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# Impact of Hydrogen Peroxide Pre-Treatment of Seed During Egyptian Wheat Cultivars Seedlings Stage Under Salinity Stress

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## Abstract

Inducing salt tolerance by seed pre-treatment is an efficient way to combat salt stress. The goal of the current study was to determine how seven Egyptian wheat cultivars i.e., Giza 171, Sakha 95, Misr 2, Misr 3, Misr 4, Sides 14 and Gemiza 12 reacted to seed priming at four different levels of hydrogen peroxide ($H_2O_2$) i.e., control, 1.0, 2.0, and 3.0% subjected to five levels of salt stress (control, 3.0, 6.0, 9.0 and 12.0 $dSm^{-1}$). Treatments were set up in a factorial experimental design using a Completely Randomised Design (CRD) with four replications. 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, cultivars responded differently to the treatments. Misr 3 cultivar came in the first rank followed by Misr 4, Misr 2, Sakha 95, Giza 171, Gemiza 12 and sides 14. Misr 3 cultivar resulted the highest values of germination parameters, RWC, TI, $K^+$ and proline contents. 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, pre-treatment of seeds with 1.5 % of $H_2O_2$ increased proline and $K^+$ in contrast to the $Na^+$ content. These results suggest that $H_2O_2$ pre-treatment helps to increase seedlings growth of Misr 3 under salinity conditions compared to the other examined wheat cultivars.

**Keyword:** wheat; cultivars; $H_2O_2$, salinity, seedlings.

# تأثير المعاملة المسبقة لبعض أصناف القمح المصري لفوق أكسيد الهيدروجين أثناء مرحلة الإنبات تحت ظروف الإجهاد الملحى

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## الملخص

الهدف من الدراسة الحالية هو تحديد إستجابة سبعة أصناف من القمح المصري، وهي جيزة 171، وسخا 95، ومصر 2، ومصر 3، ومصر 4، وسدس 14، وجميزة 12، نقع التقاوى عند أربعة مستويات مختلفة من فوق أكسيد الهيدروجين ($H_2O_2$) وهي 1.0، 2.0، و3.0٪، تحت خمسة مستويات من الإجهاد الملحى (بدون ملوحة، 3.0، 6.0، 9.0 و12.0 $dSm^{-1}$). أجريت التجربة بمعمل التقاوي وإختبارات البذور – قسم المحاصيل – كلية الزراعة – جامعة المنصورة – مصر باستخدام التجربة العاملية فى تصميم التام العشوائية (CRD) بأربعة مكررات. أظهرت النتائج أن نقع التقاوى فى $H_2O_2$ أثر معنوياً على جميع العوامل تحت الدراسة. أدى النقع بنسبة 1.5% إلى تسجيل أعلى نسبة إنبات، وصفات البادرات، والمحتوى المائي النسبي (RWC%)، ودليل تحمل الملوحة (TI%)، والبوتاسيوم ($K^+$ جزء في المليون) بالإضافة إلى مستوى البرولين بالتوازي مع أقل محتوى صوديوم. أظهر الصنف مصر 3 أكثر تفوقا يليه مصر 4، مصر 2، سخا 95، جيزة 171، جميزة 12 وسدس 14. وقد حقق الصنف مصر 3 أعلى قيم معاملات الإنبات، RWC، TI، $K^+$ ومحتويات البرولين. وفي الوقت نفسه، تم اكتشاف القيم القصوى لمحتوى $Na^+$ بترتيب عكسي. أدت زيادة مستويات إجهاد الملوحة من 3.0 إلى 12.0 ديسيسيمنز-1 إلى انخفاض معنوي في جميع الصفات المدروسة باستثناء $Na^+$ الذي ارتفع مقارنة بمعاملة الكنترول. ولذلك فإن المعالجة المسبقة للبذور بنسبة 1.5% من $H_2O_2$ أدت إلى زيادة البرولين والبوتاسيوم على عكس محتوى $Na^+$.تشير هذه النتائج إلى أن المعالجة المسبقة بـ $H_2O_2$ تساعد على زيادة صفات الإنبات والبادرات لصنف القمح مصر 3 وذلك تحت ظروف الإجهاد الملحي العالي مقارنة بأصناف القمح الأخرى التي تم فحصها.

**الكلمات المفتاحية:** القمح، الأصناف فوق أكسيد الهيدروجين, الملوحة، البادرات.

## Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops. Egypt is one of highest wheat importer in the world, therefore they try decrease they dependence from the import by increase the wheat production (Asseng *et al.* 2018). But there are two main task what need to be solve: increasing the amount of fertile land and irrigation. The irrigation causes the soil salinization not only Egypt but all in the world (Jousif 2017) that impacts to the plant production.

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The high salt concentration of soil has harmful effect on not only plant metabolism but plant physiologist and biochemicals processes. The wheat grain yield, among others, is influenced by environmental, weather and salt stress too. The effect of these factors, like too high or low temperature or high salt level influence the yield negatively (Atak *et al.* 2006). The high salt content of soil caused mostly by sodium chloride (NaCl). The hight NaCl concentration decrease the water availability by plants. also poisons the plant's metabolism and results in nutritional deficiencies (Azevedo *et al.*, 2006). With the NaCl concentration increasing the germination percentage decrease (Akbarimoghaddam *et al.* 2011).

The hydrogen peroxide ($H_2O_2$) is a chemical catalyst and has reducing and oxidizing properties as well. The $H_2O_2$ is important cellular component and has function to the aerobic organisms' development and metabolism (Bienert *et al.* 2006). It regulates among others the acclimatization and defence of plants. $H_2O_2$ plays role the plant stress tolerance improving by decrease the free radicals as a result of which the ability of plants to survive improves (Hemalatha *et al.* 2017). Several researchers reported positive effect about the seed soaking into $H_2O_2$, like seed vigour, the germination rate and plant growth mainly under unfavourable environmental conditions (Ashraf & Foolad 2005; Ashraf *et al.* 2015). The seed priming with $H_2O_2$ can be able to increase the heat and cold, drought and salt stress resistance ability of plants (Uchida *et al.* 2002). The seed priming has positive effect to the seed quality, better germination and results higher vigour (Wojtyla *et al.* 2016). The $H_2O_2$ is one of the chemical materials which level increase in under the abiotic and biotic stress of plants. This molecule activates the resistance ability to stress ability of plants (Xu *et al.* 2010). Several researches indicated that the higher $H_2O_2$ level in usually results the toxicity of cellular membrane system and harmful for plant cells (Sairam *et al.* 2002; Sairam & Tyagi 2004; Kathiresan *et al.* 2006). The plant proline content increase after water or salt stress (Vendruscolo *et al.* 2007 and Poustini *et al.* 2007). There is positive connection between the proline content and osmotic potential of plant (Bajji *et al.* 2001). The proline accumulation is increased by $H_2O_2$ treatment, which increases the plant osmotic concentration consequently the plant water potential is increasing and the water deficit of plant decrease (Ashfaque *et al.* 2014). The higher proline content of plant results in favourable $K^+/Na^+$ ratio (Colmer *et al.* 1996).

Drought and salinity stress may affect plants at any time, but the germination and seedling growth period is one of the most critical periods, and these periods are plant yield determining factors. The seed germination phase of plant life cycle is one of the most important and sensitive periods, mainly under stressful conditions (Çavusoglu & Kabar 2010) which determines the successful plant production (Hemalatha *et al.* 2017). With increasing NaCl concentration, the ratio of shoot/root dry weight increases (Akbarimoghaddam *et al.* 2011). The shoot and root length decrease by NaCl concentration increases and the shoots are more sensitive than roots (Saboora *et al.* 2006). The increase of the NaCl concentration results in the increase of $K^+$ leaking from seed.

Different plant cultivars of wheat give different responses to the different salt stresses (Öner & Kirli 2018), some of them have better, while others have less resistance to the salt stress. Consequently, it is very important to know which kind of wheat cultivars give the best answer to salt stress, and which cultivars are the safest to grow under the given conditions. The higher amount of $K^+$ can able to accumulate into the shoots and roots in salt-tolerant cultivars, compared to non-salt-tolerant cultivars, in where higher amount $Na^+$ accumulate. The salt-tolerant cultivars have higher stability of antioxidant enzyme activity and soluble sugar content, compared to non-salt-tolerant cultivars (Zheng *et al.* 2008). The proline accumulation and $Na^+$ concentration is low in salt tolerant cultivars compared to the non-salt tolerant cultivars (Poustini *et al.* 2007).

Thus, this investigation was carried out to determine the effects of pre-treating seed with $H_2O_2$ on seedlings and biochemical characteristics of certain Egyptian wheat cultivars in various salinity gradients.

## Experimental design and treatments

In January 2023, a growing chamber with 18/6 hours of day and night, a temperature of 20 ±1 °C, and a relative humidity of 70% was used to maintain controlled conditions in the Agronomy Department Laboratory of Seed Testing Faculty of Agriculture, Mansoura University, Egypt. Seven Egyptian wheat cultivars were treated to five degrees of salt stress in a laboratory experiment to determine the effects of pre-treatment of seed soaking at four levels of H2O2 on germination, seedling, and biochemical parameters. Four replicates of each treatment were used in a Factorial Experiment using a completely randomised design (CRD). Four different H2O2 concentrations, including Control (without), 1.0, 2.0, and 3.0%, were used as the initial factor. Seven Egyptian wheat cultivars, namely Giza 171, Sakha 95, Masr 2, Masr 3, Masr 4, Sides 14 and Gemiza 12, were included in the second factor and the third component consisted of four distinct salinity gradients using NaCl: 0 dSm-1 control, 3.0, 6.0, 9.0, and 12.0 dSm-1. Thus, 420 units (plastic boxes) were used throughout the entire experiment. According to Basra et al. (2003), seeds were sterilised using sodium hypochlorite (2%) for 10 minutes before being rinsed with distilled water. 25 healthy seeds of each treatment for each cultivar were permitted to grow on a filter paper in a sterile plastic box in accordance with the International Rules of Seed Testing Association (ISTA 2019). The plastic boxes were then moistened with 10 ml of water solution at various salinity stress levels, and they were observed for 9 days until the conclusion of germination.

Germination Evaluation:

-Final germination percentage (GP%):

$$GP\% = \frac{\text{No. of germinated seed at the seventh day}}{\text{total No. of germinated seeds}} \times 100$$

-Mean Daily Germination (MDG) was determined according to Rubio-Casal et al. (2003) using the following equation: GP% / number of days to final germination.

Seedling Growth Evaluation: Ten seedlings from each plastic box were randomly chosen at the conclusion of the germination phase to evaluate seedling development. Equation 1 was used to calculate the relative water content (RWC%) in accordance with Bejandi et al. (2009) to determine the seedling dry weight after measuring the shoot length (cm) and root length (cm) of the seedlings. Fresh weight and dry weight of the shoot and root (g) were also measured after the samples were dried in an oven for 72 hours at 80 C until constant weight.

$$RWC\% = \frac{Fw - Dw}{Tw - Dw} \qquad \text{Eq. 1}$$

where Fw, Dw, and Tw are the fresh, dry, and turgid weights of leaves, respectively.

Tolerance index (TI%) was estimated using equation 2.

$$TI\% = \frac{\text{Dw of seedlings under salt stress}}{\text{Dw of untreated seedlings}} \qquad \text{Eq. 2}$$

Chemical parameters

- Contents of sodium (Na+) and potassium (K+): Using a triple acid combination (10:5:4) of nitric acid, sulphuric acid, and perchloric acid according to Karmoker (1984), the Na+ and K+ concentrations were evaluated in the digested plant samples. Using a flame photometer operating at a wavelength of 767 and 589 nm, the amounts of Na+ and K+ were calculated.

- Proline content: The ninhydrin technique of Bates et al. (1970) was used to spectrophotometrically determine the proline content. 3% sulfosalicylic acid was used to homogenise 300 mg of fresh leaves. The homogenised filtrate was combined for 1 hour with 1 ml of acid ninhydrin and 1 ml of glacial acetic acid in a test tube heated to 100°C in a water bath. Toluene was used to extract the mixture, and L-proline was used as a reference to measure the absorbance at 520 nm. Using a calibration curve, the concentration of proline was calculated and represented as mg/g fresh weight of tissue.

Statistical Analysis

The IBM SPSS Statistical Software Package 21.0 version was used to evaluate all of the data that were gathered. The analysis of variance (ANOVA) technique for the factorial completely randomised design was followed, as explained by Gomez and Gomez (1984). The means of the treatments were compared using Duncan's multiple range tests, as described by Duncan (1955), with a degree of probability of 5%.

## Results and Discussion

***A. Effects of seed soaking in $H_2O_2$***

According to seed soaking data analysis of germination percentage (77.092%), mean daily germination (3.874 MDG), shoot length (21.743 cm), root length (10.775 cm), shoot and root fresh weight (0.284 mg, 0.137 mg) and dry weight (0.146 mg, 0.075 mg), relative water content (67.316%,) and tolerance index were (79.916%) higher and significantly different at the 3% treatments from the control and the other treatments (Tables 1-3). The $H_2O_2$ is able to trigger the antioxidant activity of seeds, consequently, decreases the oxidative damage, which improves the seedling growth characters and drought stress ability of plants (He *et al.* 2009). The data of germination percentage and relative water content of the 2% treatment were lower (77.035%, 67.076%) than the 3% treatment data but the differences were not significant between the 2% and 3% treatments. While the significantly highest data were resulted by the control treatment in $Na^+$ and $K^+$ and proline content (2472.485 ppm, 774.438 ppm, 110.067 mg/g), then lowest ones were recorded for the 3% $H_2O_2$ seed soaking treatment (2260.203 ppm, 558.738 ppm, 91.967 mg/g). The 1% and 2% $H_2O_2$ seed soaking treatments data of all the analysed characters were between the highest and lowest values and significantly different from the highest and lowest ones.

***B. Performance of the studied cultivars***

Significant differences were found between the data of Egyptian wheat cultivars in all the analysed characters (Tables 1-3). The obtained results detected that Misr 3 cultivar came in the first rank and recorded the highest values of germination percentage (79.628%), shoot length (20.468 cm), root length (11.215 cm), shoot and root fresh weight (0.28 and 0.122 mg) and dry weight (0.152 mg, 0.076 mg), relative water content (77.048%), and tolerance index (82.195%), which were significantly different from all the other cultivars. While Misr 3 cultivar recorded the lowest values of mean daily germination (2.666) followed by Misr 4, Misr 2, Sakha 95, Giza 171, Gemiza 12 and Sides 14. Misr 2 and Misr 4 cultivars were the second and third in terms of germination percentage (76.011%, 76.799%), shoot length (18.161 cm, 19.375 cm), root length (9.468 cm, 10.328 cm), shoot fresh weight (0.250 mg, 0.266 mg), root fresh weight (0.112 mg, 0.119 mg), shoot dry weight (0.141 mg, 0.147 mg), root dry weight (0.065 mg, 0.070 mg), relative water content (69.608%, 70.835%), and tolerance index

(74.224%, 79.803%). The germination percentage of Giza 171 (71.168%), Sakha 95 (7.669%) and Sides14 (65.434%) cultivars ranked them to the second, third, and fourth place and these values significantly differed from of other cultivars. The other characters of these three cultivars were ranked fifth, sixth or last Gemiza 12 cultivar was ranked fourth for all characters (69.530%, 15.975 cm, 8.561 cm, 0.238 mg, 0.107 mg, 0.138 mg, 0.062 mg, 67.275%, 72.356%). Significantly the lowest $Na^+$ content was measured at Misr 2 (1891.325 ppm) and Misr 3 (1971.592 ppm) cultivars. The $Na^+$ content of the other cultivars was significantly higher (Gemiza 12 2038.593 ppm; Sakha 95 2455.564 ppm; Giza 171 2618.352 ppm; Misr 4 2666.458 ppm; Sides 14 2730.396 ppm). The difference between the $K^+$ level of all of the analysed cultivars was significant. The highest $K^+$ level was 715.42ppm (Misr 2) and the lowest one was 565.892 ppm (Sides 14). The different Egyptian wheat cultivars gave significantly different response to the $H_2O_2$ treatments in terms of proline content, consequently, some of the studied cultivars have higher tolerance against salinity or salinity stress than other ones. From the point of view of cultivation, varieties with higher tolerance are more favourable and more recommended. According to the results of Khan *et al.*, (2009), wheat cultivars with higher proline content and $K^+/Na^+$ ratio produced higher yields. High $Na^+$ concentration negatively influences the $K^+$ uptake of plants (Munns *et al.*, 2000). Discrimination of the $Na^+$ and $K^+$ uptake mechanisms may be a useful criterion in case of wheat breeding for salt tolerance (Khan *et. al.*, 2009). The highest proline content was 124.310 mg/g (Sides 14) and the lowest one was 73.261 mg/g (Misr 2). The other cultivars proline contents were between: 117.116 mg/g, Misr 4; 109.203 mg/g, Giza 171; 102.236 mg/g, Sakha 95; 98.101 mg/g, Gemiza 12; 84.994 mg/g, Misr 3.

***C. Salinity stress effects***

Our research results clearly show (Tables 1-3) that there are significant effects due to salt stress on all studied characters. Between all the analysed data, the difference was statistically significant. The best results was reach by the control treatment (without any salt) while the worst ones by the highest salt concentration (12%) treatment in germination percentage (97.633%, 91.979%, 79.311%, 55.005%, 41.244%), mean daily germination (1.948 2.312 MDG, 3.072 MDG, 3.476 MDG, 3.728 MDG), shoot length (25.88 cm, 22.23 cm, 17.56 cm, 12.32 cm, 9.833 cm), root length (12.340 cm, 11.340 cm, 9.893 cm, 7.569 cm, 5.622 cm), shoot fresh weight (0.317 mg, 0.290 mg, 0.243 mg, 0.200 mg, 0.166 mg), shoot dry weight (0.138 mg, 0.129 mg, 0.115 mg, 0.091 mg, 0.068 mg, root fresh weight (0.165 mg, 0.151 mg, 0.138 mg, 0.129 mg, 0.116 mg), root dry weight (0.088 mg, 0.074 mg, 0.062 mg, 0.052 mg, 0.039 mg), relative water content (81.237%, 75.528%, 66.556%, 54.528%, 52.256%), tolerance index (100.13%, 91.467 , 77.457%, 60.180%, 41.777%) and K+ content (730.863 ppm, 671.625 ppm, 652.482 ppm, 597.911 ppm, 535.435 ppm). Na+ and proline content significantly increased with the increasing salt concentration. The Na+ and proline content was the highest in the highest salt treatment (12%) while the lowest one in the control treatment.

***D. Interaction effect***

Many significant interaction effects were found between the seed soaking in the H2O2 and the studied Egyptian wheat cultivars in the germination percentages, mean daily germination,

**Table 1.** Means of germination percentage (GP%), mean daily germination (MDG), shoot length (cm), root length (cm) and seedling vigour (SV) of seven Egyptian wheat cultivars as affected by seed soaking in $H_2O_2$, at different salinity levels and their interactions.

| Characters Treatments | Germination percentage (GP%) | Mean daily germination (MDG) | Shoot length (cm) | Root length (cm) |
|---|---|---|---|---|
| ***Seed soaking in $H_2O_2$ effects*** | | | | |
| Untreated control | 64.062c | 1.607d | 12.943d | 6.586d |
| 1% | 73.948b | 2.562c | 16.943c | 9.675c |
| 2% | 77.035a | 3.587b | 18.643b | 10.375b |
| 3% | 77.092a | 3.874a | 21.743a | 10.775a |
| F. test (at 5%) | * | * | * | * |
| Standard Error of Mean (SEM) | 0.815 | 0.026 | 0.068 | 0.031 |
| Giza 171 | 71.168c | 2.993bc | 16.655f | 8.755f |
| Sakha 95 | 72.669bc | 2.850cd | 17.648e | 8.988e |
| Misr 2 | 76.011ab | 2.823d | 18.161c | 9.468c |
| Misr 3 | 79.628a | 2.666e | 20.468a | 11.215a |
| Misr 4 | 76.799ab | 2.736de | 19.375b | 10.328b |
| Sides 14 | 65.434d | 3.091ab | 14.695g | 8.155g |
| Gemiza 12 | 69.530cd | 3.192a | 15.975 d | 8.561d |
| F. test (at 5%) | * | * | * | * |
| Standard Error of Mean (SEM) | 1.0787 | 0.0354 | 0.0905 | 0.0411 |
| Control (distilled water) | 97.633a | 1.948a | 25.88a | 12.340a |
| 3.0 | 91.979b | 2.312b | 22.23b | 11.340b |
| 6.0 | 79.311c | 3.072c | 17.56c | 9.893c |
| 9.0 | 55.005d | 3.476d | 12.32d | 7.569d |
| 12.0 | 41.244e | 3.728e | 9.833e | 5.622e |
| F. test (at 5%) | * | * | | * |
| Standard Error of Mean (SEM) | 0.9116 | 0.0299 | 0.0765 | 0.0348 |
| A×B (F. test) | * | * | NS | NS |
| A×C (F. test) | * | * | NS | NS |
| B×C (F. test) | * | * | NS | NS |
| A×B×C (F. test) | NS | NS | NS | NS |

**Table 2.** Means of shoot and root fresh and dry weight (g) and relative water content (%) of seven Egyptian wheat cultivars as affected by seed soaking in $H_2O_2$, at different salinity levels and their interactions.

| Characters / Treatments | Shoot fresh weight (mg) | Shoot dry weight (mg) | Root fresh weight (mg) | Root dry weight (mg) | Water relative content (%) |
|---|---|---|---|---|---|
| ***Seed soaking in $H_2O_2$ effects*** | | | | | |
| **Untreated control** | **0.170d** | **0.066d** | **0.131d** | **0.055d** | **64.306c** |
| **1%** | **0.250c** | **0.106c** | **0.138c** | **0.059c** | **65.386b** |
| **2%** | **0.270b** | **0.125b** | **0.144b** | **0.065b** | **67.076a** |
| **3%** | **0.284a** | **0.137a** | **0.146a** | **0.075a** | **67.316a** |
| **F. test (at 5%)** | * | * | * | * | * |
| **Standard Error of Mean (SEM)** | **0.0006** | **0.0002** | **0.0003** | **0.0003** | **0.2713** |
| ***Egyptian cultivars performances*** | | | | | |
| **Giza 171** | **0.223f** | **0.101f** | **0.133f** | **0.057f** | **60.081f** |
| **Sakha 95** | **0.229e** | **0.103e** | **0.136e** | **0.060e** | **62.828e** |
| **Misr 2** | **0.250c** | **0.112c** | **0.141 c** | **0.065c** | **69.608c** |
| **Misr 3** | **0.283a** | **0.122a** | **0.152a** | **0.076a** | **77.048a** |
| **Misr 4** | **0.266b** | **0.119b** | **0.147b** | **0.070b** | **70.835b** |
| **Sides 14** | **0.214g** | **0.096g** | **0.130g** | **0.054g** | **54.475g** |
| **Gemiza 12** | **0.238d** | **0.107d** | **0.138d** | **0.062d** | **67.275d** |
| **F. test (at 5%)** | * | * | * | * | * |
| **Standard Error of Mean (SEM)** | **0.0008** | **0.0002** | **0.0003** | **0.0003** | **0.3589** |
| ***Salinity stress levels ($dSm^{-1}$) effects*** | | | | | |
| **Control (distilled water)** | **0.317a** | **0.139a** | **0.165a** | **0.088a** | **81.237a** |
| **3.0** | **0.290b** | **0.129b** | **0.151b** | **0.074b** | **75.528b** |
| **6.0** | **0.243c** | **0.115c** | **0.138c** | **0.062c** | **66.556c** |
| **9.0** | **0.200d** | **0.091d** | **0.129d** | **0.052d** | **54.528d** |
| **12.0** | **0.1665e** | **0.068e** | **0.116e** | **0.039e** | **52.256e** |
| **F. test (at 5%)** | * | * | * | * | * |
| **Standard Error of Mean (SEM)** | **0.0007** | **0.0002** | **0.0003** | **0.0003** | **0.3033** |
| **A×B (F. test)** | **NS** | **NS** | **NS** | **NS** | * |
| **A×C (F. test)** | **NS** | **NS** | **NS** | **NS** | * |
| **B×C (F. test)** | **NS** | **NS** | **NS** | **NS** | * |
| **A×B×C (F. test)** | **NS** | **NS** | **NS** | **NS** | **NS** |

**Table 3.** Means of tolerance index (TI%), $Na^+$, $K^+$ contents (ppm) and proline content (mg/g fresh weight) of seven Egyptian wheat cultivars as affected by seed soaking in $H_2O_2$, at different salinity levels and their interactions.

| Characters / Treatments | Tolerance index (TI%) | $Na^+$ content ppm) | $K^+$ content (ppm) | Proline content (mg/g) |
|---|---|---|---|---|
| ***Seed soaking in $H_2O_2$ effects*** | | | | |
| **Untreated control** | **65.368d** | **2472.485d** | **774.438d** | **110.067d** |
| **1%** | **74.084c** | **2336.900c** | **628.638c** | **106.267c** |
| **2%** | **77.448b** | **2286.000b** | **588.838b** | **96.967 b** |
| **3%** | **79.916a** | **2260.203a** | **558.738a** | **91.967a** |
| **F. test (at 5%)** | * | * | * | * |
| **Standard Error of Mean (SEM)** | **0.1994** | **4.4970** | **2.4930** | **0.4476** |
| ***Egyptian cultivars performances*** | | | | |
| **Giza 171** | **70.490e** | **2618.352c** | **615.825e** | **109.203c** |
| **Sakha 95** | **71.394de** | **2455.564d** | **635.225d** | **102.236d** |
| **Misr 2** | **74.224c** | **1891.325g** | **715.425a** | **73.261g** |
| **Misr 3** | **82.195a** | **1971.592f** | **690.758b** | **84.994f** |
| **Misr 4** | **79.803b** | **2666.458b** | **583.025 f** | **117.116b** |
| **Sides 14** | **68.963f** | **2730.396a** | **565.892g** | **124.310a** |
| **Gemiza 12** | **72.356d** | **2038.593e** | **657.492c** | **98.101e** |
| **F. test (at 5%)** | * | * | * | * |
| **Standard Error of Mean (SEM)** | **0.2638** | **5.9489** | **3.2979** | **0.5922** |
| ***Salinity stress levels ($dSm^{-1}$) effects*** | | | | |
| **Control (distilled water)** | **100.13a** | **1337.058e** | **730.863a** | **65.539e** |
| **3.0** | **91.467b** | **2360.226d** | **671.625b** | **88.497d** |
| **6.0** | **77.457c** | **2590.902c** | **652.482c** | **104.935c** |
| **9.0** | **60.180d** | **2643.296b** | **597.911d** | **119.363b** |
| **12.0** | **41.777e** | **2763.003a** | **535.435e** | **128.25a** |
| **F. test (at 5%)** | * | * | * | * |
| **Standard Error of Mean (SEM)** | **0.2229** | **5.0278** | **2.7873** | **0.5005** |
| **A×B (F. test)** | * | **NS** | **NS** | **NS** |
| **A×C (F. test)** | * | **NS** | **NS** | **NS** |
| **B×C (F. test)** | * | **NS** | **NS** | **NS** |
| **A×B×C (F. test)** | **NS** | **NS** | **NS** | **NS** |

relative water content and tolerance index. The lowest interaction effect in germination percentage was found at the Sides 14 (57.73; 65.51333; 68.92667; 69.56667) and at the Gemiza 12 (55.23; 73.43333; 76.63333; 72.82333), while the highest interaction was found at the Misr 4 (61.29667; 79.90667; 82.75667; 83.23667) and at the Misr 3 (63.58333; 82.97333; 85.78667; 86.17) cultivars in the control and in all $H_2O_2$ seed soaking concentration treatments (Fig. 1). The lowest interaction effect in mean daily germination was found at the Giza 17 (3.632733; 3.311267; 2.286267; 1.436267) and at the Sakha 95 (3.708333; 3.389333; 2.364333; 1.482667) cultivars, while the highest at the Sides 14 (4.073867; 3.780933; 2.755933; 1.755933) and at the Gemiza 12 (4.19; 3.876867; 2.851867; 1.851867) cultivars in the control and in all $H_2O_2$ seed soaking concentration treatments (Fig. 2). The lowest interaction effect in relative water content was found at the Sides 14 (52.76; 53.84; 55.53; 55.77) and at the Giza 171 (58.36667; 59.44667; 61.13667; 61.37667) cultivars and the highest at the Misr 4 (69.12; 70.2; 71.89; 72.13) and at the Misr 3 (75.33333; 76.41333; 78.10333; 78.34333) cultivars in the control and in all $H_2O_2$ seed soaking concentration treatments (Fig. 3).

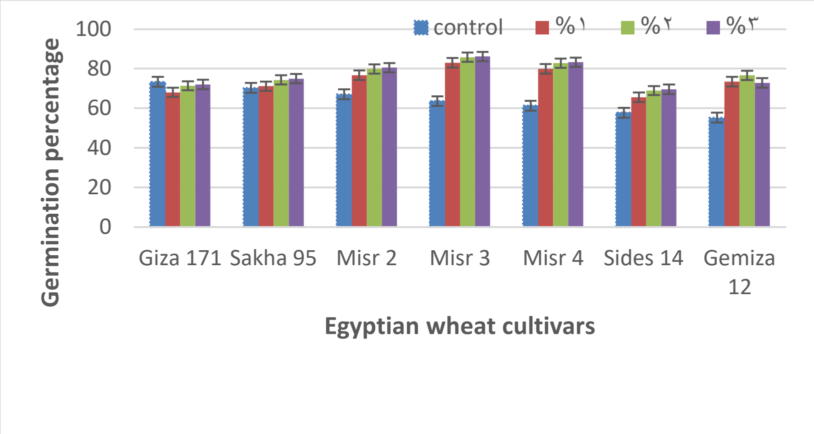


Fig. 1. Impact of the interaction between seed soaking in $H_2O_2$ and Egyptian wheat cultivars on germination percentages.

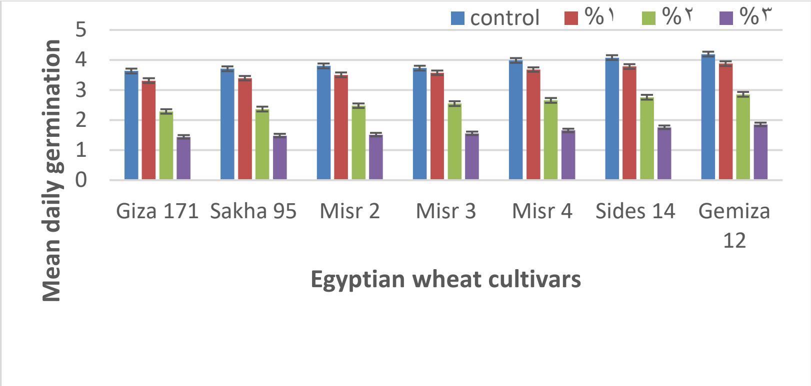


Fig. 2. Impact of the interaction between seed soaking in $H_2O_2$ and Egyptian wheat cultivars on mean daily germination.

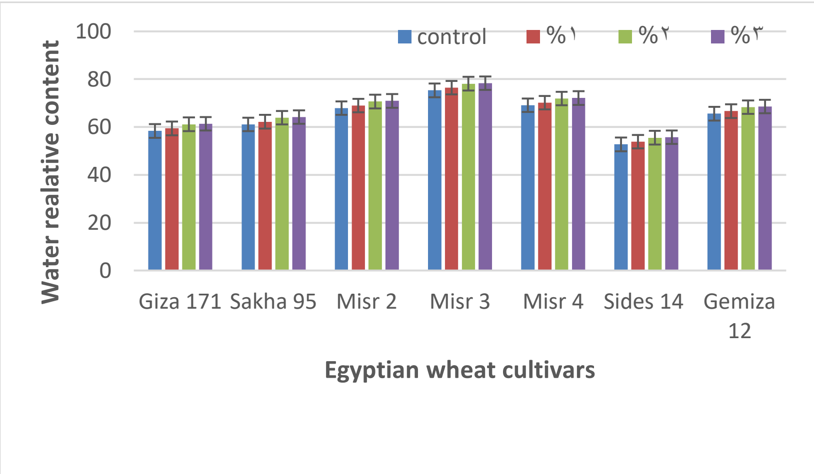


Fig. 3. Impact of the interaction between seed soaking in $H_2O_2$ and Egyptian wheat cultivars on water relative content.

The lowest interaction effect in tolerance index was found at the Sides 14 (57.48947, 68.9464; 73.22493; 76.19173) and at the Giza 171 (60.07233; 70.42653; 74.35673; 77.10813) cultivars and the highest at the Misr 4 (72.9466; 79.4866; 82.07667; 84.70547) and at the Misr 3 (76.4378; 82.10773; 84.37027; 85.8678) cultivars in the control and in all $H_2O_2$ seed soaking concentration treatments (Fig. 4).

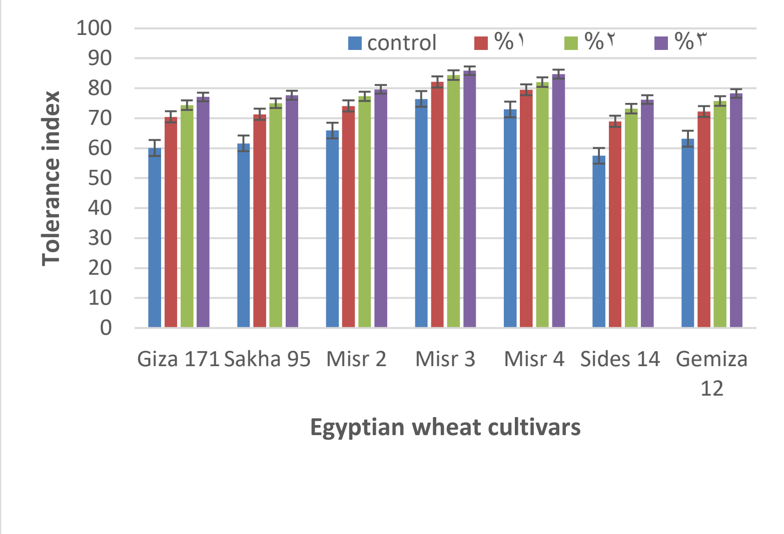


Fig. 4. Impact of the interaction between seed soaking in $H_2O_2$ and Egyptian wheat cultivars on tolerance index.

Many significant interaction effects were found between the seed soaking in the $H_2O_2$ and salinity stress levels on germination percentages, mean daily germination, relative water content and tolerance index (Fig. 5-8). The highest interaction effect were recorded at the salinity stress level in the germination percentage (control: 91.52619; 1%: 99.75238; 2%: 100; 3%: 99.25238), in the mean daily germination (control: 2.420476; 1%: 3.420476; 2% 4.445476; 3%: 4.627714) in the water relative content (control: 79.52286; 1%: 80.60286; 2% 82.29286; 3%: 82.53286) and in the tolerance index (control: 100; 1%: 100; 2% 100; 3%: 100) at the control treatments (without $H_2O_2$ seed soaking) treatments. The interaction between seed soaking in $H_2O_2$ and salinity stress level decreased with the salt level increasing compared to the control data at the germination percentage, the mean daily germination, the relative water content and the tolerance index as well. The lowest interaction data was recorded at 12 $dSm^{-1}$ salt stress level at the germination percentage (control: 27.47857; 1%: 37.77857; 2 %: 41.77857; 3 %:57.94048), the mean daily germination (control: 0.64419; 1%: 1.41919; 2%: 2.44419; 3%: 3.285048), the water relative content (control: 50.5419; 1%: 51.6219; 2%: 53.3119; 3%: 53.5519) and the tolerance index (control: 22.92471; 1%: 41.55938; 2%: 48.76791; 3%: 53.85691) as well.

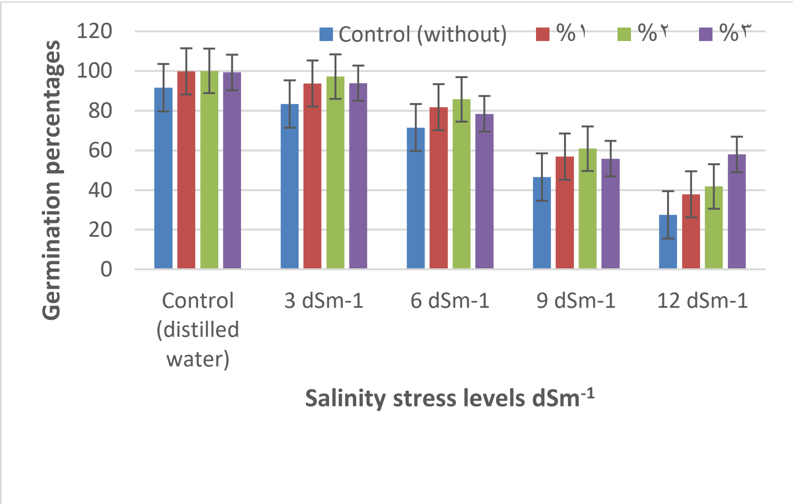


Fig. 5. Impact of the interaction between seed soaking in $H_2O_2$ and salinity stress levels on germination percentages.

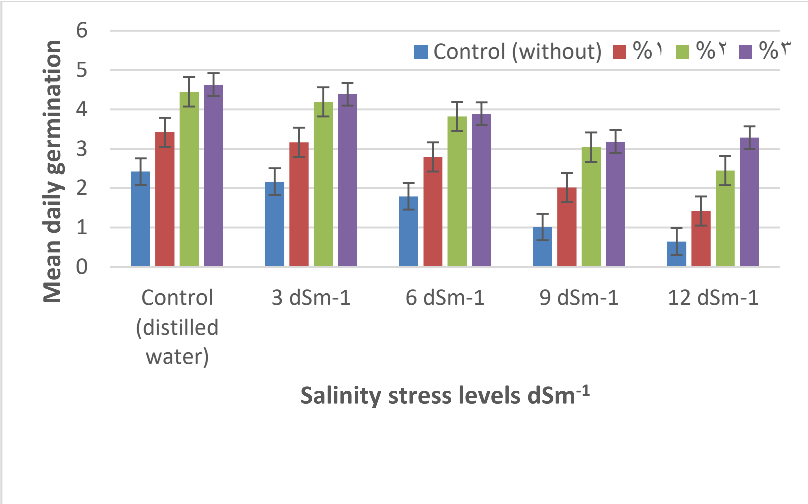


Fig. 6. Impact of the interaction between seed soaking in $H_2O_2$ and salinity stress levels on mean daily germination.

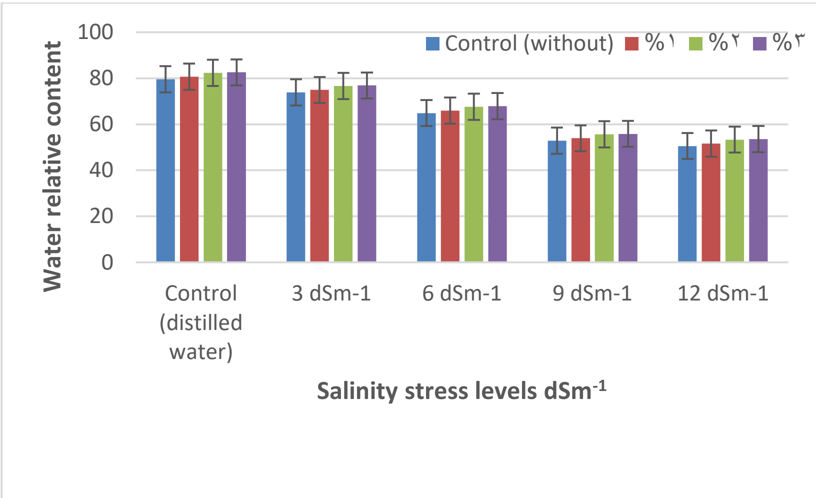


Fig. 7. Impact of the interaction between seed soaking in $H_2O_2$ and salinity stress levels on relative water contents.

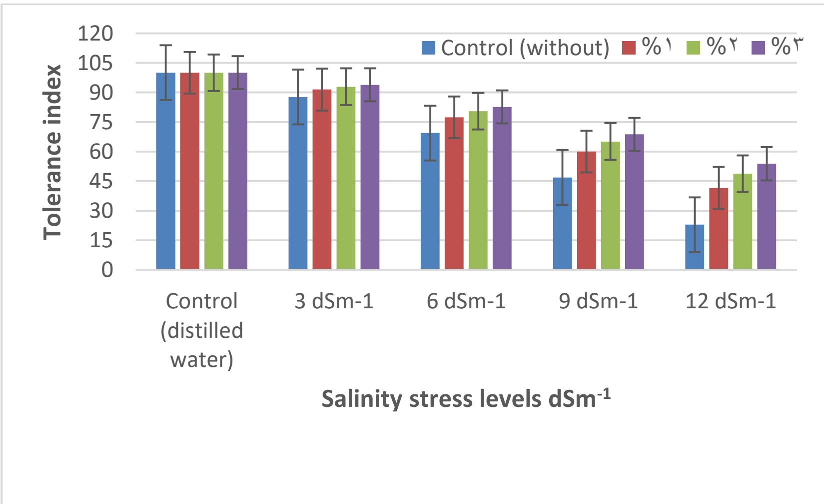


Fig. 8. Impact of the interaction between seed soaking in $H_2O_2$ and salinity stress levels on tolerance index.

Many significant interaction effects were found between the salinity stress levels and Egyptian wheat cultivars in the germination percentages, the mean daily germination, the water relative content and the tolerance index (Figures 9–12) as well. The highest one was found at the control treatments in the germination percentage (Giza 171, 97.675; Sakha 95: 98.00833; Misr 2: 98.2375; Misr 3: 97.9875; Misr 4: 98.0125; Sides 14: 96.45; Gemiza 12: 97.05833), in the mean daily germination (Giza 171, 2.052417; Sakha 95: 1.726333; Misr 2: 1.80575; Misr 3: 1.6975; Misr 4: 1.692083; Sides 14: 2.415333; Gemiza 12: 2.247667), in the water relative content (Giza 171, 74.82833; Sakha 95: 77.495; Misr 2: 86.395; Misr 3: 93.395; Misr 4: 87.96167; Sides 14: 65.495; Gemiza 12: 83.095) and in the tolerance index (Giza 171, 100; Sakha 95: 100; Misr 2: 100; Misr 3: 100; Misr 4: 100; Sides 14: 100; Gemiza 12: 100), compared with salinity stress levels. The interaction effect decreased with the salinity stress level increasing in case of each Egyptian wheat cultivar.

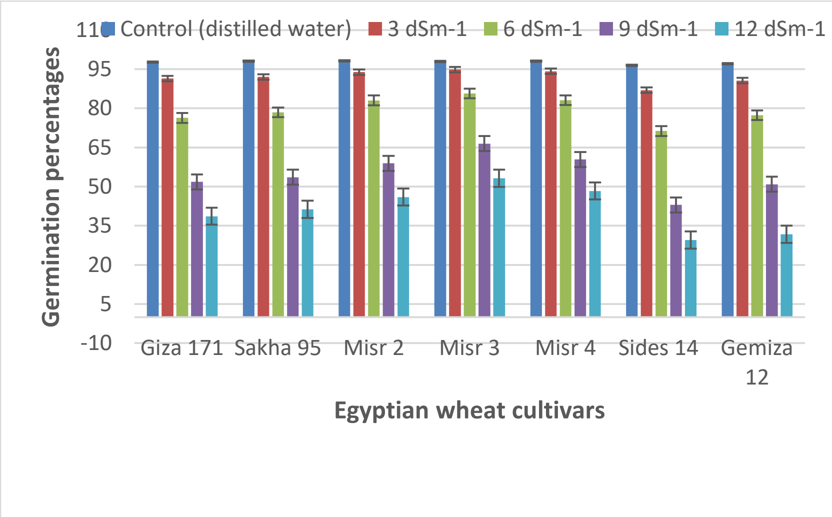


Fig. 9. Impact of the interaction between Egyptian wheat cultivars and salinity stress levels on germination percentages.

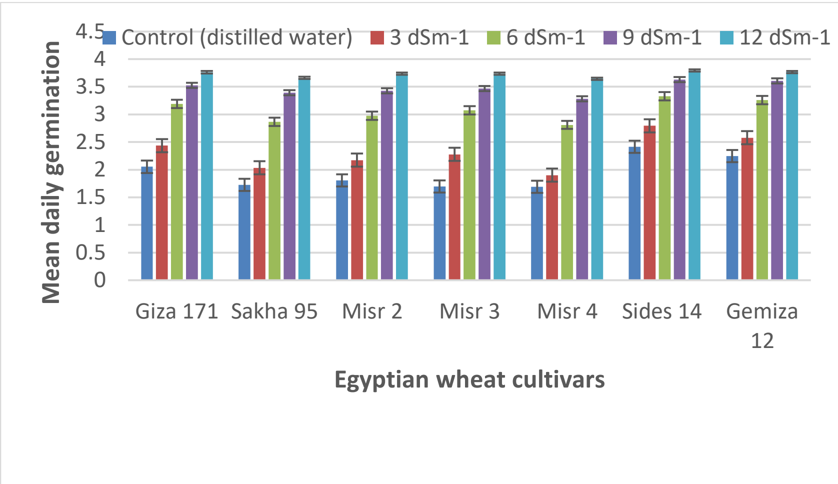


Fig. 10. Impact of the interaction between Egyptian wheat cultivars and salinity stress levels on mean daily germination.

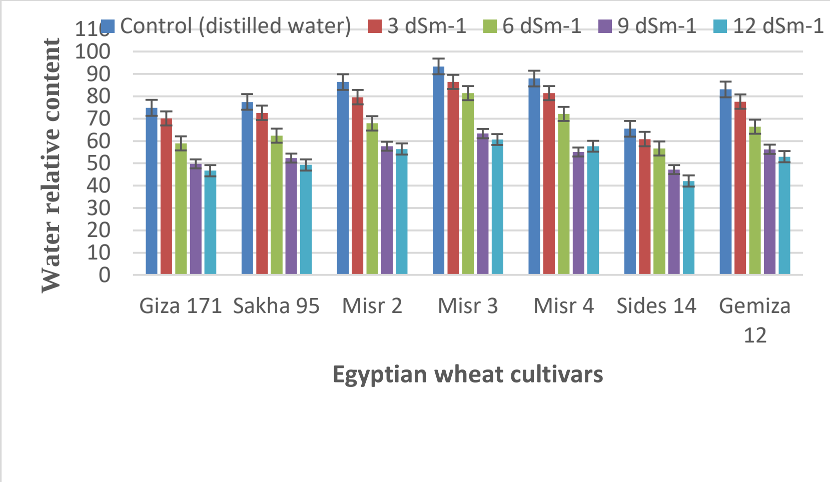


Fig. 11. Impact of the interaction between Egyptian wheat cultivars and salinity stress levels on water relative contents.

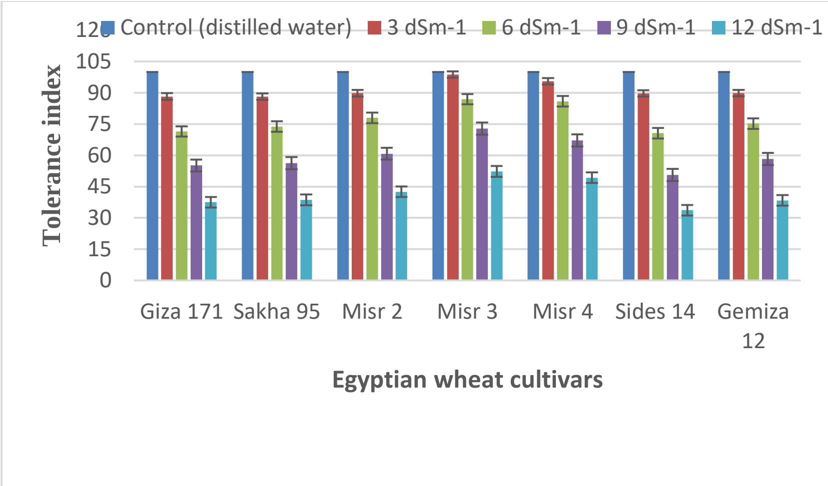


Fig. 12. Impact of the interaction between Egyptian wheat cultivars and salinity stress levels on germination index.

## Discussion

This study focused on specific Egyptian wheat cultivars grown under various salinity gradients to determine the effects of $H_2O_2$ pretreatment of seeds on seedlings and biochemical parameters. According to our findings, there were notable variances between the investigated cultivars in a number of the criteria. These results revealed that the examined Egyptian wheat cultivars had considerably variable germination characteristics, seedling parameters, RWC, TI, $Na^+$, $K^+$, and proline contents, as indicated in Tables 1-3. According to our findings (Figures 1–12), the Misr 3 cultivar came in first place and had the most promising values, followed by the Misr 4 and Misr 2 cultivars, Sakha 95, Giza 171, Gemiza 12, and Sides 14. However, at the germination stage, all of the studied wheat cultivars were vulnerable to salt stress. These results might be related to genetic differences between these cultivars, which could have a significant influence on germination features, seedling characteristics, RWC, TI and $K^+$, proline as well as $Na^+$ levels (Öner & Kirli 2018; Abido *et al.* 2021). Therefore, it is crucial to understand which types of wheat cultivars respond to salt stress the best and which cultivars are the safest to grow in the given conditions. One of the simplest methods for increasing germination rates and seedling establishment under salt stress has been pre-sowing treatments. According to Xu et al. (2010), hydrogen peroxide (H2O2) is a chemical catalyst with both oxidizing and reducing characteristics. $H_2O_2$ is a vital biological component that plays a role in the growth and metabolism of aerobic organisms (Bienert *et al.* 2006; Hussian *et al.* 2013; Wojtyla *et al.* 2016). It controls and plays a role in enhancing plant stress tolerance by reducing free radicals, which enhances plants' capacity for survival (Hemalatha *et al.* 2017).

Exogenous $H_2O_2$ use significantly affected seedling growth, germination, RWC, TI, proline, $Na^+$, and $K^+$ levels (Tables 1-3 and Figures 1–8). This may be due to the fact that $H_2O_2$ acts as a signal molecule when present in low concentrations (Li *et al.* 2011). In this regard, $H_2O_2$ has also been linked to an improvement in seedling characteristics and germination capacity by raising the $K^+/Na^+$ ratio in roots and shoots under salt stress (Jafar *et al.* 2012; Hemalatha *et al.* 2017). Additional benefits of $H_2O_2$ treatment include decreased peroxidation of membrane lipids in leaves and roots and a decrease in the negative effects of excessive salinity caused by antioxidants. Additionally, it participates in a variety of physiological processes that regulate ATP availability, early DNA replication, osmotic adjustment, enzyme activation, and proline accumulation. Proline accumulation is increased by $H_2O_2$ treatment, which increases the plant's osmotic concentration and, as a result, the plant's water potential and water deficit (Ashfaque *et al.* 2014 and Yao *et al.*, 2021).

The characteristics and indices of the Egyptian wheat cultivars' germination were much lower under saline stress conditions than they were for control treatments (Tables 1-3). According to Avusoglu and Kabar (2010), various processes caused by salt may explain why germination and seedling parameters decreased as salt dosages increased (Figures 9–12). It is well known that germination media with reduced osmotic potential make it difficult for seeds to absorb water or cause hormonal imbalances Akbarimoghaddam *et al.* (2011). Additionally, wheat plants' regular ion absorption and appropriate metabolic activities are frequently disturbed by low water potential and high sodium ion buildup (Atak *et al.*, 2006). All of these frequently raise the quantity of reactive oxygen species, which disrupts the structure and functionality of bio-membranes and, as a result, all aspects of cellular homeostasis. NaCl-induced osmotic stress ultimately causes the cell to become dehydrated, which disturbs the osmotic balance, prevents roots from absorbing water, and leads to water shortages (Azevedo *et al.* 2006; Zheng *et al.* 2008)

## Conclusion

The findings showed that pre-treatment of wheat seeds with 3.0% $H_2O_2$ enhanced proline and $K^+$ concentrations relative to $Na^+$ content, which aided in promoting seedling development of the Misr 3 cultivar under high salt levels when compared to the other studied cultivars. It is hoped that this landrace would be able to grow well in fields that are somewhat salinized because the good salt resistance qualities were able to appear in this cultivar. As a result, the Misr 3 cultivar can be a viable choice for salty soils.

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## Conflict of interest statement

There is no conflict of interest stated by the authors.

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