Revista Ciência Agronômica, v. 54, e20218253, 2023
Centro de Ciências Agrárias - Universidade Federal do Ceará, Fortaleza, CE
www.ccarevista.ufc.br

Scientific Article
ISSN 1806-6690

Effect of combined thermal and water stress on germination of wheat seeds[1]

Combinações de estresses hídricos e térmicos no processo germinativo de sementes de trigo

Altamara Viviane de Souza Sartori[2], Carolina Maria Gaspar de Oliveira[3]*, Claudemir Zucareli[2], Alessandra Rodrigues Pereira[3], Címtia Sorane Good Kitzberger[3], Elizeu David dos Santos[2], Flávia Oliveira de Araújo[3]

ABSTRACT - Combined abiotic stresses have different effects and responses than exposure to a single source of stress. The objective of this study was to evaluate the effect of the combined stress of supra-optimal temperatures and water deficit on the germination of wheat seeds with different levels of vigor. Wheat seeds from four different lots of the IPR Catuara cultivar were germinated at water potentials of 0.000, –0.075, –0.223, and –0.444 MPa, associated with temperatures of 20 °C, 24 °C, 28 °C, and 32 °C, totaling 16 stress combinations. The experimental design was completely randomized in a factorial scheme with four replications (four seed lots × 16 stress combinations), and means were compared using Scott-Knott 5%. We evaluated the germination of normal seedlings at 4 and 8 d, the activity of catalase and ascorbate peroxidase (APX), and the protein content of the aerial and root parts of wheat seedlings. Principal components analysis and hierarchical clustering were applied to the original matrix formed by the 16 combinations for the average of the four lots. Wheat seeds with higher vigor were more tolerant to combined water and thermal stress. Temperatures up to 28 °C and reduction of water potentials down to –0.223 MPa did not prejudice the germination process of wheat seeds. Regardless of temperature, a water potential of –0.444 MPa decreased the percentage and rate of germination. APX activity increased under water stress and supra-optimal temperatures, acting as a defense mechanism against oxidative stress.

Key words: *Triticum aestivum* L.. High temperature. Water deficit. Multiple stresses.

RESUMO - Estresses abióticos associados causam efeitos e respostas diferentes da exposição a uma única fonte de estresse. Objetivou-se avaliar o efeito do estresse combinado de temperatura supraótima e déficit hídrico no processo germinativo de lotes de sementes de trigo com diferentes níveis de vigor. Sementes de trigo IPR Catuara de quatro lotes foram submetidas a germinação nos potenciais hídricos 0,000, - 0,075, - 0,223 e - 0,444 MPa, associados às temperaturas 20, 24, 28 e 32 ºC, totalizando 16 combinações de estresse. O delineamento experimental foi inteiramente casualizado, com quatro repetições, em esquema fatorial: quatro lotes x 16 combinações de estresse, e comparação de médias por Scott-Knott 5%. Avaliaram-se a germinação das plântulas normais aos quatro e oito dias, o conteúdo de proteína total e a atividade da catalase e ascorbato peroxidase (APX) na parte aérea e radicular das plântulas. As análises de componentes principais e de agrupamento hierárquico foram aplicadas à matriz original formada pelas 16 combinações, para a média dos quatro lotes. Sementes de trigo de maior vigor são mais tolerantes a combinação dos estresses hídrico e térmico. Não há prejuízo ao processo germinativo de trigo nas combinações sob temperaturas supraótimas até 28 °C e redução dos potenciais hídricos até -0,223 MPa. Independente da temperatura, o potencial hídrico -0,444 MPa diminui a porcentagem e a velocidade da germinação. A atividade da APX aumenta em condições de estresses hídrico e térmico atuando como defesa ao estresse oxidativo na germinação de trigo.

Palavras-chave: *Triticum aestivum* L.. Alta temperatura. Déficit hídrico. Estresses múltiplos.

DOI: 10.5935/1806-6690.20230003
Editor-in-Article: Prof. Salvador Barros Torres - sbtorres@ufersa.edu.br
*Author for correspondence
Received for publication on 27/10/2021; approved on 06/05/2022

[1] Part of the dissertation of the first author, presented to the graduate program in Agronomy of the Universidade Estadual de Londrina

[2] Department of Agronomy, State University of Londrina, Universidade Estadual de Londrina (UEL), Londrina-PR, Brazil, maravyvyanne@gmail.com (ORCID ID 0000-0002-7138-3729), claudemircca@uel.br (ORCID ID 0000-0002-5260-0468), elizeudavid.santos@uel.br (ORCID ID 0000-0003-2055-6746)

[3] Paraná Rural Development Institute (IAPAR-EMATER), Londrina-PR, Brazil, carolina@idr.pr.gov.br (ORCID ID 0000-0001-6908-1601), alessandrapereira53@hotmail.com (ORCID ID 0000-0001-6512-3325), cintia_kitzberger@idr.pr.gov.br (ORCID ID 0000-0001-7506-8761), flaviaodearaujo@gmail.com (ORCID ID 0000-0002-2668-8320)

INTRODUCTION

The germination and initial growth of plants are affected by several abiotic stressors that occur during the initial phase of plant development. Soil water availability is one of the factors affecting the initiation of germination and is directly and indirectly involved in all subsequent metabolic processes (AL-SHEBLY; AL-EBRAHEMI; ALI, 2020). Thus, a lack of water during the seed imbibition phase can change the rate, percentage, and uniformity of germination (GRZYBOWSKI *et al*., 2019; VIÇOSI *et al*., 2017). Temperature acts on the biochemical reactions that govern the entire germination process; the temperature range varies with the species, which, in the face of other ideal conditions, provides maximum germination and seedling emergence in the shortest time (AL-SHEBLY; AL-EBRAHEMI; ALI, 2020). The optimal temperature recommended for wheat seed germination is 20 °C (BRASIL, 2009).

Restriction in water availability is also associated with high temperatures, and the simultaneous combination of these factors, rather than individually, has more negative effects on physiological processes (AHMED *et al*., 2013). High temperatures associated with drought are the most important environmental factors that can reduce plant survival and crop productivity (PRAMANIK; SIKDER, 2020). Although several studies on abiotic stress in wheat have been conducted (ALMAGHARABI, 2012; AL-SHEBLY; AL-EBRAHEMI; ALI, 2020; BURIRO *et al*., 2011), relatively little information is available on the behavior of wheat seed germination and initial seedling growth under conditions of combined water deficit and heat stress (HAMPSON; SIMPSON, 1990).

The initiation of germination is associated with an increase in mitochondrial respiration, which naturally produces reactive oxygen species (ROS) that are continuously eliminated by a continuous antioxidant defense system (EL-MAAROUF-BOUTEAU; BAILLY, 2008). In response to abiotic stress, metabolic changes cause the accumulation of ROS, resulting in oxidative stress, which causes damage to cell membranes (lipid peroxidation), proteins, RNA, and DNA molecules, thus culminating in cell death (MIGNOLET-SPRUYT *et al*., 2016) and impairing the germination process and the formation of normal seedlings.

One of the plants' immediate responses to oxidative stress is to remove ROS using biochemical and molecular mechanisms, including the stimulation of enzymatic and non-enzymatic antioxidant systems that play a key role in ROS detoxification (MIGNOLET-SPRUYT *et al*., 2016). The intensity of cell damage depends on the efficiency of the plants in activating antioxidant enzymes (ESPOSITO *et al*., 2018). Among the enzymes capable of acting in favor of the antioxidant defense system, ascorbate peroxidase and catalase are prominent. Thus, plant species that respond to oxidative stress with increased enzymatic activity and ROS neutralization are able to tolerate stress and maintain their development (GILL; TUTEJA, 2010; ZHENG *et al*., 2009).

Evaluation of the response of seeds and seedlings to abiotic stress, whether during drought periods and/or under high temperature conditions, facilitates better elucidation of the strategies adopted by seeds to maintain the germination and establishment of seedlings. The objective of this study was to evaluate the effect of the combined stress of supra-optimal temperature and water deficit on the germination process of wheat seed lots with different vigor levels, and to evaluate the activity of the defense system in the subsequent seedlings.

MATERIAL AND METHODS

The study was carried out at the Instituto de Desenvolvimento Rural do Paraná – IAPAR-EMATER (IDR-Paraná) in Londrina-PR. Four lots of wheat seeds from the IPR Catuara cultivar (RNC/MAPA, Nº. 27495), harvested in 2019 (L1, L2, L3, and L4), were provided by IDR-Paraná, with initial germination within the commercialization standards for the species. The lots were previously characterized according to their physical and physiological qualities using the following tests:

1000-seed weight: Eight replications of 100 seeds each were weighed on a balance (precision 0.001 g), and the results were expressed in grams per sample (BRASIL, 2009), with adjustment of the water content to 13%.

Seed water content: This was determined by the oven method at 105 ± 3 °C for 24 h using two replications of 100 seeds each (BRASIL, 2009), and the results were expressed as percentages (fresh weight basis).

Germination: This was performed with four replications of 50 seeds each, sown in rolls of paper towels moistened with water in the amount of 2.5 times the weight of the dry paper. The rolls were maintained in a germinator at 20 °C. The percentage of normal seedlings on the fourth (first count - PC) and eighth (total germination - G) days after initiation of experiment was evaluated after subtracting the total number of abnormal seedlings and dead seeds (BRASIL, 2009).

Seedling emergence was conducted with four replicates of 50 seeds, distributed at a depth of 1 cm, in plastic boxes (11 × 11 × 3.5 cm) containing washed sand. Daily counts of the number of emerged seedlings (greater than 5 mm) were performed until stabilization, which occurred between 7 and 11 d after initiation of experiment, and the emergence percentage was determined. The formula proposed by Maguire (1962) was used to calculate the emergency speed index (IVE).

Data from tests of initial characterization of physical and physiological parameters were subjected to analysis of variance and comparison of means using the Scott-Knott test at 5% (Table 1). The lots showed similar germination rates according to the results of the initial characterization. However, there were differences in vigor, as verified by the tests of first count, emergence in sand, emergence speed index, and mass of a thousand seeds, with lots 2 and 3 being more vigorous than lots 1 and 4.

To evaluate the effect of combined stress of supra-optimal temperature and water deficit on germination and on the activity of the seedling defense system, wheat seeds from the four lots were germinated following the same procedures as those described for the germination test, in 16 combinations of temperature and water stress. For water stress, the substrate paper was moistened with solutions of different concentrations of polyethylene glycol 6000 (PEG 6000) to simulate predetermined water potentials: 0.000 (control, distilled water), –0.075, –0.223, and –0.444 MPa (MICHEL; KAUFMANN, 1973). The PEG solutions were prepared by diluting PEG 6000 in distilled water at 20 °C. For temperature stress, the seeds were subjected to germination at temperatures of 20 °C, considered optimal (BRASIL, 2009), and at temperatures of 24 °C, 28 °C, and 32 °C, considered supra-optimal temperatures.

The stress combinations were evaluated by the percentage of normal seedlings on the fourth (first count - PC) and eighth (total germination - G) days after initiation of experiment (BRASIL, 2009), and by protein content, catalase (CAT) enzyme activity, and ascorbate peroxidase (APX) activity from wheat seedlings collected 8 d after initiation of experiment.

The crude extracts for the analysis of protein content and CAT and APX activities were obtained by separating the wheat seedlings into shoots and roots, with the removal of approximately 2 g of the sample. The plant material was then packed in paper bags, frozen in liquid nitrogen, and stored in an ultra-freezer at –80 °C. Subsequently, 0.5 g of plant material was macerated in 10 mL of 50 mM potassium phosphate buffer (pH 7.0) and 1% (w/v) of polyvinylpolypyrrolidone, previously cooled, and centrifuged for 10 min at 785.4 rad sec^{-1} at 4 °C. The supernatant was transferred to 2-mL Eppendorf's microtubes and kept in a freezer at –14 °C until analysis in an Evolution 300 UV-VIS spectrophotometer to read the reactions.

Total soluble protein aliquots of 30 µL (shoot) and 120 µL (roots) of the extracts were transferred to test tubes, and 570 and 480 µL of distilled water were added, respectively, followed by 2.4 mL of Bradford reagent (BRADFORD, 1976). After stirring, the absorbance was measured at 595 nm wavelength. The concentration of total protein was obtained by comparing the readings with those obtained from a standard curve obtained with bovine serum albumin (BSA 0–15 µg µL^{-1}). The results were expressed in mg of protein per g of fresh matter (FM).

CAT and APX activities were determined according to the methodology proposed by Peixoto *et al.* (1999) with modifications. For CAT, 200 µL of the enzyme extract was homogenized with 2.8 mL of 50 mM potassium phosphate buffer (pH 7.0 + 0.1 mM EDTA) containing 15 mM oxygen peroxide (0.5 M). Specific CAT activity was determined by following the decrease in absorbance at 240 nm in Kinect mode with eight cycles of 30 s, using an extinction coefficient of H_2O_2 of 36.0 M^{-1} cm^{-1} nm. The enzyme activity was expressed in mmol H_2O_2 min^{-1} mg protein^{-1} (CAT) when considering the protein content and in mmol H_2O_2 min^{-1} g FM^{-1} (CAT MF) when expressed as fresh mass. For APX activity, 100 µL of enzyme extract was homogenized with 2.8 mL of 50 mM potassium phosphate buffer containing 0.5 mM L-ascorbic acid. The reading of the enzyme activity was started after the addition of 100 µL H_2O_2 1.0 mM to the reaction medium, observing the decrease in the reading in the

Table 1 - Characterization of physical and physiological quality from four wheat seed lots, cultivar IPR Catuara

Tests	Lots				CV (%)
	L1	L2	L3	L4	
1000-seed weight (g)	52 a*	50 b	47 c	51 b	2.32
Seed Water content (%)	10.9	10.5	10.4	11.0	1.57
Germination first count (%)	92 b	99 a	97 a	97 a	2.98
Total Germination (%)	96	99	97	97	2.31
Emergency speed index	15 a	14 a	15 a	13 b	8.21
Seedlings emergency (%)	94 b	100 a	100 a	96 b	1.87

* Averages followed by the same lowercase letter in the row do not differ statistically from each other by the Scott-Knott averages test at 5% probability. CV = coefficient of variation

interval of 15 s for 2 min at an absorbance of 290 nm. The calculation of APX activity considered an extinction coefficient of 2.8 mM^{-1} cm^{-1}, and the values were expressed in UA min^{-1} g FM^{-1} (unit-activity - UA).

The experimental design was completely randomized with four replications. The analysis of variance was performed in a 4 × 16 factorial, with four lots with different levels of initial seed vigor and 16 combinations of temperature (T) and water potentials (ψ_h), with the comparison of means by the Scott-Knott test at 5 % probability. Principal component analysis (PCA) was applied to the original matrix formed by the 16 stress combinations, considering the average of the four lots to simultaneously verify the effect of the combined stress. For the PCA, the variations of catalase expressed in protein content (CAT) and fresh mass (CAT MF) were used, considering the importance of removing the effect of protein content from the catalase calculation in order to verify only the effect of enzyme activity on fresh matter. A hierarchical cluster analysis was performed using Euclidean distance and Ward's algorithm to identify the formation of groups between the stress combinations using the statistical software XLStat (ADDINSOFT, 2010).

RESULTS AND DISCUSSION

The analysis of variance carried out to evaluate the effect of the combined stress of supra-optimal temperature and water deficit on the germination process and on the activity of the stress defense system of seedlings of wheat seed cultivar IPR Catuara from lots with different vigor showed significant interactions between stress combinations (thermal and water) and seed lots with different vigor levels (Table 2), indicating that the physiological potential of the seeds altered their response to the stress combinations.

The difference in vigor between seed lots reflects the ability of seeds to germinate and produce normal seedlings under adverse conditions, and its influence is important in all aspects of the germination process, resulting in rapid and uniform germination in a wide range of environments (SIMONI *et al*., 2011). Grzybowski *et al.* (2019) also found that seeds with lower vigor were more affected by water deficit, probably because of their deterioration and inability to tolerate the effects of abiotic stresses.

Regarding total germination (Table 3), it is important to highlight the stress combinations that resulted in a lower percentage of normal seedlings compared to the ideal conditions of temperature (°C) and water potential (MPa) for the germination test of the species (BRASIL, 2009), represented by the combination 20/0.000. Thus, there was lower germination in all lots in the combinations 32/–0.075, 32/–0.223, 20/–0.444, 24/–0.444, 28/–0.444, and 32/–0.444. The combination 32/0.000 resulted in lower germination only for lots L1 and L4, and the combinations of temperatures of 20 °C, 24 °C, and 28 °C at –0.223 MPa only resulted in lower germination for L4.

Considering the effect of temperature only, the combinations with water potential of 0.000 MPa exhibited no reduction in the percentage of germination at all temperatures, until at 28 °C for all lots. At 32 °C, only lots L2 and L3 maintained a germination percentage similar to the one at 20 °C, indicating the importance of vigorous seeds in situations of more accentuated stress (Table 3). This reduction in germination for L1 and L4 was due to the negative effects of high temperature on cell metabolism, resulting in changes in the pattern of protein synthesis, which inactivates enzymes, damages membranes, and impairs cell division process

Table 2 - Analysis of variance of four wheat seed lots, cultivar IPR Catuara, with different vigor levels under 16 stress combinations (temperature and water potential)

Sources	Df	Mean Square							
		First Cont.	Germ	PTN Sh	PTN R	CAT Sh	CAT R	APX Sh	APX R
Lot	3	2164.889323*	1753.358073*	2.137588*	0.894458*	0.000943	0.018888*	131.874459*	26.397230*
Comb	15	12043.285156*	1846.055990*	3.625912*	0.184060*	0.010607*	0.014056*	216.338711*	76.914901*
Lot*Comb	45	152.250434*	119.049740*	1.444589*	0.166720*	0.005011*	0.008788*	25.668534*	18.884239*
Residuals	192	32.040365	19.511719	0.17176	0.013161	0.000509	0.000454	4.325838	4.162088
Total	255								
CV (%)		7.17	4.95	15.72	14.63	15.63	26.19	17.63	22.03
Mean		78.99	89.27	2.64	0.78	0.14	0.08	11.8	9.26

* significant at 5%. Comb = combinations; CV = coefficient of variation; Df = degrees of freedom; Cont. = First counting of germination; Germ = total germination; PTN Sh = total protein shoots; PTN R = total protein roots; CAT Sh = catalase shoots; CAT R = catalase roots; APX Sh = ascorbate peroxidase shoots; APX R = ascorbate peroxidase roots

Table 3 - Total germination (%) of four wheat seed lots, from cultivar IPR Catuara, under different 16 stress combinations of temperature (T, ºC) e and water potentials (ψ_h, MPa)

Combinations (T/ψ_h)	Lots (L)			
	L1	L2	L3	L4
20/0.00	96.5 Aa*	99.5 Aa	97.5 Aa	97.0 Aa
24/0.00	95.5 Aa	99.5 Aa	99.5 Aa	94.5 Aa
28/0.00	91.0 Ab	98.0 Aa	97.5 Aa	91.5 Ab
32/0.00	83.8 Bb	97.5 Aa	96.0 Aa	86.0 Bb
20/–0.075	94.5 Aa	98.5 Aa	96.0 Aa	94.0 Aa
24/–0.075	92.0 Aa	98.5 Aa	95.0 Aa	95.5 Aa
28/–0.075	91.5 Ab	98.5 Aa	98.5 Aa	93.5 Ab
32/–0.075	82.5 Bb	94.5 Ba	92.0 Ba	86.0 Bb
20/–0.223	89.5 Ab	97.5 Aa	98.0 Aa	89.5 Bb
24/–0.223	88.0 Ab	96.0 Aa	96.5 Aa	85.0 Bb
28/–0.223	93.5 Aa	95.5 Aa	98.5 Aa	88.5 Bb
32/–0.223	91.5 Aa	94.0 Ba	92.5 Ba	76.8 Cb
20/–0.444	86.5 Ba	87.0 Ca	83.0 Da	78.0 Cb
24/–0.444	78.5 Bb	92.0 Ba	91.5 Ba	80.5 Cb
28/–0.444	81.0 Bb	92.5 Ba	88.0 Ca	70.5 Dc
32/–0.444	42.0 Cc	60.8 Cb	79.0 Da	30.0 Ed

* Means followed by the same uppercase letter in the column and lowercase letter in the row do not differ from each other by the Scott-Knott test at 5% probability

(BURIRO *et al*., 2011). Buriro *et al.* (2011) found that at 20 °C and 30 °C, the wheat seeds maintained germination and vigor. Otherwise, Souza and Ramalho (2001) observed a reduction in the germination of wheat seeds under high soil temperatures at an average of 27 °C. They highlighted that every carbon source is dependent on the reserve of this element in the endosperm of the seed, and when temperatures are high, much of the carbon is lost through respiration.

When the effect of water stress was verified only at the optimal temperature for the species (20 °C), a decrease in germination was observed only at a water potential of –0.223 MPa for lots L1, L2, and L3, and for L4, only at a water potential of –0.075 MPa. Under the most drastic water stress, –0.444 MPa, the germination process was compromised in all the lots (Table 3). These results agree with those in the literature indicating that the availability of water is one of the essential factors for the initiation of germination as it is involved in all stages of germinal metabolism, including cell cycle reactivation and growth (PARTHEEBAN *et al*., 2017; VIÇOSI *et al*., 2017). Drastic limitations of this factor can compromise germination performance, even in vigorous seed lots. Almagharabi (2012) did not observe shoot growth in wheat seedlings at water potentials lower than –0.444 MPa. In addition, several studies have demonstrated a decrease in germination under water stress, such as Partheeban *et al.* (2017) in corn and Viçosi *et al.* (2017) in corn, soybeans, and beans.

Germination was reduced for all the lots under both stresses when subjected to extreme conditions (32 °C and –0.444 MPa), and this combination resulted in additive effects, indicating the restriction of the seed germination process and establishment of wheat seedlings. Supra-optimal temperatures, together with water stress, reduced metabolic activity and germination rate, causing a decrease in germination and an increase in abnormal seedlings and dead seeds in all the evaluated lots (AHMED *et al.*, 2013), justifying the additive effect observed. In one of the first studies with combined stresses in wheat, Hampson and Simpson (1990) found that at temperatures below 10 °C and above 30 °C, and at osmotic potentials below –0.3 MPa, germination was reduced, delayed, and eventually inhibited. Temperature stress intensified the effect of osmotic stress on germination and vice versa; this is in line with the results obtained in our study.

When one of the factors was in the ideal condition, in other words, temperature of 20 °C or water potential of 0.000 Mpa seeds with lower vigor exhibited lower performance than those with higher vigor, and this behavior is even more limited under combined abiotic stress conditions, possibly because less vigorous seeds tend to have greater difficulty in overcoming adverse conditions, consequently demonstrating lower performance (GRZYBOWSKI *et al*., 2019), especially under multiple stress conditions.

Similar to the germination results, in the first count, the percentage of normal seedlings decreased with decreasing water potential and increasing temperature (Table 4). Considering that this test is used to evaluate the vigor of seed lots by rate of germination, seed germination (Table 3) was not only affected by the combination of stresses but also by the speed of the process (Table 4).

In the combinations of supra-optimal temperature and water potential of 0.000 MPa, there was no reduction in germination rate until 28 °C for all the lots. At 32 °C, only the L2 and L3 lots, with greater vigor, presented values statistically similar to those at 20 °C, which is the standard temperature for germination tests in wheat seeds (BRASIL, 2009).

In the combination of water stress at 20 °C, there was a reduction in the germination rate with decrease in water potential, highlighting that at –0.444 MPa, none of the lots presented normal seedlings in the first count of the germination test. Otherwise, germination was observed under the combination of increasing temperatures at constant water potentials; however, the values were below those obtained in the combination 20/0.000 (°C/MPa), which is the ideal condition for the germination of wheat seeds (BRASIL, 2009). The results showed that a decrease in water potential resulted in a lower germination rate. Hence, of note, when the seeds germinate faster, they are less exposed to adverse field conditions, ensuring better establishment of the crop.

Water stress in wheat seeds, in addition to affecting imbibition, germination rate, and germination percentage, impairs germinative development of seeds and seedling vigor (DHANDA; SETHI; BEHL, 2004; GIROTTO *et al*., 2012). Water promotes the softening of the tegument and benefits the penetration of oxygen, providing the development of the embryo and reserve tissues, stimulating basic metabolic activities, and favoring the growth of the embryonic axis (AL-SHEBLY; AL-EBRAHEMI; ALI, 2020).

Water stress delays germination, as it reduces the flow of water to the cell, until the extreme point at which water diffusion through the symplast interrupts the process, resulting in a reduction in germination rate and the percentage of germinated seedlings (VIÇOSI *et al*., 2017). In the germination process, the most negative water potentials, especially at the beginning of imbibition, influence water absorption by seeds, making the sequence of events, such

Table 4 - First counting of the germination test (%) of four wheat seed lots, from cultivar IPR Catuara, under different 16 stress combinations of temperature (T, ºC) e and water potentials (ψ_h, MPa)

Combinations (T/ψ_h)	Lots (L)			
	L1	L2	L3	L4
20/0.00	92.0 Aa*	99.0 Aa	97.5 Aa	97.0 Aa
24/0.00	95.0 Aa	99.5 Aa	99.5 Aa	92.0 Aa
28/0.00	90.5 Aa	98.0 Aa	97.5 Aa	91.5 Aa
32/0.00	76.0 Bc	97.5 Aa	94.0 Aa	84.5 Bb
20/–0.075	88.0 Ab	97.0 Aa	86.0 Bb	72.0 Cc
24/–0.075	88.5 Aa	98.0 Aa	95.0 Aa	95.5 Aa
28/–0.075	91.5 Aa	98.5 Aa	98.5 Aa	93.5 Aa
32/–0.075	80.0 Bb	94.5 Aa	92.0 Aa	86.0 Bb
20/–0.223	89.5 Ab	97.5 Aa	98.0 Aa	89.5 Bb
24/–0.223	88.0 Ab	96.0 Aa	96.5 Aa	85.0 Bb
28/–0.223	93.5 Aa	95.5 Aa	98.5 Aa	88.5 Ba
32/–0.223	91.5 Aa	94.0 Aa	92.5 Aa	76.8 Cb
20/–0.444	0.0 Fa	0.0 Ea	0.0 Da	0.0 Fa
24/–0.444	40.5 Dc	56.0 Cb	62.0 Ca	51.5 Db
28/–0.444	66.0 Cb	79.5 Ba	84.0 Ba	54.0 Dc
32/–0.444	24.5 Ec	34.0 Db	61.5 Ca	15.5 Ed

* Means followed by the same uppercase letter in the column and lowercase letter in the row do not differ from each other by the Scott-Knott test at 5% probability

as the breakdown of reserves and cell division, unfeasible. In addition, delay and decrease in the percentage and rate of germination presented by Partheeban *et al*. (2017) are in line with the results obtained in this study for the lowest water potential evaluated (–0.444 MPa).

The combinations of 24/–0.075 and 28/–0.075 MPa resulted in higher germination speed than at 20/–0.075 (°C/MPa) for seeds from lots L1, L3, and L4 (Table 4), demonstrating that supra-optimal temperatures contribute to mitigating the negative effect of the lack of water on germination speed. These temperatures accelerate not only the physical hydration process but also the breakdown of reserves and embryo growth (ZIMMER, 2012). An increase in temperature increases water energy and diffusion pressure, increases metabolic activity, and decreases the internal potential of the seed, allowing greater water absorption and accelerating the germination process (ESSEMINE, AMMAR; BOUZID, 2010).

However, the first count and germination for all stress combinations showed significantly lower values at 32 °C, demonstrating that this temperature affected the behavior of the lots, especially those with lower vigor, thus limiting wheat seed germination. Ribeiro and Borghetti (2014) also found a positive effect of adequate temperatures on the seed germination process but highlighted that there is an optimal limit for each species.

Shoot and root protein content did not show a response pattern in the values as a result of the increase in temperature and/or decrease in water potential (Table 5).

During germination, reserve proteins are consumed and used as a source of energy for the development of the seedling (BURIRO *et al*., 2011) and therefore, a lower protein content is expected in the seedlings grown under the stress combinations that exhibit a higher percentage of germination (Table 3). However, here, this was not the case, and one of the reasons is that in addition to being a reserve for the embryo and subsequent seedling development, proteins are involved in protection against different biotic and abiotic stresses by, for instance, protecting cellular and subcellular structures against oxidative damage (RODRIGUES *et al*., 2019). In addition, reserve mobilization is completely altered at negative water potentials, as the low availability of water affects the translocation of metabolized products from the scutellum to the embryo, consequently changing the manner in which germination occurs (TEIXEIRA *et al*., 2011).

Table 5 - Total protein content (mg protein g FM^{-1}) of shoots and roots of wheat seedlings from cultivar IPR Catuara, of four lots (L) under different 16 stress combinations of temperature (T, ºC) e and water potentials (ψ_h, MPa)

(T/ψ_h)	Shoots							
	L1	L2	L3	L4	L1	L2	L3	L4
	Protein content							
20/0.000	1.39 Db*	2.16 Da	2.34 Ea	2.43 Ba	0.88 Ca	0.64 Cb	0.85 Aa	0.86 Ca
24/0.000	2.48 Ba	2.53 Da	1.59 Fb	2.19 Ca	1.23 Aa	0.23 Dd	0.65 Bc	1.05 Bb
28/0.000	3.17 Aa	2.87 Ca	2.14 Eb	2.84 Aa	0.61 Db	0.82 Ba	0.69 Bb	0.58 Db
32/0.000	2.22 Cb	2.43 Db	2.95 Da	1.98 Cb	0.54 Dc	0.77 Bb	0.75 Bb	0.93 Ca
20/–0.075	3.13 Aa	2.21 Db	2.76 Da	2.62 Ba	0.76 Ca	0.50 Cb	0.61 Bb	0.85 Ca
24/–0.075	2.29 Ba	1.48 Eb	2.48 Ea	1.54 Cb	0.67 Da	0.62 Ca	0.63 Ba	0.73 Da
28/–0.075	1.68 Dc	3.37 Cb	4.73 Aa	2.80 Ab	0.84 Ca	0.56 Cb	0.59 Bb	0.83 Ca
32/–0.075	2.16 Cb	3.01 Ca	2.25 Eb	2.23 Cb	1.00 Ba	0.79 Ba	0.90 Aa	0.91 Ca
20/–0.223	2.86 Ab	3.12 Cb	3.79 Ba	3.39 Aa	0.79 Cb	0.76 Bb	0.76 Bb	1.01 Ba
24/–0.223	2.72 Ba	1.77 Eb	1.72 Fb	2.59 Ba	0.39 Ec	0.36 Dc	0.76 Bb	0.93 Ca
28/–0.223	2.60 Bc	4.62 Aa	3.25 Cb	2.50 Bc	1.00 Ba	1.00 Aa	0.92 Aa	0.74 Db
32/–0.223	1.80 Db	3.15 Ca	2.56 Ea	2.59 Ba	0.60 Dc	0.48 Cc	0.92 Ab	1.33 Aa
20/–0.444	2.92 Aa	3.54 Ca	2.91 Da	3.19 Aa	0.78 Ca	0.39 Db	0.95 Aa	0.90 Ca
24/–0.444	2.34 Bb	2.92 Ca	1.34 Fc	2.08 Cb	0.55 Dc	0.77 Bb	0.75 Bb	1.19 Aa
28/–0.444	2.41 Bc	3.97 Ba	3.94 Ba	3.24 Ab	0.25 Ec	0.84 Bb	1.01 Aa	1.08 Ba
32/–0.444	2.49 Bb	2.58 Db	2.13 Eb	3.30 Aa	0.98 Bc	1.07 Ab	0.92 Ac	1.15 Aa

* Means followed by the same uppercase letter in the column and lowercase letter in the row do not differ from each other by the Scott-Knott test at 5% probability

The protein content related to antioxidants changes significantly during the stress process, with some levels of protein expression being increased, while others are decreased (RODRIGUES *et al*., 2019). The lower levels of protein at lower water potentials can be attributed to the fact that water scarcity reduces the ability of plant enzymes to carry out protein synthesis, leading to a lower protein content in the plant (AL-SHEBLY; AL-EBRAHEMI; ALI, 2020).

Under conditions of supra-optimal temperature, when wheat is subjected to heat stress, there is a reduction in protein synthesis, influencing their inhibition and degradation; however, some proteins, called heat shock proteins (HSPs), are induced, often improving performance when exposed to high temperatures (BURIRO *et al*., 2011; XU, ZHAN; HUANG, 2011). These proteins, along with their heat shock transcription factors (HSFs), are involved in the molecular mechanisms underlying high-temperature tolerance. Essemine, Ammar and Bouzid (2010) also showed a decrease in the total protein content of wheat seeds after 22 h of germination at high temperatures, as the degradation of normal proteins is faster and vital compared to the synthesis of HSPs.

There was a significant interaction between the combinations of stresses (thermal and water) and seed lots for catalase (CAT) (Table 6) in both the shoots and roots of wheat seedlings, indicating that the lots responded differently to the stress combinations; however, it is not possible to infer that the vigor of the lots had an influence on CAT content.

CAT activity was higher in the shoots than in the roots of wheat seedlings. In addition, in the shoots, it was possible to verify the increase in CAT activity when exposed to the combined stresses of water restriction and high temperature, compared with the combination that simulates the ideal conditions for wheat germination (20 °C/ 0.000 MPa). The highest CAT values were obtained for the combinations 24/–0.223, 24/–0.075, 24/–0.444, 32 /–0.075, 32/ 0.000, and 32/–0.223 (Table 5).

Despite the records of greater activation of CAT under stress conditions (BARAKAT *et al.*, 2013), there is also evidence of a negative regulation by increasing the intensity of stress or the maintenance of activity without significant change (MHAMDI; NOCTOR; BAKER, 2012), as recorded in this study for the combination of 32 °C and –0.444 MPa, which, despite having similar CAT values to other combinations, had the lowest percentage of germination (Table 3).

Table 6 - Catalase activity (mmol H_2O_2 min^{-1}mg protein^{-1}) of wheat seedlings from cultivar IPR Catuara (shoots and roots) of four lots (L) under different 16 stress combinations of temperature (T, ºC) e and water potentials (ψ_h, MPa)

(T/ψ_h)	Shoots				Roots			
	L1	L2	L3	L4	L1	L2	L3	L4
	Catalase							
20/0.00	0.12 Da*	0.11 Ca	0.12 Ca	0.11 Ba	0.05 Ea	0.07 Da	0.06 Ba	0.07 Aa
24/0.00	0.06 Fb	0.15 Bb	0.20 Aa	0.17 Ab	0.31 Aa	0.28 Aa	0.07 Bb	0.06 Bb
28/0.00	0.13 Db	0.14 Bb	0.21 Aa	0.15 Ab	0.06 Eb	0.07 Db	0.11 Aa	0.07 Ab
32/0.00	0.18 Ba	0.17 Aa	0.11 Cb	0.19 Aa	0.09 Ea	0.05 Ea	0.07 Ba	0.06 Ba
20/–0.075	0.10 Eb	0.18 Aa	0.09 Db	0.08 Cb	0.06 Eb	0.12 Ca	0.07 Bb	0.06 Bb
24/–0.075	0.16 Ca	0.19 Aa	0.17 Ba	0.17 Aa	0.08 Ea	0.07 Da	0.06 Ba	0.06 Ba
28/–0.075	0.22 Aa	0.13 Bc	0.08 Dd	0.17 Ab	0.06 Eb	0.06 Db	0.10 Aa	0.07 Ab
32/–0.075	0.18 Ba	0.14 Ba	0.17 Ba	0.17 Aa	0.06 Ea	0.05 Ea	0.08 Ba	0.05 Ba
20/–0.223	0.07 Fb	0.13 Ca	0.07 Db	0.07 Cb	0.04 Ea	0.07 Da	0.06 Ba	0.07 Aa
24/–0.223	0.15 Cb	0.21 Aa	0.21 Aa	0.13 Bb	0.12 Db	0.15 Ba	0.09 Ac	0.05 Bd
28/–0.223	0.13 Db	0.12 Cb	0.14 Cb	0.17 Aa	0.06 Ea	0.03 Eb	0.04 Bb	0.09 Aa
32/–0.223	0.20 Ba	0.12 Cc	0.16 Bb	0.17 Ab	0.15 Ca	0.10 Cb	0.07 Bc	0.04 Bd
20/–0.444	0.10 Ea	0.12 Ca	0.12 Ca	0.12 Ba	0.06 Eb	0.13 Ba	0.07 Bb	0.05 Bb
24/–0.444	0.13 Dc	0.13 Bc	0.23 Aa	0.18 Ab	0.10 Da	0.08 Da	0.10 Aa	0.04 Bb
28/–0.444	0.15 Ca	0.10 Cb	0.12 Cb	0.14 Ba	0.25 Ba	0.06 Db	0.04 Bb	0.06 Bb
32/–0.444	0.16 Ca	0.15 Ba	0.17 Ba	0.12 Bb	0.07 Eb	0.04 Eb	0.06 Bb	0.10 Aa

* Means followed by the same uppercase letter in the column and lowercase letter in the row do not differ from each other by the Scott-Knott test at 5% probability

In general, the results for ascorbate peroxidase (APX) (Table 7) show that at high negative water potentials and an increase in temperature, there was a significant increase in APX activity, although in some combinations, these values were statistically similar to those under the combination 20/ 0.000.

It is possible to highlight the effect of combinations 28/0.000, 28/–0.075, 32/–0.075, 24/–0.223, 28/–0.223, 32/–0.223, 28/–0.444, and 32/–0.444 in the aerial part, with the values of APX two to three times higher than at 20/0.000. Generally, as a consequence of oxidative stress, damaged tissues present an increase in the activity of their antioxidant systems, such as the induction of CAT and APX enzymes (KILIC; KAHRAMAN, 2016).

Different results provided in a review detailed the response to individual and combined stresses in the detoxification mechanisms of ROS in several species, and showed that drought stress induces CAT and heat stress and the activity of APX (ZANDALINAS *et al.*, 2018). With increased stress, the formation of ROS intensifies, and their elimination must constantly occur to avoid oxidative stress. Thus, the synchronized action of enzymes responsible for the removal of ROS is one of the factors that confer greater tolerance in plants to stress conditions (ZHENG *et al*., 2009).

The ability to maintain high levels of APX and CAT activities under environmental stress conditions is essential to maintain a balance between the formation and removal of H_2O_2 from the intracellular environment (ZHANG *et al.*, 2017).

Signaling by H_2O_2 accumulation is intrinsically related to the activity of APX and CAT enzymes, and because of its high affinity for H_2O_2, APX reacts to very small changes in its concentration and is responsible for the final regulation of the signal; CAT activity, Otherwise, is induced at higher concentrations of H_2O_2 (GILL; TUTEJA, 2010). One of the aggravating causes of combined stresses is the increase in the generation of ROS, which can result in secondary oxidative stress when the defense system is not efficiently activated, and damage to cell membranes can occur as a result of lipid peroxidation (GILL; TUTEJA, 2010). As shown in the present study, the combined stress increased ROS, detected through the increased APX and CAT activities.

In the PCA, the dispersion of stress combinations obtained with the first two components (F1 and F2) explained 59.97% of the total variance between them (Figure 1). The main components F1 and F2 contributed 42.35% and 17.62%, respectively. The F1 component

Table 7 - Ascorbate peroxidase activity (UA min^{-1} g FM^{-1}) of wheat seedlings from cultivar IPR Catuara (shoots and roots) of four lots (L) under different 16 stress combinations of temperature (T, °C) and water potentials (ψ_h, MPa)

(T/ψ_h)	Shoots				Roots			
	L1	L2	L3	L4	L1	L2	L3	L4
	Ascorbate Peroxidase							
20/0.00	4.45 Db*	3.52 Db	4.20 Db	7.47 Da	8.58 Ba	9.18 Ca	6.69 Ba	9.50 Ba
24/0.00	9.28 Ba	10.95 Ba	5.79 Db	8.81 Da	5.68 Ca	5.09 Da	6.85 Ba	7.50 Ba
28/0.00	6.60 Cc	15.42 Ab	14.81 Bb	21.10 Aa	3.82 Cc	14.07 Aa	4.38 Cc	9.31 Bb
32/0.00	9.49 Ba	9.24 Ba	10.89 Ca	8.64 Da	8.38 Bb	8.38 Cb	12.19 Aa	12.38 Aa
20/–0.075	4.35 Da	6.43 Ca	5.05 Da	6.13 Da	7.35 Ba	7.32 Ca	6.89 Ba	7.69 Ba
24/–0.075	11.17 Ba	9.04 Ba	9.66 Ca	12.30 Ca	6.09 Ca	2.41 Da	4.09 Ca	5.15 Ba
28/–0.075	9.92 Bb	14.06 Aa	14.14 Ba	14.04 Ba	8.02 Bb	12.98 Aa	6.86 Bb	8.38 Bb
32/–0.075	13.20 Aa	12.26 Aa	9.06 Cb	13.31 Ca	12.24 Aa	9.85 Bb	14.47 Aa	10.62 Ab
20/–0.223	7.70 Ca	7.22 Ca	8.11 Ca	9.69 Da	9.15 Ba	9.85 Ba	10.67 Aa	11.97 Aa
24/–0.223	13.02 Ab	9.21 Bc	8.96 Cc	17.26 Ba	6.33 Cb	7.81 Cb	3.82 Cc	11.86 Aa
28/–0.223	11.01 Bc	16.33 Ab	20.86 Aa	20.15 Aa	13.19 Ab	15.17 Aa	10.99 Ab	12.00 Ab
32/–0.223	15.83 Aa	14.84 Aa	14.86 Ba	15.63 Ba	9.67 Ba	10.37 Ba	8.51 Ba	10.89 Aa
20/–0.444	12.07 Ba	15.18 Ab	9.51 Cb	15.18 Ba	12.52 Aa	7.28 Cb	9.65 Ab	8.98 Bb
24/–0.444	11.98 Bb	17.21 Aa	11.11 Cb	12.62 Cb	6.66 Cb	11.19 Ba	12.10 Aa	10.69 Aa
28/–0.444	14.00 Ab	14.20 Ab	18.99 Aa	21.54 Aa	9.05 Ba	10.48 Ba	10.88 Aa	11.96 Aa
32/–0.444	10.46 Bb	13.23 Ab	16.57 Ba	15.80 Ba	12.20 Aa	8.89 Cb	12.56 Aa	12.87 Aa

* Means followed by the same uppercase letter in the column and lowercase letter in the row do not differ from each other by the Scott-Knott test at 5% probability

consisted of the total protein content of the roots (0.65), CAT MF shoots (0.52), CAT MF roots (0.38), APX shoots (0.94), APX roots (0.77), germination (−0.83), first count (−0.81), and horizontal separation. The F2 component was formed by CAT shoots (–0.75) and CAT roots (0.62), and vertically separated the combinations in the biplot.

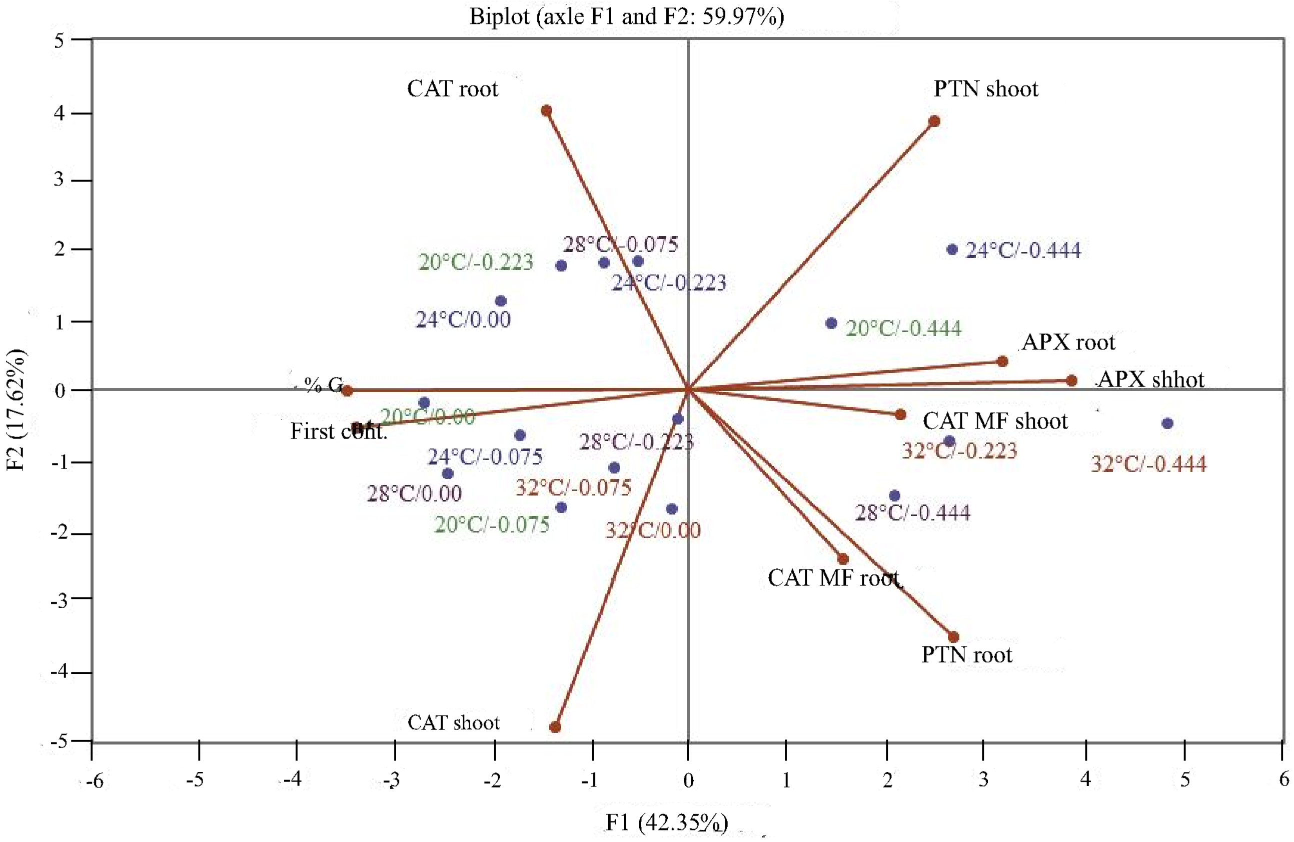
Most combinations with the three lowest temperatures and three highest water potentials were allocated to the left side of the biplot (F1-). Therefore, it can be seen that the predominant influence was related to water potentials, for which, all temperature combinations with a water potential of –0.444 MPa formed a single cluster allocated on the right side of the biplot (F1+).

The combinations with a temperature of 20 °C, considered ideal for wheat, were mostly allocated in the lower left quadrant (F1-) for the highest percentages of germination and first count. It can be seen that the combinations 20/0.000 and 20/–0.075 still showed higher levels of shoot CAT (F2-) and lower levels of root CAT, indicating an induction of shoot CAT as a way to avoid oxidative damage; however, root CAT was not efficient in removing H_2O_2 for these combinations.

Regarding the combinations of the different water potentials at a temperature of 24 °C, most of them were allocated in (F1-) by the highest percentages of germination, first count, and at the temperature of 20 °C, but the combinations 24/0.000 and 20/–0.223 showed the highest levels of root CAT and the lowest levels of shoot CAT. At 28 °C, the combinations 28/0.000 and 28/–0.223 were allocated in the lower left quadrant (F1- and F2-) due to the highest germination and first count values and the lowest total protein content in the shoots and roots, CAT MF shoot, CAT MF root, APX shoot and root.

The highest levels of total protein in shoots and roots, CAT MF shoots and roots, and APX shoots and roots were observed at 32 °C, with the exception of the 32/0.000 combination. The other combinations of the lowest temperatures and highest water potentials

Figure 1 - Biplot of principal component analysis of 16 combinations of temperature (T) (20, 24, 28 and 32 ºC) and water potentials (ψ_h) (0.00, –0.075, –0.223 e –0.444 MPa), for the average of four wheat seed lots of cultivar IPR Catuara



CAT shoot = catalase activity in shoots; CAT root = catalase activity in roots; PTN shoot = total protein content in shoots; PTN root = total protein content in roots; CAT MF shoot = catalase activity in shoots based on fresh matter; CAT MF root = catalase activity in roots based on fresh matter; APX shoot = ascorbate peroxidase activity in shoots; APX root = ascorbate peroxidase activity in roots; %G = germination percentage; first cont. = First count of germination test showed the same behavior as at 32/0.000, indicating that water deficit had a more pronounced effect on the tolerance of the seeds to stress. In combinations with different temperatures and a water potential of –0.223 MPa, damage to seeds occurred, regardless of the temperature. For a more precise definition of the groups formed by their response to water and heat stress, hierarchical cluster analysis was applied (Figure 2).

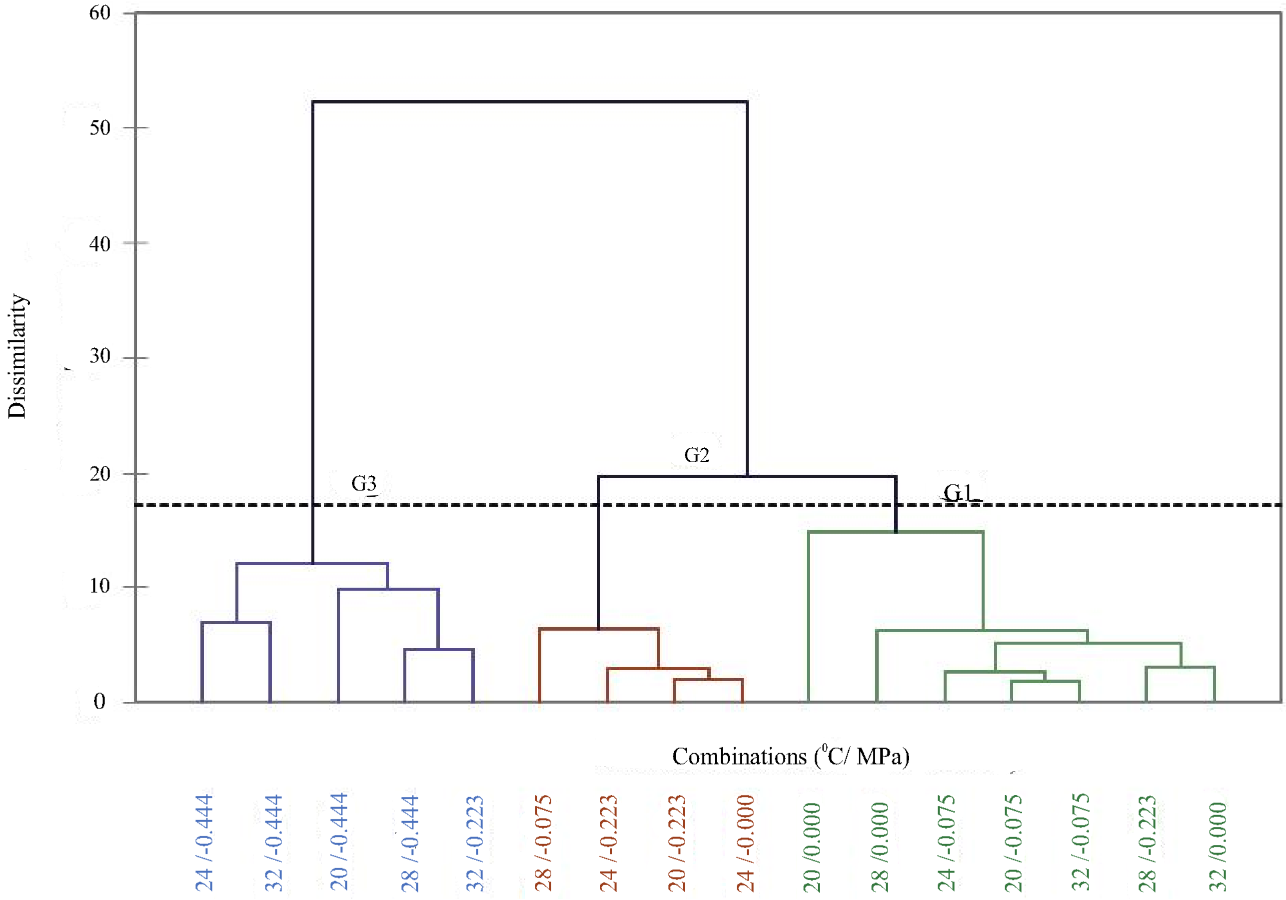
Thus, from the data of the combinations of different temperatures and water potentials (averages of four lots), it was found that three groups were formed by the dissimilarities between their respective variables. The first group, G1, was prominent for the highest percentage of germination and first count and lower values of root CAT, total protein content of the shoot, APX shoot and root, and was constituted of the combinations 20 /0.000, 20 /–0.075, 24 /–0.075, 28 /0.000, 28 /–0.223, 32/0.000, and 32/–0.075.

Group G2 was formed by four combinations, namely 20/–0.223, 24/0.00, 24/–0.223, and 28/–0.075, due to the higher percentages of germination, first count, and root CAT, and lower values of total protein content of the root part and APX shoot. Finally, G3, formed by the combinations 20/–0.444, 24/–0.444, 28/–0.444, 32/–0.223, and 32/–0.444, showed the highest values of total protein content in shoots and roots, and APX shoots and roots, and lowest values of root CAT, percentage of germination, and first count.

Therefore, in general, there was an increase in the protein content of both shoots and roots and in the activity of the APX enzyme in response to the lower germination percentage, which in turn was a result of the stress caused by the combination of higher temperatures and lower water potentials.

Wheat tolerates temperatures of up to 28 °C and reductions in water potentials down to –0.223 MPa without compromising germination; however, temperatures above 28 °C are detrimental to germination, as they cause an increase in seed mortality and growth of abnormal seedlings. As the water stress increases, the imbibition rate decreases. The combination 32/–0.223 decreased the germination percentage and increased the number of abnormal seedlings and dead seeds. Regardless of temperature, a water potential of –0.444 is harmful to wheat seed germination.

Figure 2 - Dendrogram for the 16 combinations of temperature (T) and water potentials (yh), obtained from hierarchical cluster analysis using the Euclidean distance method



Abiotic stresses, especially water deficit, cause an increase in antioxidant enzymes to prevent oxidative damage. The increase in APX enzymatic activity minimizes the effects of oxidative stress through the removal of H_2O_2 when the plants are subjected to a combination of drought and heat, or to either of the two stress conditions applied individually.

The results of the present study emphasize the growing need for studies on the effects of different combinations of abiotic stresses on plant germination and development, as well as on defense mechanisms. This is because different combinations of stress require unique adaptation of metabolic and signaling responses, including antioxidant mechanisms, hormones, and osmolyte synthesis (PANDEY; RAMEGOWDA; SENTHIL-KUMAR, 2015).

Studies focusing on abiotic factors in isolation do not represent the responses of plants to a combination of different stresses in the field. It is important that the combination of stresses be treated as a new state of abiotic stress in plants, resulting in different acclimatization responses and causing disturbances in the entire plant metabolism, which are mainly represented by a variety of physiological changes to prevent oxidative damage.

CONCLUSIONS

1. Wheat seeds tolerate supra-optimal temperatures of up to 28 °C and water potentials not lower than –0.223 MPa without adverse effects to the germination process;
2. The combination of a temperature of 32 °C and a water potential of –0.223 MPa reduced germination, and regardless of temperature, a water potential of –0.444 MPa caused a reduction in both the percentage and rate of germination;
3. APX enzyme activity increases under combinations of water stress and supra-optimal temperatures, acting as a defense mechanism against oxidative stress in wheat seeds.

ACKNOWLEDGEMENTS

The authors thank the National Council for Scientific and Technological Development (CNPq) and the Coordination for the Improvement of Higher Education Personnel (CAPES) for their financial support in performing this study.

REFERENCES

ADDINSOFT. **XLSTAT**: Software for Statistical Analysis. Versão 2010.19.01. Paris, 2010. 1 CD-ROM.

AHMED, I. M. *et al*. Genotypic differences in physiological characteristics in the tolerance to drought and salinity combined stress between Tibetan wild and cultivated barley. **Plant Physiology and Biochemistry**, v. 63, p. 49-60, 2013.

ALMAGHARABI, O. A. Impact of drought stress on germination and seedling growth parameters of some wheat cultivars. **Life Science Journal**, v. 9, n. 1, p. 590-598, 2012.

AL-SHEBLY, H. A. A.; AL-EBRAHEMI, F. K. K.; ALI, A. A. Effect of water quantity and coverage on seeds germination and their emergence in wheat crop *Triticum aestivum L*. **Plant Archives**, v. 20, n. 1, p. 2091-2094, 2020.

BARAKAT, N. *et al*. Antioxidant potential and oxidative stress markers in wheat (*Triticum aestivum*) treated with phytohormones under salt-stress condition. **International Journal of Agriculture and Biology**, v. 15, n. 5, p. 843-849, 2013.

BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, v. 72, n. 1/2, p. 248-254, 1976.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. **Regras para Análise de Sementes**. Brasília, DF: Mapa/ACS, 2009. 395 p.

BURIRO, M. *et al*. Wheat seed germination under the influence of temperature regimes. **Sarhad Journal Agriculture**, v. 27, n. 4, p. 539-543, 2011.

DHANDA, S. S.; SETHI, G. S.; BEHL, R. K. Indices of drought tolerance in wheat genotypes at early stages of plant growth. **Journal of Agronomy and Crop Science**, v. 190, n. 1, p. 6-12, 2004.

EL-MAAROUF-BOUTEAU, H.; BAILLY, C. Oxidative signaling in seed germination and dormancy. **Plant Signaling & Behavior**, v. 3, n. 3, p. 175-182, 2008.

ESPOSITO, M. P. *et al*. Oxidant-antioxidant balance and tolerance against oxidative stress in pioneer and non-pioneer tree species from the remaining Atlantic Forest. **Science of the Total Environment**, v. 625, n. 1, p. 382-393, 2018.

ESSEMINE, J.; AMMAR, S.; BOUZID, S. Impact of heat stress on germination and growth in higher plants: physiological, biochemical and molecular repercussions and mechanisms of defence. **Journal of Biological Sciences**, v. 10, n. 6, p. 565-572, 2010.

GILL, S. S.; TUTEJA, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. **Plant Physiology and Biochemistry**, v. 48, n. 12, p. 909-930, 2010.

GIROTTO, L. *et al*. Tolerância à seca de genótipos de trigo utilizando agentes indutores de estresse no processo de seleção. **Revista Ceres**, v. 59, n. 2, p. 192-199, 2012.

GRZYBOWSKI, C. R. de S. *et al.* Efeito do potencial osmótico na interação genótipo e vigor de sementes de milho. **Applied Research & Agrotechnology**, v. 12, n. 3, p. 65-73, 2019.

HAMPSON, C. R.; SIMPSON, G. M. Effects of temperature, salt, and osmotic potential on early growth of wheat (*Triticum aestivum*). I. Germination. **Canadian Journal of Botany**, v. 68, n. 3, p. 524-528, 1990.

KILIC, S.; KAHRAMAN, A. The mitigation effects of exogenous hydrogen peroxide when alleviating seed germination and seedling growth inhibition on salinity-induced stress in barley. **Polish Journal Environmental Studies**, v. 25, n. 3, p. 1053-1059, 2016.

MAGUIRE, J. D. Speed of germination-aid in selection and evaluation for seedling emergence and vigor. **Crop Science**, v. 2, n. 2, p. 176-177, 1962.

MHAMDI, A.; NOCTOR, G.; BAKER, A. Plant catalases: peroxisomal redox guardians. **Archives of Biochemistry and Biophysics**, v. 525, n. 2, p. 181-194, 2012.

MICHEL, B. E.; KAUFMANN, M. R. The osmotic potential of Polyethylene Glycol 6000. **Plant Physiology**, v. 51, n. 5, p. 914-916, 1973.

MIGNOLET-SPRUYT, L. *et al.* Spreading the news: subcellular and organellar reactive oxygen species production and signalling. **Journal of Experimental Botany**, v. 67, n. 13, p. 3831-3844, 2016.

PANDEY, P.; RAMEGOWDA, V.; SENTHIL-KUMAR, M. Shared and unique responses of plants to multiple individual stresses and stress combinations: physiological and molecular mechanisms. **Frontiers in Plant Science**, v. 6, n. 723, p. 1-14, 2015.

PARTHEEBAN, C. *et al.* Effect of PEG induced drought stress on seed germination and seedling characters of maize (*Zea mays* L.) genotypes. **International Journal of Current Microbiology and Applied Sciences**, v. 6, n. 5, p. 1095-1104, 2017.

PEIXOTO, P. H. P. *et al.* Aluminum effects on lipid peroxidation and activities of enzymes of oxidative metabolism in sorghum. **Revista Brasileira de Fisiologia Vegetal**, v. 11, n. 3, p. 137-143, 1999.

PRAMANIK, S. K.; SIKDER, S. Thermal unit requirement and grain yield of wheat under non-irrigated dry warmer condition. **Asian Journal of Research in Botany**, v. 4, n. 2, p. 1-10, 2020.

RIBEIRO, L. C.; BORGHETTI, F. Comparative effects of desiccation, heat shock and high temperatures on seed germination of savanna and forest tree species. **Austral Ecology**, v. 39, n. 3, p. 267-278, 2014.

RODRIGUES, G. A. G. *et al.* Drought stress effects on germination and reserve degradation of *Aspidosperma polyneuron* seeds. **Revista Brasileira de Ciências Agrárias**, v. 14, n. 4, p. 1-10, 2019.

SIMONI, F. D *et al.* Sementes de *Sorghum bicolor* L. - Gramineae, submetidas ao estresse hídrico simulado com PEG (6000). **Revista de Biologia e Ciências da Terra**, v. 11, n. 1, p. 188-192, 2011.

SOUZA, M. A. de; RAMALHO, M. A. P. Controle genético e tolerância ao estresse de calor em populações híbridas e em cultivares de trigo. **Pesquisa Agropecuária Brasileira**, v. 36, n. 10, p. 1245-1253, 2001.

TEIXEIRA, R. N. *et al.* Germinação e vigor de sementes de crambe sob estresse hídrico. **Irriga**, v. 16, n. 1, p. 42-51, 2011.

VIÇOSI, K. A. *et al.* Estresse hídrico simulado em genótipos de feijão, milho e soja. **Revista de Agricultura Neotropical**, v. 4, p. 36-42, dez. 2017. Suplemento 1.

XU, Y.; ZHAN, C.; HUANG, B. Heat shock proteins in association with heat tolerance in grasses. **International Journal of Proteomics**, v. 2011, n. 1, p. 1-11, 2011.

ZANDALINAS, S. I. *et al.* Plant adaptations to the combination of drought and high temperatures. **Physiologia Plantarum**, v. 162, n. 1, p. 2-12, 2018.

ZHANG, G. *et al.* Wheat TaPUB1 modulates plant drought stress resistance by improving antioxidant capability. **Scientific Reports**, v. 7, n. 1, p. 1-13, 2017.

ZHENG, C. *et al.* Exogenous nitric oxide improves seed germination in wheat against mitochondrial oxidative damage induced by high salinity. **Environmental and Experimental Botany**, v. 67, n. 1, p. 222-227, 2009.

ZIMMER, P. D. Fundamentos da qualidade da semente. *In*: PESKE, S. T.; VILLELA, F. A.; MENEGHELLO, G. E. **Sementes**: fundamentos científicos e tecnológicos. 3. ed. rev. e ampl. Pelotas: Ed. Universitária/UFPel, 2012. p. 105-160.