na^+ exclusion and compartmentalization in vacuoles, which reduce ionic toxicity in metabolically active tissues (Rehman *et al*., 2025). Differences in antioxidant defense systems may also contribute to cultivar variability, since abiotic stresses frequently induce the overproduction of ROS, which can damage cellular structures if not efficiently detoxified (Darko *et al*., 2017; Hussain *et al*., 2022).

Although PEG and NaCl solutions were adjusted to the same osmotic potential (−0.30 MPa), these agents impose different types of stress on plants. Polyethylene glycol primarily induces osmotic stress by reducing water availability without penetrating plant tissues, whereas NaCl causes both osmotic stress and ionic toxicity due to the accumulation of Na^+ and Cl^- ions in plant cells. Consequently, plant responses to PEG are mainly associated with water deficit tolerance mechanisms, while NaCl responses involve additional processes related to ion exclusion, ion compartmentalization, and maintenance of ionic balance. This difference may explain the distinct performance of some cultivars under drought and saline stress conditions observed in the present study. The contrasting responses observed among cultivars suggest differences in traits such as root growth capacity, water uptake efficiency, and early seedling vigor, as also reported by Zuffo *et al*. (2025a, b) in studies evaluating tropical wheat genotypes under abiotic stress conditions.

The ranking method shows a high correlation (0.89, $p<0.001$) with the index associated with root length, moderate correlation with the TLSTI (0.75, $p<0.01$) and GSTI (0.68, $p<0.05$) indices. Studies have linked the reduction in shoot and root growth in wheat seedlings to decreased water uptake by seeds due to salinity (Al-Tabbal & Al-Zboon, 2021), as well as the fact that seed germination speed can be delayed by salt toxicity under saline stress conditions, which affects cell division and differentiation, enzymatic activity, and nutrient uptake and distribution during the germination process (Rahman *et al.*, 2016). The allelic variability of the *TaALMT1* and *TaMATE1B* genes in a Brazilian wheat collection and its association with root growth in acidic soil has been demonstrated (Pereira *et al.*, 2015; Aguilera *et al.*, 2016). These reports highlight the potential of wheat genotypes regarding salinity resistance and position them as one of the best international sources.

In general, the strategy employed allows the selection of genotypes for each studied stress environment and, therefore, promising for implementation in areas characterized by the analyzed particularities. These genotypes can be recommended to compose breeding programs where current commercial cultivars can be improved with those selected for one or more abiotic stress environments. The proposed methods assist in the selection of wheat genotypes under control conditions and water and salt stress, due to the use of selection indices.

CONCLUSION

The results obtained under controlled conditions indicate variability among wheat cultivars in their responses to drought and saline stress during the early growth stage. Cultivars such as TBIO Sintonia and TBIO Calibre showed greater tolerance to osmotic stress, while TBIO Convicto presented better performance under saline conditions. Although these findings provide useful insights into the early-stage response of tropical wheat cultivars to abiotic stresses, additional studies under field conditions and different stress intensities are necessary to confirm the stability of these responses and their potential use in breeding programs.

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