Pretreatment of Seed With Hydrogen Peroxide for Mitigating Salt Stress of Some Hungarian Wheat Landraces at Seedlings Stage

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Abstract

Salt stress is a stringent problem limiting germination and productivity of wheat all over the world, seed pretreatment can effectively induce salt tolerance. The present experiment conducted to investigate the germination, seedling and chemical parameters response of seven Hungarian wheat Landraces (Szentesi, Nyirádi, Kiszombori, Háromfai, Tapiószelei, Nagykállói and Szajlai) to seed soaking at four levels of hydrogen peroxide (H2O2) i.e. control, 0.5, 1.0 and 1.5% subjected to five levels of salt stress (control, 3.0, 6.0, 9.0 and 12.0 dSm−1). Treatments were arranged in Factorial Experimental based on Completely Randomized Design (CRD) with four replications. Under non-saline and salt stress H2O2 soaking significantly influenced all parameters. Soaking at 1.5% resulted the highest germination ability, seedling parameters, relative water content (RWC%), tolerance index (TI%) and potassium (K+) ppm as well proline level in parallel with the lowest sodium content. However, wheat landraces responded differently to the treatments. Háromfai landrace came in the first rank followed by Tapiószelei, Kiszombori, Szajlai, Nyirádi, Szentesi and Nagykállói landraces. Háromfai landrace resulted the highest values of germination parameters, RWC, TI, K+ and proline contents. At the same time, the maximum values of Na+ content was detected in reverse order. Increasing salinity stress levels from 3.0 to 12.0 dSm−1 significantly decreased all studied character except Na+ which was increased as compared with control treatment. Therefore, pretreatment of seeds with 1.5 % of H2O2 increased proline and K+ in contrast to the Na+ content. These results suggest that H2O2 pretreatment helps to increase seedlings growth of Háromfai landrace under high salt stress compared to the other examined wheat landraces.

Keywords: Soaking, H2O2, Salt stress, Seedlings, Sodium, Potassium, proline parameters.

1.Introduction

Wheat (Triticum aestivum L.) is the second most produced food among the cereal crops after maize plant, it is consumed as a staple food by 36% of world population and it has 55% carbohydrates and about 20% of food calories [1]. As it has been reported, wheat landraces are important factor influencing germination performance of the seeds and there are genetic alterations between bread wheat landraces in terms of seedling growth under salt stress [2]. Similarly, seedlings and biochemical features for Hungarian landraces also have different responses to salt stress [3-5]. Around 30% of the agricultural lands are affected by salt stress [6], not only on irrigated areas [7], but also on non-irrigated fields due to the global climate change and other anthropogenic activities, reducing the growth and agricultural yield [8]. High salinity is caused predominantly by NaCl. As reviewed by many authors, under saline conditions water availability decreases causing osmotic stress, but ionic stress also occurs if the ion components of NaCl, especially Na+ ions, reach a toxic level which affect many important life processes during germination and growth [9-13]. The nutritional imbalance disturbs the normal metabolism, cellular homeostasis, membrane functions, activities of several enzymes and hormonal balance. Parallel with these phenomena, photosynthesis is hindered by the secondary effects of disturbed water and ion homeostasis leading to oxidative damage [14-17].

Germination is the crucial and pivotal phase of plants life cycle, and it is the determinant of the following growth and yield characteristics of plants. Salinity of the media up to 125 mM usually causes longer germination phase with lower germination rate, shoot and root length and weight, RWC due to the secondary effects of higher osmotic potential causing changes for water uptake by grains, hormonal balance, enzyme activities in nucleic acid/protein metabolism and food reserves of seeds. Parallel with this, TI is decreased than seeds germinated under non-saline condition [18,19]. According to [20,21] they found...
that wheat germination percentage, growth and yield starts to decrease at stress 6 to 8 dSm\(^{-1}\) level. Same results were reported by [22-25]. Their findings indicate that from 12.0 to 16.0 dSm\(^{-1}\) stress level germination characters, seedling parameters and K\(^+\) were decrease, while Na\(^+\) and proline contents were increased compared to non-salt stress conditions. Furthermore, proline content and higher ratio of potassium to sodium in tissue of wheat plants plays an important protective role under high salinity [4,26].

Several research works have shown that among various strategies, pre-sowing treatments is one of the easiest technique for improving germination percentage and seedling establishment under salt stress, like seed soaking for wheat plants. Pretreatment of seeds by oxidants such as H\(_2\)O\(_2\) are more effective for decreasing adverse consequences of salinity on pre-germination metabolites, enhanced activities of antioxidants and reduced peroxidation of membrane lipids in leaves and roots. It is also effective to regulate in a broad range of physiological processes, greater availability of ATP, early DNA replication, osmotic adjustments, enzyme activation and membrane reorganization [27], acts as a second messenger in response to salt stress in plants [28]. At low levels of H\(_2\)O\(_2\) plays as a signal molecule and involved in acclamatory signaling molecule triggering tolerance against salt stress and increase K\(^+\)/Na\(^+\) ratio in roots and shoots under salt stress, which led to improve germination and seedling parameters [29,30].

Therefore, this study was conducted to identify the impact of pretreatment of seed with H\(_2\)O\(_2\) on seedlings and biochemical parameters of some Hungarian wheat landraces under different salinity gradient.

2. Materials and Methods

2.1 Germination Experiment

Under controlled condition at Research Institute of Nyiregyhaza, Debrecen University (USF), Hungary during December 2020 using growth chamber at 20 ±1 °C, relative humidity 70% and with an 18/6 h day/night period. A laboratory experiment was conducted to indentify the impact of pretreatment of seed soaking at four levels of H\(_2\)O\(_2\) on germination, seedling and biochemical parameters of seven Hungarian wheat landraces subjected to five levels of salt stress. All treatments were arranged in Factorial Experiment based on Completely Randomized Design (CRD) with four replicates. The first factor included four concentrations of pretreatment of seed soaking with H\(_2\)O\(_2\) i.e. Control (without), 0.5, 1.0 and 1.5%, the second factor was included seven of Hungarian wheat landraces i.e. (Szentesi, Nyirádi, Kiszombori, Háromfai, Tapiószelei, Nagykállói and Szajlai) and the third factor was four different salinity gradients i.e. control without (0), 3.0, 6.0, 9.0 and 12.0 dSm\(^{-1}\) using NaCl. Thus, the whole experiment included 420 units (plastic box). Seeds were sterilized using sodium hypochloride (2%) for 10 minutes after that washed with distilled water according to [31]. According to the International Rules of Seed Testing Association [32], 25 healthy seeds of each treatment for each landrace were allowed to germinate on a filter paper in sterile plastic box. Then was moistened with 10 ml of water solution at different salinity stress levels and the plastic boxes were monitored for 9 days to the end of germination.

2.2 Germination Assessment

The following parameters were assessed using [32];
- Final germination percentage (FGP%): 
\[
\text{FGP\%} = \frac{A}{B} \times 100
\]
Where: No of normally germinated seeds and B is the total No of seeds sown.
- Mean Daily Germination (MDG); according to [33]
\[
\text{MDG} = \frac{\text{FGP\%}}{\text{number of days to final germination}}\]

2.3 Seedling Growth Assessment

At the end of the germination period, ten seedlings were randomly selected from each plastic box to assess seedling growth assessment. After selecting seedlings, shoot length (cm) and root length (cm) were measured; seedling vigor index was calculated by multiplying means of GP\% by means of seedling length according to [34]; fresh weight and dry weight of shoot and root (g) after drying the samples in an oven for 72 h at 80 °C till constant weight to resulted seedling dry weight; water relative content (RWC\%), it was calculated according to [35] using the following equation:
\[
\text{RWC\%} = \frac{Fw - Dw}{Tw - Dw} \times 100
\]
Where, Fw, Dw and Tw were the fresh, dry, and turgid weight of leaves, respectively. Tolerance index (TI\%), it was estimated using this equation:

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2.4 Chemical parameters

-Sodium (Na⁺) and potassium (K⁺) contents: Na⁺ and K⁺ contents were determined in the digested plant samples using Tri acid mixture (10:5:4) of nitric acid, sulphuric acid and perchloric acid according to [36]. The content of Na⁺ and K⁺ were estimated using flame photometer at a wavelength (767 and 589 nm).

- Content of proline: Proline content was determined spectrophotometrically by adopting the ninhydrin method of [37]. Fresh leaves (300 mg) were homogenized in 3 ml of 3% sulphosalicylic acid. The homogenate filtrate was reacted with 1 ml each of acid ninhydrin and glacial acetic acid for 1h in a test tube placed in a water bath at 100°C. The mixture was extracted with toluene and the absorbance was measured at 520 nm using L-proline as a standard. Concentration OF proline was determined using calibration curve and expressed as mg/g fresh weight of tissue.

2.5 Statistical Analysis

Overall, all experimental results were statistically analyzed using analysis of variance (ANOVA) in accordance with factorial experiment based on Completely Randomized Design, using MSTAT statistical package according to [38], and means were separated with LSD test at P < 0.05 [39].

3. Results

3.1 Pretreatment of Seed with H₂O₂ Effects

Seed soaking pretreatment using H₂O₂ concentrations had significant positive effect on different aspects of germination characters, seedlings parameters, RWC, TI and chemical analysis as presented in Tables 1, 2 and 3. Soaking seeds in H₂O₂ significantly increased germination and seedlings parameters as compared with the control treatment (without soaking).

Highest final germination percentage (74.69 %), mean daily germination (1.26 day), shoot length (20.07 cm), root length (9.09 cm), seedling vigor index (23.48), shoot fresh weight (0.249 g), dry weight of shoot (0.126 g), fresh weight of root (0.099 g) and dry weight of root (0.057 g), RWC (65.33 %), TI (76.71 %), K⁺ content (729.43 ppm) and content of proline (105.41 mg/g fresh weight) were resulted from seed soaking in H₂O₂ at 1.5%. While, the lowest values of germination characters, seedling parameters and K⁺ and proline contents were obtained under the control treatment (without seed soaking). It could be summarized that FGP %, shoot length, root length, SVI, fresh weight of shoot, dry weight of shoot, fresh weight of root, dry weight of root, RWC, TI, K⁺ and contents of proline were increased by (16.93, 22.62 and 22.82 %), (51.49, 50.57 and 78.08 %), (63.06, 77.34 and 85.51 %), (56.10, 77.25 and 96.32 %), (59.25, 74.07 and 84.44 %), (72.72, 107.27 and 129.09 %), (8.33, 15.47 and 17.85 %), (10.81, 27.02 and 54.05 %), (1.73, 4.44 and 4.82 %), (5.85, 13.60 and 41.98 %), (5.72, 16.37 and 20.73 %) and (14.00, 19.40 and 23.22 %) with pretreatment in 0.5, 1.0 and 1.5 % of H₂O₂ as compared with control treatment, respectively.

While MDG and Na⁺ were decreased by (8.21, 37.11 and 64.30 %) and (5.68, 7.82 and 8.90 %) with increasing H₂O₂ concentrations from 0.5, 1.0 to 1.5 % as compared with control treatment, respectively.

3.2 Hungarian Wheat landraces Performance

Significant differences were found in germination characters, seedlings parameters, RWC, TI, Na⁺, K⁺ and contents of proline between wheat landraces (Tables 1, 2 and 3). The obtained results detected that Háromfai landrace came in the first rank and recorded the highest values of FGP (79.70 %), shoot length (18.798 cm), root length (9.53 cm), SVI (24.26), fresh weight of shoot (0.248 g), dry weight of shoot (0.126 g), fresh weight of root (0.105 g), dry weight of root (0.058 g), RWC (75.06 %), TI (79.11 %). K⁺ content (670.42 ppm) and proline content (119.66 mg/g fresh weight), followed by Tapiószelei, Kiszombori, Szajlai, Nyírádi, Szentesi and Nagykállói landrace came in the last rank. On the other the lowest values of MDG (2.327 day) and Na⁺ (1802.32 ppm) were resulted with Háromfai landrace as compared with the other landraces under studies. As our results the increases in Háromfai landrace surpassed Tapiószelei, Kiszombori, Szajlai, Nyírádi, Szentesi and Nagykállói landrace by (4.052, 7.99, 13.73, 15.15, 19.28 and 22.38 %), (5.81, 12.26, 15.00, 20.28, 23.90 and 30.71 %), (9.33, 18.36, 23.39, 25.81, 27.80 and 32.10 %), (9.31, 18.17, 24.64, 29.51, 34.33 and 40.76 %), (6.45, 13.31, 17.74, 23.79 and 27.82 %), (9.52, 9.52, 24.60, 24.60, 56.35 and 56.35 %), (4.76, 9.52, 12.38, 15.24 and 20.95 %), (8.62, 18.97, 22.41, 27.59, 31.03 and 37.93 %), (8.27, 9.91, 13.02, 18.94, 22.60 and 30.07 %), (3.31, 10.11, 12.48, 13.69, 14.84 and 16.76 %), (3.68, 8.64, 11.96, 14.86, 19.75 and 42.66 %) and (6.02, 12.63, 18.45, 21.90, 32.86 and 42.66) for FGP, (shoot and root length), SVI, (shoot fresh and shoot dry weight), (shoot and root dry weight), RWC, TI, K⁺ and contents of proline, respectively. On the contrary, MDG and Na⁺ contents were decreased by (3.05, 6.70, 7.90, 14.09, 18.30 and 22.64 %) and (4.45, 8.17, 31.31,
40.34, 43.01 and 46.55%) in Háromfai landrace as compared with Tapiószelei, Kiszombori, Szajlai, Nyirádi, Szentesi and Nagykállói landraces, respectively.

3.3 Salt Stress Effects

Our results clearly in (Tables 1, 2 and 3) showed that there are significant effects due to salt stress on germination, seedlings and chemical parameters. All studied parameters were decreased with increasing salt stress up to 12 dSm\(^{-1}\). The reduction of FGP, (shoot and root length), (shoot fresh and shoot dry weight), (root fresh and root dry weight), SVI, RWC, TI, K\(^+\) contents were reached about (7.66, 21.08, 46.31 and 60.28%), (15.08, 34.37, 56.01, 44.84 and 63.29%), (9.38, 22.98, 44.84 and 63.04%), (19.53, 44.84, 73.53 and 84.02%), (9.54, 26.15, 41.70 and 53.36%), (7.75, 19.38, 37.21 and 55.04%), (11.86, 22.03, 30.51 and 41.53%), (19.72, 36.62, 50.70 and 69.01%), (7.21, 18.52, 33.70 and 36.57%), (12.40, 26.44, 43.71 and 62.12%), (8.64, 11.43, 19.38, 28.49%) with salt stress 3.0, 6.0, 9.0 and 2 dSm\(^{-1}\), respectively. While the increases in MDG, Na\(^+\) and proline contents were reached (23.13, 70.63, 95.63 and 111.88%), (81.98, 100.46, 104.66 and 114.25%), and (37.71, 64.72, 88.42 and 103.02%) with salt stress 3.0, 6.0, 9.0 and 2 dSm\(^{-1}\) in comparison with control treatments.

3.4 Interactions Effect

Under this study we found many significant effects of the interactions among factors under studies as showed in Tables 1, 2 and 3. For that reason, we focused herein on the significant of the triple interactions only. Data graphically illustrated in Figs from 1 to 6 clearly showed that the highest averages of GP % (Fig. 1), SVI (Fig. 2), RWC (Fig. 3), TI (Fig. 4), K\(^+\) content (Fig. 5) and proline content (Fig. 6) were resulted from pretreatment of Háromfai seeds at 1.5% of H\(_2\)O\(_2\) under non salt stress, followed by Tapiószelei, Kiszombori, Szajlai, Nyirádi, Szentesi and Nagykállói landrace under the same condition of seeds soaking and control treatment. While the lowest values of these above-mentioned characters and Na\(^+\) content (Fig. 7) were recorded with Nagykállói landrace besides non soaking in H\(_2\)O\(_2\) under the highest levels of salinity stress.

**Table 1.** Effect of seed soaking in H\(_2\)O\(_2\), Hungarian wheat landraces, salinity levels and their interactions on germination percentage (GP%), mean daily germination (MDG), (shoot and root length cm) and seedling vigor index (SVI).

| Source of variance | Germination percentage (GP%) | Mean daily germination (MDG) | Shoot length (cm) | Root length (cm) | Seedling vigor index (SVI) |
|--------------------|-------------------------------|-----------------------------|-------------------|-----------------|--------------------------|
| Control (without)  | 60.81                         | 3.53                        | 11.27             | 4.90            | 11.96                    |
| 1.0                | 71.11                         | 3.24                        | 15.27             | 7.99            | 18.67                    |
| 2.0                | 74.57                         | 2.22                        | 16.97             | 8.69            | 21.20                    |
| 3.0                | 74.69                         | 1.26                        | 20.07             | 9.09            | 23.48                    |
| LSD at 5 %         | 2.41                          | 0.08                        | 0.18              | 0.08            | 0.45                     |

Hungarian landraces performances

| Source of variance | Germination percentage (GP%) | Mean daily germination (MDG) | Shoot length (cm) | Root length (cm) | Seedling vigor index (SVI) |
|--------------------|-------------------------------|-----------------------------|-------------------|-----------------|--------------------------|
| Szentesi           | 64.33                         | 2.753                       | 14.305            | 6.88            | 15.93                    |
| Nyirádi            | 67.62                         | 2.655                       | 14.985            | 7.07            | 17.10                    |
| Kiszombori         | 73.33                         | 2.483                       | 16.492            | 7.78            | 19.85                    |
| Háromfai           | 79.70                         | 2.327                       | 18.798            | 9.53            | 24.26                    |
| Tapiószelei        | 76.47                         | 2.398                       | 17.705            | 8.64            | 22.00                    |
| Nagykállói         | 61.86                         | 2.854                       | 13.025            | 6.47            | 14.37                    |
| Szajlai            | 68.75                         | 2.511                       | 15.978            | 7.30            | 18.28                    |
| LSD at 5 %         | 3.18                          | 0.10                        | 0.23              | 0.11            | 0.71                     |

Salinity stress levels (dSm\(^{-1}\))

| Source of variance | Germination percentage (GP%) | Mean daily germination (MDG) | Shoot length (cm) | Root length (cm) | Seedling vigor index (SVI) |
|--------------------|-------------------------------|-----------------------------|-------------------|-----------------|--------------------------|
| Control (distilled water) | 96.38                       | 1.60                        | 24.21             | 10.66           | 33.85                    |
| 3.0                | 89.00                         | 1.97                        | 20.56             | 9.66            | 27.24                    |
| 6.0                | 76.06                         | 2.73                        | 15.89             | 8.21            | 18.67                    |
| 9.0                | 51.75                         | 3.13                        | 10.65             | 5.88            | 8.96                     |
| 12.0               | 38.28                         | 3.39                        | 8.16              | 3.94            | 5.41                     |
| LSD at 5 %         | 2.69                          | 0.08                        | 0.19              | 0.09            | 0.59                     |

Interactions (F. test)

| Interactions | F. test | P-value |
|--------------|---------|---------|
| A×B          | NS      | NS      |
| A×C          | *       | NS      |
| B×C          | *       | *       |
Table 2. Effect of seed soaking in H$_2$O$_2$, Hungarian wheat landraces, salinity levels and their interactions on shoot & root fresh and dry weight (g) and water relative content (%).

| Source of variance | Shoot fresh weight (mg) | Shoot dry weight (mg) | Root fresh weight (mg) | Root dry weight (mg) | Water relative content (%) |
|--------------------|-------------------------|-----------------------|------------------------|----------------------|---------------------------|
| Control (without)  | 0.135                   | 0.055                 | 0.084                  | 0.037                | 62.32                     |
| 1%                 | 0.215                   | 0.095                 | 0.091                  | 0.041                | 63.40                     |
| 2%                 | 0.235                   | 0.114                 | 0.097                  | 0.047                | 65.09                     |
| 3%                 | 0.249                   | 0.126                 | 0.099                  | 0.057                | 65.33                     |
| LSD at 5 %         | 0.011                   | 0.012                 | 0.001                  | 0.011                | 0.211                     |

Hungarian landraces performances

| Source of variance | Shoot fresh weight (mg) | Shoot dry weight (mg) | Root fresh weight (mg) | Root dry weight (mg) | Water relative content (%) |
|--------------------|-------------------------|-----------------------|------------------------|----------------------|---------------------------|
| Szentesi           | 0.189                   | 0.055                 | 0.087                  | 0.040                | 58.10                     |
| Nyirádi            | 0.194                   | 0.095                 | 0.090                  | 0.042                | 60.84                     |
| Kiszombori         | 0.215                   | 0.114                 | 0.095                  | 0.047                | 67.62                     |
| Háromfai           | 0.248                   | 0.126                 | 0.095                  | 0.058                | 75.06                     |
| Tapiószelei        | 0.232                   | 0.114                 | 0.090                  | 0.053                | 68.85                     |
| Nagykállói         | 0.179                   | 0.055                 | 0.083                  | 0.036                | 52.49                     |
| Szajlai            | 0.204                   | 0.095                 | 0.089                  | 0.045                | 65.29                     |
| LSD at 5 %         | 0.002                   | 0.001                 | 0.001                  | 0.001                | 0.81                      |

Salinity stress levels (dSm$^{-1}$)

| Source of variance | Tolerance index (TI %) | K$^+$ content (PPM) | Proline content (mg/g fresh weight) | Na$^+$ content (PPM) |
|--------------------|------------------------|---------------------|-------------------------------------|----------------------|
| Control (distilled water) | 62.25                 | 513.73              | 87.31                               | 2383.48              |
| 3.0                | 70.97                  | 543.83              | 87.31                               | 2247.90              |
| 6.0                | 74.33                  | 583.63              | 101.61                              | 2197.00              |
| 9.0                | 76.71                  | 729.43              | 105.41                              | 1949.59              |
| 12.0               | 79.11                  | 670.42              | 119.66                              | 1802.32              |
| LSD at 5 %         | 0.48                   | 6.30                | 0.91                                | 12.01                |

Hungarian landraces

| Source of variance | Tolerance index (TI %) | K$^+$ content (PPM) | Proline content (mg/g fresh weight) | Na$^+$ content (PPM) |
|--------------------|------------------------|---------------------|-------------------------------------|----------------------|
| Szentesi           | 67.37                  | 538.02              | 80.34                               | 2577.45              |
| Nyirádi            | 68.28                  | 570.82              | 93.45                               | 2529.35              |
| Kiszombori         | 71.11                  | 612.49              | 104.55                              | 1949.59              |
| Háromfai           | 79.11                  | 670.42              | 119.66                              | 1802.32              |
| Tapiószelei        | 76.49                  | 645.75              | 112.46                              | 1882.39              |
| Nagykállói         | 65.85                  | 520.89              | 68.61                               | 2641.39              |
| Szajlai            | 69.24                  | 590.22              | 97.58                               | 2366.56              |
| LSD at 5 %         | 0.64                   | 8.34                | 1.21                                | 15.89                |
Figure 1. Effect of seed soaking in H₂O₂, Hungarian wheat landraces and salinity levels on germination percentages (GP%).

Figure 2. Effect of seed soaking in H₂O₂, Hungarian wheat landraces and salinity levels on seedling vigor index (SVI).

Figure 3. Effect of seed soaking in H₂O₂, Hungarian wheat landraces and salinity levels on water relative content (RWC %).
Figure 4. Effect of seed soaking in H$_2$O$_2$, Hungarian wheat landraces and salinity levels on tolerance index (TI%).

Figure 5. Effect of seed soaking in H$_2$O$_2$, Hungarian wheat landraces and salinity levels on potassium content (K$^+$ ppm).

Figure 6. Effect of seed soaking in H$_2$O$_2$, Hungarian wheat landraces and salinity levels on proline content (mg/fresh weight).
Figure 7. Effect of seed soaking in H$_2$O$_2$, Hungarian wheat landraces and salinity levels on sodium content (Na$^+$ ppm).

4. Discussion

In this paper the identification of the impact of H$_2$O$_2$ pretreatment of seeds on seedlings and biochemical parameters was aimed on some Hungarian wheat landraces under different salinity gradient. From our results it can be noticed that significant differences were found in the different parameters between the examined lines. In connection with this, [2] have reported, that seeds of wheat landraces have different germination performance and there are genetic alterations between bread wheat landraces too in terms of seedling growth under salt stress. According to these findings, the germination characters, seedlings parameters, RWC, TI, Na$^+$, K$^+$ and proline contents were significantly different between the studied wheat landraces as shown in Tables 1, 2 and 3. Háromfai landrace came in the first rank and showed the most promising values followed by Tapiószelei, Kiszonombori, Szajlai, Nyirádi, Szentesi and Nagykállói landrace came in the last rank. However, all the examined wheat landraces were susceptible to salt stress during germination stage. As it has recently been reported, these findings might be in relation to the genetic differences between these landraces, which might have significant effects on germination characters, seedlings parameters, RWC, TI and K$^+$, proline as well as Na$^+$ contents. In this context, it has also been documented that there are significant differences between several Hungarian wheat landraces in seedling and chemical parameters under salt stress [2,4,5].

Salinity also has a significant effect on the germination ability and growth [20]. It is not surprising, then, the Hungarian landraces generally germinated in shorter times and resulted the highest values of germination and seedling parameters under control circumstances than the stressed plants (Table 1, 2 and 3). The decrease in germination and seedling parameters parallel with the increase in salt doses may be due to some salt created phenomena. It is known that lower osmotic potential of germination media hampers the imbibition of water by seeds, or it results hormonal imbalance. This might cause an inhibition of normal activities of enzymes responsible for nucleic acid and protein metabolism and deteriorates the food reserves of seeds [18]. Furthermore, low water potential and excess sodium ion accumulation often disturb the normal ion uptake and adequate metabolic processes of wheat plants [10,11,13]. All these often increase the level of reactive oxygen species, which disturb biomembrane structure and function thus all of the cellular homeostasis. NaCl induced osmotic stress ultimately results in dehydration of the cell which disrupts the osmotic equilibrium and hinders water uptake by roots and finally ends up to water deficits [16,17]. On the other hand, the osmotic stress, also leads to a cascade of events, in whole seedling level to synthetize sugars and other osmolytes, through the process known as osmotic adjustment, in order to decrease cellular osmotic potential, thereby helping the diffusion of water into the leaf resulting in increase in leaf turgor [9].

Although, under salt stress the germination characters and seedling parameters are decreased [4,5,19,23,26], these protective mechanisms against the osmotic stress have been considered to play a significant role in the different tolerance of wheat landraces (Fig. 4) against the osmotic effects [3]. This is supported by the fact that K$^+$ content was decrease, while Na$^+$ and protecting osmolyte contents were increased during the stress (Fig. 7). However, these changes were altered for the examined landraces, thus the role of different regulating/protecting mechanisms is also not negligible.

Pre-sowing treatments has shown one of the easiest techniques for improving germination percentage and seedling establishment under salt stress [27]. Using of exogenous H$_2$O$_2$ had significant effects on germination, seedlings, RWC, TI, K$^+$, proline and Na$^+$ contents (Tables 1, 2 and 3). This is possibly related to the fact that H$_2$O$_2$ at low concentration operates as
a signal molecule [28], triggering tolerance against salt stress. In this context, H$_2$O$_2$ has also reported increasing K$^+$/Na$^+$ ratio in roots and shoots under salt stress, which led to improve germination ability and seedling parameters [29,30]. In addition, H$_2$O$_2$ treatment plays a crucial role for decreasing adverse effects of high salinity by antioxidants and reduced peroxidation of membrane lipids in leaves and roots. Furthermore, it also takes part in a broad range regulation of physiological processes, greater availability of ATP, early DNA replication, osmotic adjustment, enzyme activation and membrane reorganization [27]. According to the above-mentioned facts, pretreatment of seeds for Háromfai landrace with 1.5 % of H$_2$O$_2$ had promising effects for improving GP % (Fig. 1), SVI (Fig. 2), RWC (Fig. 3), TI (Fig. 4), K$^+$ content (Fig. 5) and proline content (Fig. 6) under salt stress. These results might be attributed to the positive effects of H$_2$O$_2$ as a signal molecule. It is possibly involved in the acclamatory signaling and triggering tolerance against salt stress and increase K$^+$/Na$^+$ ratio in roots and shoots under salt stress, which led to increase germination and seedling parameters [29,30].

**Conclusion**

In conclusion, the results proved that pretreatment of wheat seeds with 1.5 % of H$_2$O$_2$ increased proline and K$^+$ contents as compared with Na$^+$ content, which helps to increase seedlings growth of Háromfai landrace under high salt stress compared to the other examined landraces. As the good salt tolerance traits were able to manifest in this genotype thus it is hoped that this landrace will adequately be cultivable in moderately salty fields. Therefore, Háromfai landrace may be a good candidate for sowing to saline soils in Hungary.

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