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*Original Scientific Paper*

# Physiological responses of bread and durum wheat seeds to osmotic stress and salinity in the early germination stage

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**ABSTRACT:**
Determining the mechanisms underlying tolerance to osmotic stress and salinity during the germination period is an essential task in order to improve agricultural production in arid and semi-arid areas. In this work, the seeds of bread and durum wheat cultivars were treated with different concentrations of polyethylene glycol (PEG)-6000 and NaCl for 1 week, and half-maximal inhibitory concentrations ($IC_{50}$) of germination were calculated. The seeds were then exposed to $IC_{50}$ values of NaCl and PEG-6000 for 2 days in order to assess their physiological and biochemical properties. Alpha and beta amylase enzyme activities, the reducing sugar, total sugar, proline, protein, and $H_2O_2$ contents, and DPPH radical scavenging activity of the seeds were determined by spectrophotometric methods. The results showed that the bread wheat seeds had a much higher tolerance to excess salt and osmotic stress than the durum wheat seeds. In particular, the average $IC_{50}$ value of NaCl for the bread wheat cultivars was almost twice that for the durum wheat cultivars. The imbibition test revealed that the water uptake capacity of the seeds did not explain the difference in tolerance to these stress conditions. On the other hand, the bread wheat seeds exhibited constitutively higher proline, total sugar, and $H_2O_2$ contents as well as antioxidant capacity compared to the durum wheat seeds ($p < 0.05$). In conclusion, the current findings suggest that the interplay of oxidative metabolism and compatible solutes may contribute to improving germination tolerance under water deficit and salinity conditions in wheat.

**Keywords:**
salinity, osmotic stress, germination, wheat, reactive oxygen species, compatible solutes

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## INTRODUCTION

Soil salinity and drought are considered to be among the most important challenges facing agriculture today, which directly affect every stage of plant growth and development from seed germination to yield formation (Ma *et al.* 2020; Negacz *et al.* 2022). The devastating effects of these stress conditions on crop production and the economy have been reported in many parts of the world (Qadir *et al.* 2014; Madadgar *et al.* 2017). Considering that the intensity and frequency of extreme heat events is likely to increase in the future (Liu *et al.* 2017) and more than 67% of salinized soils are located in arid areas (FAO 2022), determining drought- and salt-tolerant crop cultivars is essential in order to improve agricultural production.

Seeds and juvenile seedlings are more frequently exposed to salinity and drought than mature plants because germination usually takes place at the soil surface where high salt accumulation and low water availability are evident (Almansouri *et al.* 2001; Orsenigo *et al.* 2017). In this context, many germination experiments have been carried out to determine salinity- and/or osmotic stress-tolerant cereal cultivars under laboratory-controlled conditions (Sayar *et al.* 2010; Zhang *et al.* 2010a; Abdel-Ghani *et al.* 2020; Mwando *et al.*

2020; Hasseb *et al.* 2022; Mahpara *et al.* 2022). These studies suggest that there is substantial genotypic variation in germination tolerance to salt and osmotic stress in cereals, such as barley and wheat (Mwando *et al.* 2020; Mahpara *et al.* 2022). In addition, low concentrations of sodium chloride (NaCl) and polyethylene glycol (PEG) treatments were found to have a much greater influence on mean germination time compared with the total germination percentage (Abdel-Ghani *et al.* 2020; Hasseb *et al.* 2022).

Seed germination consists of a number of physical and metabolic events, such as imbibition of water, respiration, protein synthesis, DNA repair, and reserve mobilisation, which eventually lead to the appearance of the embryo (Nonogaki *et al.* 2010). It has been documented that salt and osmotic stresses can interfere with one or more of these events. For instance, El-Hendawy *et al.* (2019) showed that increasing levels of salinity caused a significant reduction in the water uptake rate of wheat seeds. In addition, excess salt was also found to have a negative impact on the alternative oxidase pathway for mitochondrial respiration in *Brassica juncea* seeds (Pandey & Penna 2017). Moreover, Dell'Aquila & Spada (1992) revealed that protein synthesis in germinating wheat seeds was markedly reduced in response to drought and salt stress. Lastly, low osmotic potential has been shown to reduce the activities of hydrolytic enzymes, including protease, α-amylase, and β-amylase involved in reserve mobilisation (Ashraf *et al.* 2002; Liu *et al.* 2019; Wang *et al.* 2022).

Bread wheat (*Triticum aestivum* L., AABBDD – 2n = 6x = 42) and durum wheat (*T. turgidum* L. subsp. *durum* (Desf.), AABB – 2n = 4x = 28) are two closely related species providing 17% of the calories consumed by the human population (Ashikari & Ma 2015; Mastrangelo & Cattivelli 2021). When compared to tetraploid durum wheat, hexaploid bread wheat is known to have a higher adaptive capacity to different environmental conditions and exhibit enhanced tolerance to salinity and aluminium toxicity (Dubcovsky & Dvorak 2007; Han *et al.* 2016). Many comparative studies have been performed to investigate the physiological and yield responses of these wheat species to excess salt and water deficit (Moayedi *et al.* 2010; Wu *et al.* 2018). However, these works have mostly focused on different vegetative stages and less attention has been given to metabolic processes during seed germination. Therefore, the objectives of this research were (i) to ascertain whether there is any variation in germination response to salinity and osmotic stress between and within wheat species, and (ii) to identify the mechanisms involved in tolerance to these two stresses in the germination process. In order to achieve these goals, two bread wheat cultivars (Bayraktar-2000 and Bezostaja-1) and two durum wheat cultivars (Ç-1252 and Kızıltan-91) were subjected to different concentrations of PEG-6000 and NaCl solutions during the germination process. The half-maximal inhibitory concentrations ($IC_{50}$) of NaCl and PEG-6000 in suppressing germination were calculated to represent the concentrations for further assays. Spectrophotometric methods were used for those assays in which several physiological and biochemical traits associated with sugar metabolism, oxidative status, antioxidant capacity, and osmotic balance were determined.

## MATERIALS AND METHODS

**Plant material.** In this study, the seeds of two bread (*Triticum aestivum* L. cv. Bayraktar-2000 and cv. Bezostoja-1) and two durum (*Triticum turgidum* L. subsp. *durum* (Desf.) cv. Ç-1252 and cv. Kızıltan-91) wheat cultivars were used to determine the effects of salinity and osmotic stress on seed germination. The seeds were obtained from the Republic of Turkey's General Directorate of Agricultural Enterprises (TIGEM). The mean seed weight of the wheat cultivars ranged from 0.033 to 0.048 g.

**Seed germination protocol.** The wheat seeds were sterilised with 20% (v/v) bleach-water solution (1% NaClO) for 20 min and washed with sterile distilled water. Following drying in autoclaved paper towels, the seeds were placed in Petri plates containing two sheets of filter paper (Whatman, Grade 1) dampened with 10 mL of the test solutions. The same volume of sterile distilled water was used as the control. The Petri plates were then incubated at 20 ± 0.5°C in the dark for one week. At the end of the germination assay, the seeds were categorised as germinated and ungerminated. The emergence of a 5 mm radicle was taken as the germination criterion, and the cut test was used for assessing the viability of the ungerminated seeds (Çatav *et al.* 2015). Six groups of 25 seeds were used per treatment in the germination assays.

**Experiment 1: The effect of excess salt on seed germination.** In this experiment, the seeds of the durum wheat cultivars were subjected to five different concentrations (75, 150, 225, 300, and 375 mM) of sodium chloride (Merck, Darmstadt, Germany). Based on the preliminary results, the bread wheat seeds were found to be more tolerant to salinity than the durum wheat seeds. Therefore, 450, 525, and 600 mM NaCl treatments were also included in the germination experiment of the bread wheat cultivars.

**Experiment 2: The effect of osmotic stress on seed germination.** In this experiment, PEG-6000 (Sigma-Aldrich) was used for the simulation of osmotic stress on seed germination. Briefly, a 50% (w/v) stock solution was prepared by dissolving PEG-6000 in the presence of heat. The stock solution was diluted to working concentrations (20, 25, 30, 35, and 40%) with distilled water

(Zhang *et al.* 2010b). The bread and durum wheat seeds were then exposed to these PEG-6000 solutions.

**Biochemical analyses.** Before biochemical analyses, the $IC_{50}$ values of NaCl and PEG-6000 for germination inhibition of the studied cultivars were calculated (Figs. 1 & 2). Subsequently, Bezostaja-1 and Kızıltan-91 were chosen as the bread and durum wheat cultivars to study the mechanisms underlying the differences in osmotic stress and salinity tolerance among wheat species. Surface-sterilised seeds (approx. 1 g) of both cultivars were subjected to $IC_{50}$ concentrations of NaCl (255 and 457 mM) and PEG-6000 (27 and 31%) for 2 days according to the conditions described in the section on "seed germination protocol". Afterwards, the seeds were dried with autoclaved paper towels and stored in a freezer at -30°C prior to use. The selected exposure time corresponds to phase II in the germination process in which metabolism reactivation takes place (Dong *et al.* 2015; He *et al.* 2015). Three replicates of seed samples were used per treatment for each biochemical analysis.

The frozen seeds were ground in a pre-chilled mortar containing 10 mL of ice-cold $dH_2O$, and the homogenate was centrifuged at 5031 *g* for 20 min at 4°C. The resultant supernatants were used for the estimation of the protein content and α- and β-amylase activities (Kishorekumar *et al.* 2007). The protein content of the wheat seeds was measured according to the protein-dye binding method (Bradford 1976). In order to determine the α-amylase activity of the seeds, 375 µL of 3 mM $CaCl_2$ was added to 625 µL of supernatant, and the mixture was incubated in a heating block at 70°C for 5 min. 0.7 mL of the hot mixture, 0.2 mL of 1 mM citrate buffer (pH 5.0), and 1.1 mL of 3.64% (w/v) soluble starch solution were mixed and incubated at 30°C for 5 min. Next, 2 mL of 3,5-dinitrosalicylic acid (DNS) reagent was added, and the reaction mixture was maintained at 95°C for 5 min. Six mL of $dH_2O$ was added after the termination of the reaction in an ice bath. Finally, the absorbance of the mixture was read at 540 nm. For the assessment of β-amylase activity, 375 µL of 0.1 M Titriplex III (pH 3.4) was added to 625 µL of supernatant. 0.7 mL of this enzyme solution was mixed with 0.2 mL of 1 mM citrate buffer (pH 3.4) and 1.1 mL of 3.64% (w/v) soluble starch and incubated at 30°C for 5 min. The DNS assay was performed as outlined above. A standard graph was prepared using D-(+)-maltose monohydrate (Sigma-Aldrich), and the amylase enzyme activity was expressed as unit $mg^{-1}$ protein. One unit was defined as the amount of enzyme releasing 1 mg of maltose from starch per minute (Zheng *et al.* 2009).

The wheat seeds were homogenized in hot ethanol (80%) as previously described (Çatav *et al.* 2022), and the DNS assay and sulfuric acid-phenol reaction were used for the quantification of reducing and total sugar contents, respectively (Dubois *et al.* 1956; Miller 1959). The data were expressed as mg glucose per gram of fresh weight (FW).

The hydrogen peroxide ($H_2O_2$) content and free radical scavenging capacity of the wheat seeds were estimated by the KI oxidation and DPPH methods, respectively (Çatav *et al.* 2021). Briefly, the supernatants of 0.1% (w/v) trichloroacetic acid- and methanol-homogenized seeds were exposed to 1 M KI and 50 µM 2,2-diphenyl-1-picrylhydrazyl for the $H_2O_2$ and DPPH assays, respectively, and the absorbance of the reaction mixtures was recorded with a spectrophotometer. The acid-ninhydrin method was performed to determine the free proline content, and the data were expressed as nmol $g^{-1}$ FW (Shabnam *et al.* 2016).

**Imbibition test.** One gram of seeds from the Kızıltan-91 and Bezostaja-1 wheat cultivars was incubated in Petri dishes with two layers of filter paper and 10 mL of the test solutions for 48 h. The seeds were then dried with filter papers and weighed on an analytical balance. This procedure was repeated three times and the weight increase (%) was calculated for each treatment (Jain *et al.* 2008).

**Data analysis.** The $IC_{50}$ values of NaCl and PEG-6000 for germination inhibition of the bread and durum wheat cultivars were determined by linear and nonlinear regression analyses, respectively. Dose-response inhibition curves were generated using the [inhibitor] vs. response – variable slope model in GraphPad Prism 7.

Prior to statistical analyses, the arcsine square-root transformation was applied to the proportional data obtained from the germination and imbibition assays. These data were then analysed by one-way ANOVA and Tukey's post-hoc test. The parametric assumptions of the data from the biochemical analyses were checked using Anderson-Darling and Bartlett's tests. Two-way ANOVA was performed to ascertain the separate and interactive effects of species and treatment on the tested parameters. All the analyses were conducted at the 0.05 significance level.

## RESULTS

**The germination response of the wheat cultivars to excess salt and osmotic stress.** The germination percentages ranged from 63.2 to 68.0 for the durum wheat cultivars and from 87.9 to 97.4 for the bread wheat cultivars under control conditions (Tables 1 & 2). Significant reductions in the germination percentages of the wheat cultivars started to be observed at treatments of 150 and 225 mM NaCl. High concentrations of NaCl (300 mM and above) led to dramatic decreases in the germination of the studied cultivars (Table 1). There were no marked differences in the germination percentages between low concentrations of PEG-6000 treatments (20 and 25%)

**Table 1.** The effect of different concentrations of NaCl solutions on the germination of the bread and durum wheat cultivars. The data are presented as mean ± SEM of sextuplicate determinations. Values with different superscript letters within the same column demonstrate significant differences ($p < 0.05$) among the treatments for each wheat cultivar. "-" : not tested.

| | Bread wheat | | Durum wheat | |
|---|---|---|---|---|
| NaCl (mM) | Bayraktar-2000 | Bezostaja-1 | Kızıltan-91 | Ç-1252 |
| 0 | 97.4 ± 1.7$^{a}$ | 89.9 ± 1.4$^{a}$ | 63.2 ± 2.0$^{a}$ | 67.8 ± 4.2$^{a}$ |
| 75 | 93.3 ± 1.3$^{ab}$ | 87.3 ± 1.2$^{ab}$ | 56.0 ± 2.7$^{ab}$ | 65.5 ± 1.9$^{a}$ |
| 150 | 88.5 ± 3.4$^{b}$ | 86.0 ± 1.7$^{ab}$ | 49.3 ± 3.7$^{bc}$ | 51.3 ± 6.1$^{a}$ |
| 225 | 86.0 ± 2.3$^{b}$ | 80.0 ± 2.1$^{b}$ | 38.3 ± 4.5$^{c}$ | 29.6 ± 3.4$^{b}$ |
| 300 | 68.7 ± 2.4$^{c}$ | 63.3 ± 1.6$^{c}$ | 25.3 ± 1.3$^{d}$ | 13.4 ± 2.2$^{c}$ |
| 375 | 53.3 ± 3.2$^{cd}$ | 56.0 ± 3.7$^{cd}$ | 12.6 ± 2.0$^{e}$ | 8.0 ± 2.9$^{c}$ |
| 450 | 39.3 ± 5.0$^{de}$ | 45.3 ± 2.5$^{d}$ | - | - |
| 525 | 29.3 ± 3.0$^{ef}$ | 34.0 ± 2.9$^{e}$ | - | - |
| 600 | 16.8 ± 1.7$^{f}$ | 26.0 ± 1.7$^{e}$ | - | - |

and the control in the bread and durum wheat cultivars. A considerable drop in germination was detected in response to 30% PEG-6000, but this was more pronounced in the durum wheat cultivars ($p < 0.05$). Finally, PEG-6000 at concentrations of 35 and 40% induced dramatic reductions (3.9- to 34-fold) in the germination percentages of all the wheat cultivars (Table 2).

**$IC_{50}$ values of NaCl and PEG-6000.** The $IC_{50}$ values of NaCl for germination inhibition of the bread wheat cultivars were found to be significantly greater than those of the durum wheat cultivars (Fig. 1). For instance, the Bezostaja-1 seeds displayed a 2.1-fold higher tolerance to NaCl when compared with Ç-1252 seeds. The inhibitory effect of PEG-6000 against germination also differed between wheat species (Fig. 2). The average $IC_{50}$ values of PEG-6000 for the durum and bread wheat cultivars were calculated as 27.18% and 31.24%, respectively.

**Imbibition test.** Imbibition in distilled water for 48 h produced a 46% increase in the weight of both the Bezostaja-1 and Kızıltan-91 seeds. Only minor differences were noted in the water uptake of the seeds between two wheat species in response to osmotic and salt stresses (Tables 3 & 4). There was a sharp decline in the water uptake of the wheat seeds after NaCl and PEG-6000 treatments ($p < 0.05$). Interestingly, even though the 255 mM NaCl and 27% PEG-6000 solutions had almost similar osmotic potential, the weight increase of the wheat seeds subjected to these solutions differed significantly from each other (Table 3). Overall, the water uptake of the PEG-6000-treated seeds was less than that of the NaCl-treated seeds.

***α*- and β-amylase activities.** All the NaCl and PEG-6000 treatments gave rise to a remarkable reduction ($p < 0.05$) in the α-amylase activity of the bread and durum wheat seeds (Fig. 3A). In particular, 56.2 to 77.5% decreases were observed in the α-amylase activities of the seeds treated with high concentrations of NaCl and PEG-6000. A similar pattern of change was recorded in the β-amylase activity of the Bezostaja-1 and Kızıltan-91 seeds exposed to osmotic stress and salinity (Fig. 3B). The two-way ANOVA results indicated that there were only slight differences in the α- and β-amylase activities between the two cultivars (Table 4).

**Table 2.** The effect of different concentrations of PEG-6000 solutions on the germination of the bread and durum wheat cultivars. The data are presented as mean ± SEM of sextuplicate determinations. Values with different superscript letters within the same column demonstrate significant differences ($p < 0.05$) among the treatments for each wheat cultivar.

| | Bread wheat | | Durum wheat | |
|---|---|---|---|---|
| PEG-6000 (%) | Bayraktar-2000 | Bezostaja-1 | Kızıltan-91 | Ç-1252 |
| 0 | 93.9 ± 2.0$^{a}$ | 87.9 ± 2.2$^{a}$ | 66.0 ± 3.8$^{ab}$ | 68.0 ± 4.4$^{ab}$ |
| 20 | 92.6 ± 1.6$^{a}$ | 88.7 ± 2.2$^{a}$ | 78.0 ± 5.6$^{a}$ | 76.0 ± 2.7$^{a}$ |
| 25 | 86.0 ± 1.4$^{a}$ | 84.7 ± 2.4$^{a}$ | 58.7 ± 2.0$^{b}$ | 52.0 ± 5.1$^{b}$ |
| 30 | 66.0 ± 5.5$^{b}$ | 58.7 ± 3.5$^{b}$ | 21.3 ± 2.2$^{c}$ | 18.7 ± 4.9$^{c}$ |
| 35 | 13.3 ± 3.2$^{c}$ | 22.7 ± 2.9$^{c}$ | 12.8 ± 1.6$^{cd}$ | 5.3 ± 0.8$^{cd}$ |
| 40 | 6.0 ± 1.7$^{c}$ | 12.0 ± 1.0$^{d}$ | 8.7 ± 0.7$^{d}$ | 2.0 ± 0.9$^{d}$ |

**Table 3.** The weight increases (%) of the bread and durum wheat seeds exposed to NaCl and PEG-6000 treatments for 48 h. The data are presented as mean ± SEM of triplicate determinations. Values with different superscript letters demonstrate significant differences ($p < 0.05$) among the treatments.

| | | Weight increase (%) | |
|---|---|---|---|
| Treatment | Osmotic potential (MPa) | Bezostaja-1 | Kızıltan-91 |
| $dH_2O$ | 0.00 | 45.9 ± 0.8$^{a}$ | 46.0 ± 1.3$^{a}$ |
| PEG-6000 (27%) | -1.02 | 24.5 ± 1.5$^{cd}$ | 21.5 ± 0.8$^{de}$ |
| PEG-6000 (31%) | -1.36 | 23.1 ± 0.5$^{de}$ | 19.4 ± 0.8$^{e}$ |
| NaCl (255 mM) | -1.06 | 32.4 ± 0.4$^{b}$ | 29.7 ± 0.4$^{b}$ |
| NaCl (457 mM) | -2.13 | 28.3 ± 0.2$^{bc}$ | 25.4 ± 1.0$^{cd}$ |

**Total and reducing sugar contents.** The results showed that the 457 mM NaCl and 31% PEG-6000 treatments caused a prominent reduction in the total sugar content of both cultivars (Fig. 4A). Bezostaja-1 exhibited significantly higher levels of total sugars than Kızıltan-91 (Table 4). The reducing sugar content of the non-treated seeds for Bezostaja-1 and Kızıltan-91 was equivalent to 18.4% and 21.4% of the total sugar content, respectively. All the NaCl and PEG-6000 treatments decreased the reducing sugar content of the tested cultivars by approx-

**Table 4.** Two-way ANOVA results for the individual and interactive effects of "species" and "treatment" on the tested parameters. % stands for the percentage of the total variation.

| | Species | | Treatment | | Interaction | |
|---|---|---|---|---|---|---|
| **Parameter** | **%** | ***p*** | **%** | ***p*** | **%** | ***p*** |
| α-amylase | 2.24 | 0.039 | 82.94 | < 0.0001 | 5.66 | 0.039 |
| β-amylase | 2.07 | 0.044 | 74.93 | < 0.0001 | 14.04 | 0.0006 |
| DPPH | 86.83 | < 0.0001 | 6.42 | 0.0006 | 2.61 | 0.037 |
| $H_2O_2$ | 63.96 | < 0.0001 | 26.65 | < 0.0001 | 2.78 | 0.119 |
| Reducing sugar | 0.43 | ns | 92.55 | < 0.0001 | 1.27 | ns |
| Proline | 71.13 | < 0.0001 | 16.63 | < 0.0001 | 11.26 | < 0.0001 |
| Protein | 6.15 | 0.012 | 72.65 | < 0.0001 | 5.13 | ns |
| Total sugar | 47.87 | < 0.0001 | 42.36 | < 0.0001 | 4.56 | 0.011 |
| Water imbibition | 2.14 | 0.0001 | 95.26 | < 0.0001 | 0.64 | ns |

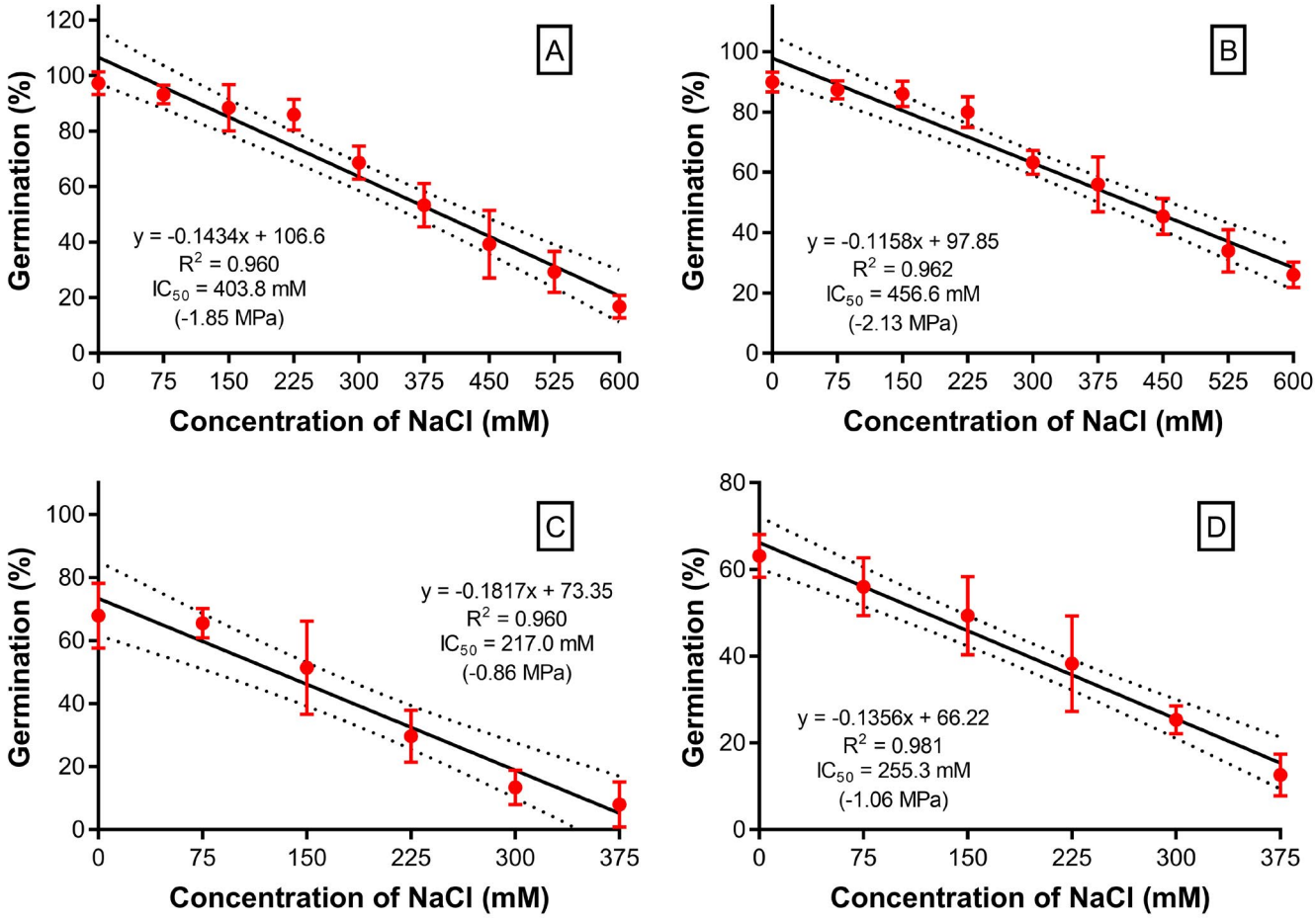


**Fig. 1.** The results of the regression analysis for NaCl concentration and germination percentage. A) Bayraktar-2000, B) Bezostaja-1, C) Ç-1252, and D) Kızıltan-91. The water potential (MPa) of the NaCl solutions were calculated theoretically by using the $IC_{50}$ values according to Sun (2002).

imately half (Fig. 4B). Lastly, no difference was apparent in the reducing sugar content among the bread and durum wheat cultivars (Table 4).

**Protein and proline contents.** In Bezostaja-1, the protein content of the non-treated seeds was 46 to 53% lower than that of the PEG-6000- and NaCl-treated seeds (Fig.

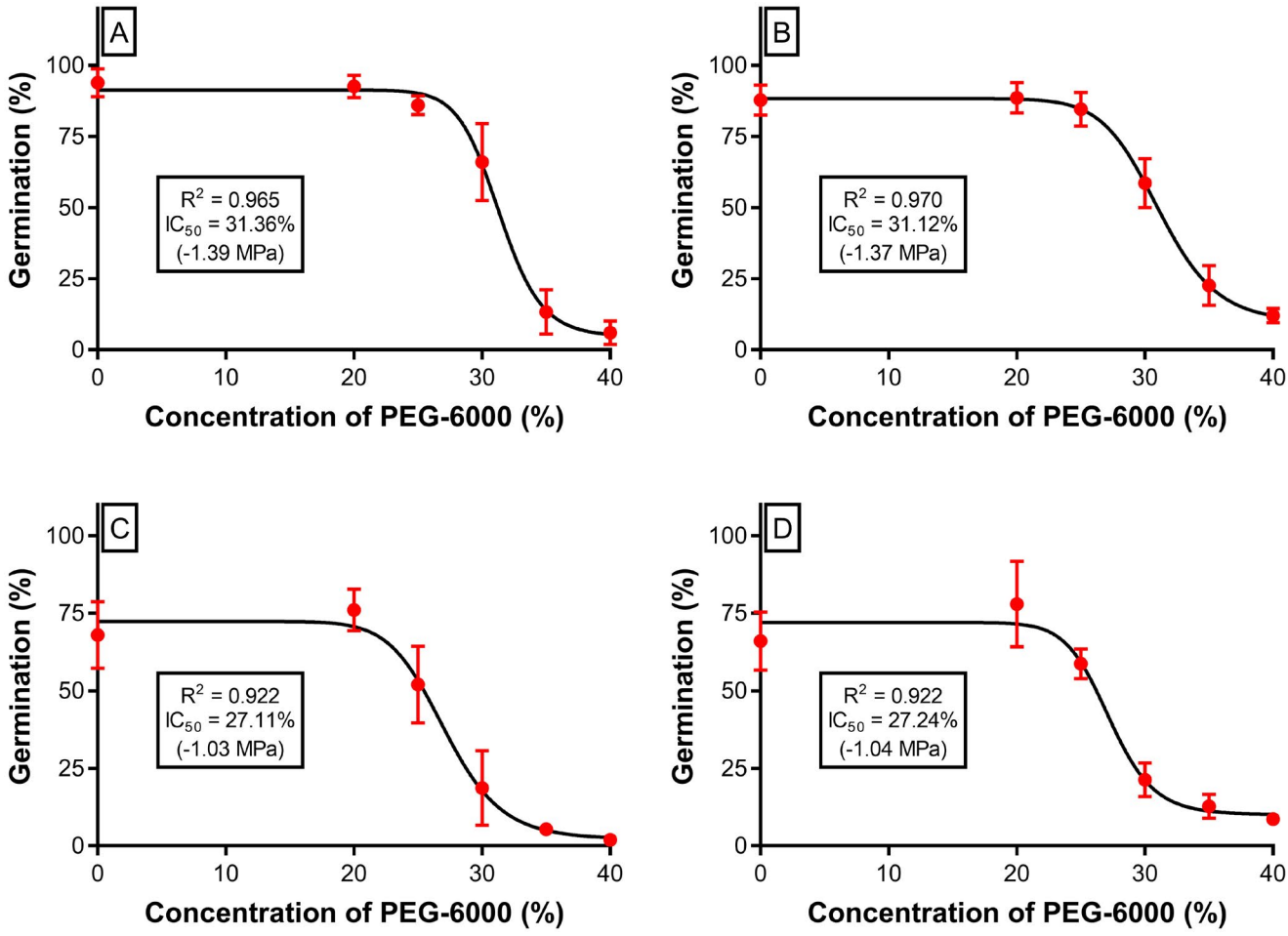


**Fig. 2.** The results of the regression analysis for PEG-6000 concentration and germination percentage. A) Bayraktar-2000, B) Bezostaja-1, C) Ç-1252, and D) Kızıltan-91. The water potential (MPa) of the PEG-6000 solutions were calculated theoretically by using the $IC_{50}$ values according to Money (1989).

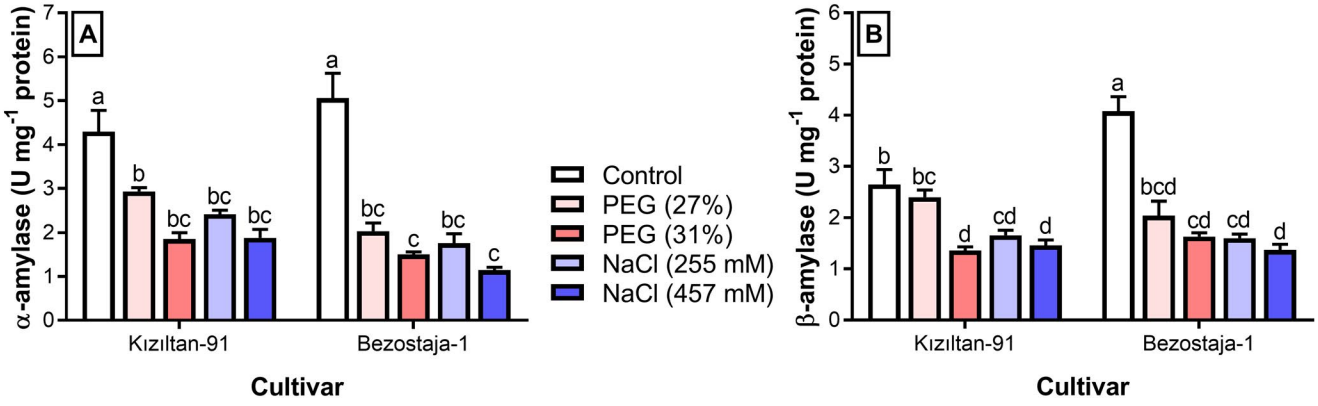


**Fig. 3.** The effects of the NaCl and PEG-6000 treatments on the A) alpha-amylase and B) beta-amylase enzyme activities of the bread (Bezostaja-1) and durum (Kızıltan-91) wheat seeds. The bars and error bars in the graph represent the mean ± SEM of triplicate measurements, respectively. Different letters above the error bars demonstrate significant differences ($p < 0.05$) among the treatments.

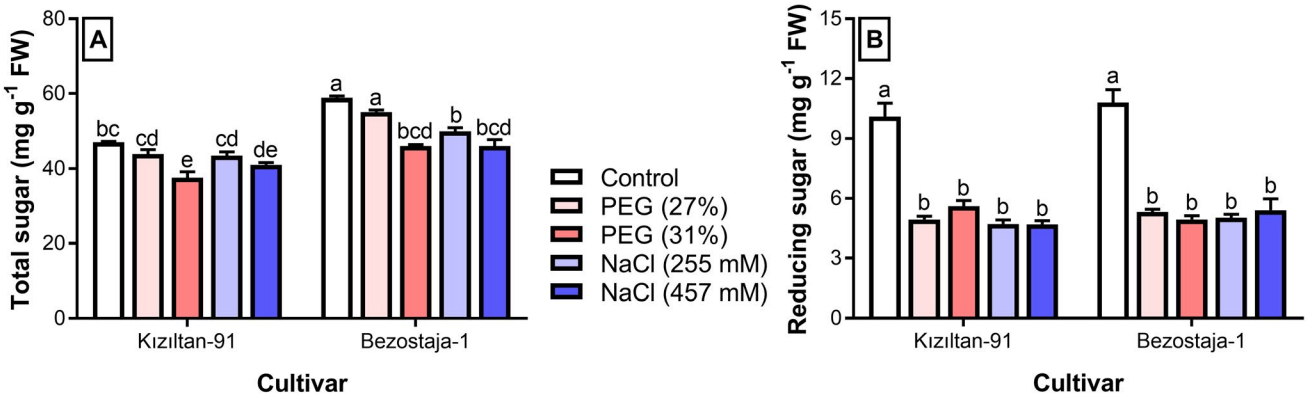


**Fig. 4.** The effects of the NaCl and PEG-6000 treatments on the A) total sugar and B) reducing sugar contents of the bread (Bezostaja-1) and durum (Kızıltan-91) wheat seeds. The bars and error bars in the graph represent the mean ± SEM of triplicate measurements, respectively. Different letters above the error bars demonstrate significant differences ($p < 0.05$) among the treatments.

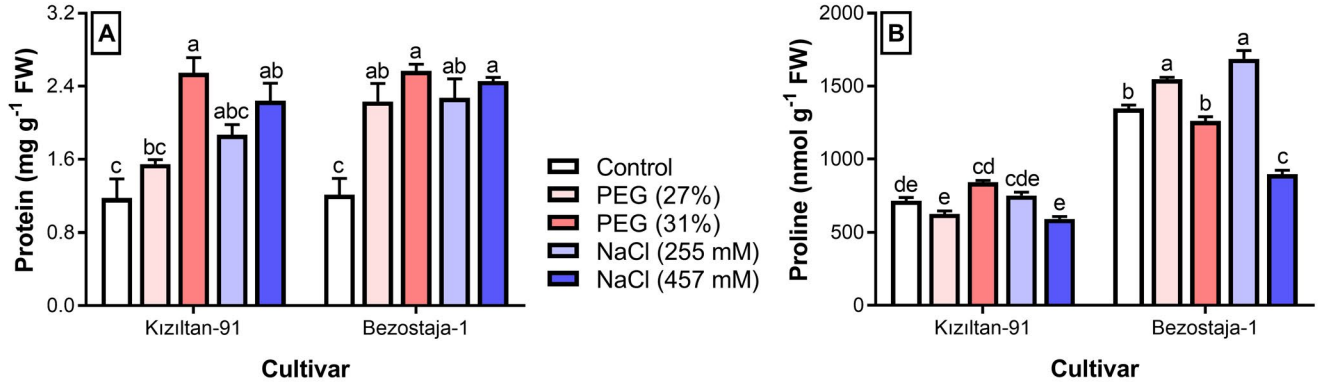


**Fig. 5.** The effects of the NaCl and PEG-6000 treatments on the A) soluble protein and B) proline contents of the bread (Bezostaja-1) and durum (Kızıltan-91) wheat seeds. The bars and error bars in the graph represent the mean ± SEM of triplicate measurements, respectively. Different letters above the error bars demonstrate significant differences ($p < 0.05$) among the treatments.

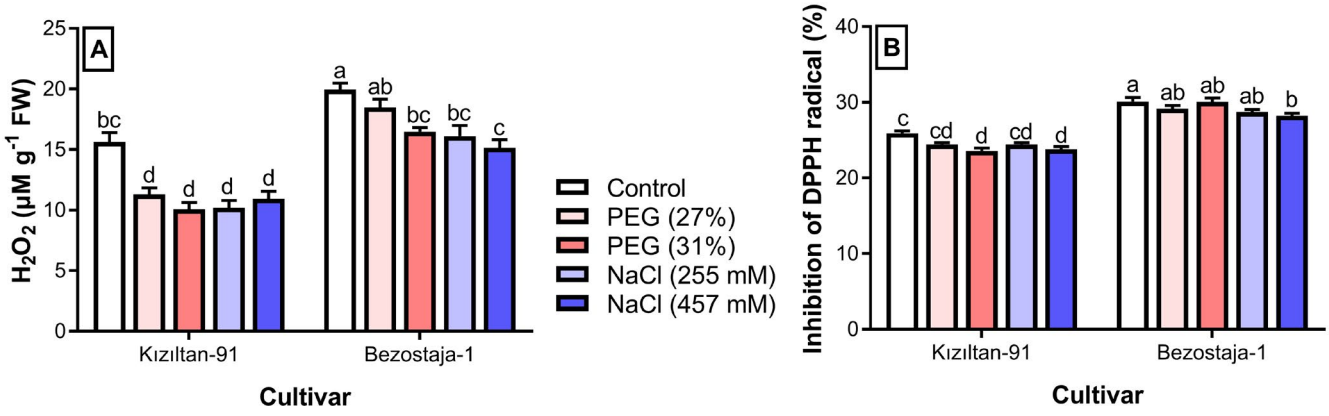


**Fig. 6.** The effects of the NaCl and PEG-6000 treatments on the A) $H_2O_2$ content and B) DPPH scavenging activity of the bread (Bezostaja-1) and durum (Kızıltan-91) wheat seeds. The bars and error bars in the graph represent the mean ± SEM of triplicate measurements, respectively. Different letters above the error bars demonstrate significant differences ($p < 0.05$) among the treatments.

5A). The Kızıltan-91 seeds subjected to high concentrations of NaCl and PEG-6000 also had higher soluble protein content compared to the control ($p < 0.05$). Species type was found to explain most of the variation (71.1%) in the proline level (Table 4). For example, the proline content of the bread wheat (Bezostaja-1) seeds was almost equal to twice that of the durum wheat (Kızıltan-91) seeds under control conditions (Fig. 5B). The 255 mM NaCl and 27% PEG-6000 treatments significantly increased the proline level of the Bezostaja-1 seeds ($p < 0.05$). On the other hand, 457 mM NaCl application resulted in a 33% fall in the proline content of the seeds in comparison to the control. Finally, a slight insignificant increase was observed in the proline level of the Kızıltan-91 seeds after exposure to 31% PEG-6000.

**$H_2O_2$ content and DPPH radical scavenging activity.** The findings demonstrate that in Kızıltan-91, the $H_2O_2$ content of the control seeds is 1.38- to 1.55- times greater than that of the seeds incubated in the NaCl and PEG-6000 solutions (Fig. 6A). Similar but less pronounced changes were also detected for the $H_2O_2$ content of the Bezostaja-1 seeds under salinity and osmotic stress. High concentrations of NaCl and PEG-6000 induced a notable decline in the DPPH radical scavenging activity of the Kızıltan-91 seeds (Fig. 6B). A small but non-negligible decrease was also seen in the radical scavenging capacity of the Bezostaja-1 seeds treated with 457 mM NaCl relative to the control group ($p < 0.05$). Lastly, the two-way ANOVA results showed that Bezostaja-1 had significantly higher $H_2O_2$ content as well as DPPH radical scavenging activity than Kızıltan-91 (Table 4; Fig. 6).

## DISCUSSION

Soil salinity and water deficit are among the most important abiotic stresses, which inhibit the seed germination of crop species, including wheat (Llanes *et al.* 2016; Yan *et al.* 2020). In this research, different concentrations of PEG-6000 and NaCl solutions were used in order to ascertain the physiological and biochemical responses of bread and durum wheat seeds to excess salt and osmotic stress. In agreement with previous findings (Sayar *et al.* 2010; Jovović *et al.* 2018), the current results show that high NaCl and PEG-6000 concentrations lead to drastic decreases in the germination percentage of wheat cultivars when compared to control conditions. Furthermore, it has been reported that considerable variation can be observed in germination response to these stress conditions among and within wheat species (Płażek *et al.* 2013; Vardar *et al.* 2014; Öztürk *et al.* 2016; Mahpara *et al.* 2022). In this study, there were slight or no differences in the $IC_{50}$ values of NaCl and PEG-6000 which suppress the germination of cultivars belonging to the same *Triticum* species. The lack of genotypic variation in germination response to salinity and osmotic stress might result from the fact that only two cultivars were used per species. On the other hand, meaningful differences were found in the $IC_{50}$ values of NaCl and PEG-6000 for germination inhibition between the bread and durum wheat cultivars. The seeds of the bread wheat cultivars exhibited a much higher tolerance to excess salt compared to those of the durum wheat cultivars. The present results support the findings of Vardar *et al.* (2014) that salinity generally induces more severe germination inhibition in *T. turgidum* subsp. *durum* genotypes than in *T. aestivum* genotypes.

The low osmotic potential of soil solution is known to limit or prevent water uptake by seeds (El-Hendawy *et al.* 2019). It has been demonstrated that NaCl-treated seeds have more capacity to uptake water than PEG-treated seeds (Zhang *et al.* 2010a; Irving & Zhang 2021). The reason for this is that in contrast to PEG, sodium ions can be absorbed by seeds and this, in turn, leads to a drop in the internal osmotic potential of seeds, thus enhancing water uptake (Almansouri *et al.* 2001). In accordance with these observations, the current results show that the bread and durum wheat seeds exposed to 255 mM NaCl (-1.06 MPa) uptake more water than those exposed to 27% PEG-6000 (-1.02 MPa). On the other hand, there was no remarkable difference in water uptake between the Kızıltan-91 and Bezostaja-1 seeds subjected to the same concentration of PEG-6000 or NaCl. This clearly indicates that the higher tolerance of the bread wheat seeds to salinity and osmotic stress, as compared with the durum wheat seeds, is not related to seed water uptake capacity.

The imbibition of water by seeds initiates a series of metabolic events that ultimately results in the appearance of the radicle or plumule. Reserve mobilisation is one of these events which has a vital function during germination and early seedling growth periods (Nonogaki *et al.* 2010). In cereal species, the synthesis and secretion of hydrolytic enzymes, such as protease and α-amylase from aleurone cells, are induced by gibberellic acid ($GA_3$) released from the embryo. These enzymes play a crucial role in the degradation of reserves stored in the endosperm into smaller subunits (e.g. maltose and peptides), which are subsequently mobilised to the growing embryo (Kathpalia & Bhatla 2018; Lemmens *et al.* 2019). The effect of low osmotic conditions on the α- and β-amylase enzyme activities of cereal seeds have been investigated in a number of studies (Almansouri *et al.* 2001; Hua-Long *et al.* 2014; Debez *et al.* 2020; Yan *et al.* 2020; Song *et al.* 2023). The findings of these works generally show that both water deficit and excess salt can induce significant decreases in α-, β-, and total-amylase activities depending upon the severity and duration of the stress. The current results, indicating that NaCl and PEG-6000 treatments cause a prominent decrease in the α- and β-amylase activities of bread and durum wheat seeds, are also consistent with these data.

Reactive oxygen species (ROS), like $H_2O_2$ and the hydroxyl radical, are important signalling molecules involved in the regulation of seed dormancy and germination in a certain concentration range, also defined as the oxidative window (Diaz-Vivancos *et al.* 2013). It is known that water uptake promotes the generation of ROS in seeds through metabolism activation. The mitochondrial electron transport chain, NADPH oxidases, and peroxisomes are the main sources of ROS in germinating seeds. ROS have been shown to have a direct or indirect impact on several processes during germination, including protein oxidation, nuclear gene expression, ABA degradation, GA biosynthesis, cell wall loosening, and programmed cell death in the aleurone layers (Oracz & Karpiński 2016; Bailly 2019; Bailly & Merendino 2021). In addition, the antioxidant defence system plays a critical role in the prevention of oxidative damage by maintaining ROS homeostasis (Dumanović *et al.* 2021). In this study, the control seeds of both wheat cultivars had markedly higher $H_2O_2$ content than the PEG-6000- and NaCl-treated seeds, suggesting that stress-exposed seeds exhibit lower metabolic activity due to decreased water uptake. Moreover, it was found that the bread wheat (Bezostaja-1) seeds had greater $H_2O_2$ content and DPPH radical scavenging activity compared to the durum wheat (Kızıltan-91) seeds under both control and stress conditions. This is interesting because several studies have indicated a strong relationship between basal ROS levels and abiotic stress tolerance in plants, such as *Medicago truncatula*, wheat, and rice (Puckette *et al.* 2007; Saini *et al.* 2018; Yadav *et al.* 2019). For instance, Saini *et al.* (2018) found that salt-tolerant rice plants (cv. Luna Suvarna) exhibited higher $H_2O_2$ levels and NADPH oxidase, catalase, superoxide dismutase, and peroxidase activities than salt-sensitive rice plants (cv. IR64) under normal growth conditions. Taken together, it seems that ROS are not only important for the completion of seed germination, but also for improving stress tolerance during the germination period by a possible priming effect (Ellouzi *et al.* 2021).

The amino acid proline and simple sugars (e.g. fructose and sucrose) are compatible solutes which regulate osmotic balance in seeds and plants subjected to abiotic stresses, such as water deficit and salinity (An & Liang 2013; Çatav *et al.* 2022). It has been reported that proline also participates in scavenging free radicals and increasing protein stability (Kaul *et al.* 2008; Norouzi *et al.* 2020). In this study, the proline and total sugar contents of the bread wheat seeds were found to be significantly higher than those of the durum wheat seeds under both stress and non-stress conditions. In particular, Bezostaja-1 accumulated 2.25- and 2.58-fold higher proline compared to Kızıltan-91 in seed treatments with 255 mM NaCl and 27% PEG-6000, respectively. A similar but less pronounced pattern was also observed for the total sugar content of the studied cultivars. Interestingly, previous studies suggested that high $H_2O_2$ levels promoted the accumulation of proline in *Brassica napus* seeds and maize seedlings by increasing the activity of pyrroline-5-carboxylate synthetase, an essential enzyme in the synthesis of proline (Yang *et al.* 2009; Kubala *et al.* 2015). The present results support these findings by showing that bread wheat seeds with high $H_2O_2$ content accumulate more proline than durum wheat seeds with low $H_2O_2$ content.

## CONLUSION

This work provides evidence that seeds of bread wheat cultivars display a higher tolerance to salinity and osmotic stress than those of durum wheat cultivars in the early stage of germination. The water uptake capacity of the bread and durum wheat seeds during germination did not explain the variation in tolerance to these stress conditions. A comparative analysis of their biochemical properties revealed that the bread wheat seeds had intrinsically higher total sugar, proline, and $H_2O_2$ contents and antioxidant activity than the durum wheat seeds. Overall, the results suggest that interaction between oxidative metabolism and compatible solutes may play a pivotal role in improving the germination of wheat seeds exposed to excess salt and water deficit.

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**REZIME**

Botanica SERBICA

# Fiziološki odgovori semena hlebne i durum pšenice na osmotski stres i salinitet u ranoj fazi klijanja

Şükrü Serter Çatav

Utvrđivanje mehanizama koji leže u osnovi tolerancije na osmotski stres i salinitet tokom perioda klijanja je suštinski zadatak za poboljšanje poljoprivredne proizvodnje u sušnim i polusušnim područjima. U ovom radu seme sorti hlebne i durum pšenice tretirano je različitim koncentracijama polietilen glikola (PEG)-6000 i NaCl tokom 1 nedelje i izračunate su polumaksimalne inhibitorne koncentracije (IC50) klijanja. Seme je zatim izloženo IC50 vrednostima NaCl i PEG-6000 tokom 2 dana da bi se procenila fiziološka i biohemijska svojstva. Spektrofotometrijskim metodama određivane su aktivnosti enzima alfa i beta amilaze, redukcioni sadržaj šećera, ukupnog šećera, prolina, proteina i $H_2O_2$, kao i aktivnost uklanjanja DPPH radikala u semenu. Rezultati su pokazali da seme hlebne pšenice ima mnogo veću toleranciju na višak soli i osmotski stres od semena durum pšenice. Konkretno, prosečna vrednost IC50 NaCl za sorte hlebne pšenice bila je skoro dvostruko veća od sorte durum pšenice. Test imbibicije je otkrio da kapacitet semena da upija vodu ne objašnjava razliku u toleranciji na ove stresne uslove. S druge strane, seme hlebne pšenice imalo je konstitutivno veći sadržaj prolina, ukupnog šećera i $H_2O_2$, kao i antioksidativni kapacitet u poređenju sa semenom durum pšenice ($p < 0,05$). U zaključku, trenutni nalazi sugerišu da međudejstvo oksidativnog metabolizma i kompatibilnih rastvora može doprineti poboljšanju tolerancije klijanja u uslovima deficita vode i saliniteta pšenice.

**Ključne reči:** salinitet, osmotski stres, klijanje, pšenica, reaktivne vrste kiseonika, kompatibilne rastvorene supstance