Primers of *Silybum marianum* (L.) Gaertn seeds with H$_2$O$_2$ and magnetic field ameliorates seawater stress

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**ARTICLE INFO**

**Keywords:**
- Plant biology
- Biophysics

**ABSTRACT**

*Silybum marianum* (L.) Gaertn is an important medicinal plant and has been used as a traditional medicine for diseases of the liver and biliary tract. The effects of seed priming by H$_2$O$_2$ (Halopriming) and magnetic field (Magnetopriming, MF) on the impacts of seawater concentration were tested using *S. marianum* at the vegetative stage. These plant species accumulate flavonoids especially sylimarine that is used in liver treatment. Some soaked *S. marianum* seeds were subjected to 0.18 T MF for different time durations (0, 10, 20 and 30 min) and other seeds were soaked in different concentrations of H$_2$O$_2$ (0, 80, 160 and 240 μM) for 8h. H$_2$O$_2$ priming increased growth and development under water irrigation more than under sea water stress. Moreover, our results uncovered statistical evidence that the priming seeds with H$_2$O$_2$ and MF increased the tolerance of *S. marianum* to salinity. In summary, we provide clear evidence that seawater stress caused a highly significant reduction in the growth parameters and stimulation in proline and phenolic compounds. It was concluded that, application of H$_2$O$_2$ and MF of *S. marianum* could scavenge or alleviate the harmful effects of salinity stress at early seedling stage and alleviate the oxidative damage leading to improvements in physiological attributes for the plant growth under sea water stress.

1. Introduction

Milk thistle (*Silybum marianum, S. marianum*) is one of the chemotypes specific medicinal plants whose seeds' effective substance is used in the production of medicines for liver diseases (Davazdah and Majnoon, 2008). There are medical confirmations for anticarcinogenic and hepatoprotective activities of *S. marianum*. *S. marianum* is used vigorously in cirrhosis, prostate, skin and breast cancer, cervical cells and kidney ailments (Noreen, 2017).

Seed priming is a pre-sowing treatment in different ways so as to cause early germination and obtain better seed vigor (Evenari, 1984). Priming improves seed viability, synchronizes and accelerates germination and sprouting, increases stress resistance and antioxidant activity, and improves plant productivity and growth (McDonald, 2000). Specifically under stress conditions, it induces the germination changes and which is to maintain the germination rate and uniformity in the seedling emergence (Ashraf and Foolad, 2005). Salinity is one of the most important factors that limit plant growth and productivity (Mahajan and Tuteja, 2005). Saline water was used to be considered unusable for irrigation but research efforts during the past two decades have brought into practice some large irrigation schemes which depend on saline water (Hamdy et al., 1993). The effects of salinity and drought stress on seed germination characteristics of *S. marianum* showed that radicle and plumule length decreased by increasing of salinity and drought stress (Yazdani et al., 2010).

Using high vigor plant seeds is important in the dry parts of the world like Egypt that faces natural stresses as a result of the decrease in water availability for germination and growth. Seed priming with H$_2$O$_2$ having the capacity to enhance the multi-resistance to heat, drought, chilling and salt stress (Uchida et al., 2002). H$_2$O$_2$ is one of the main chemicals which are induced to elevate in plants by biotic and abiotic stresses. Environmental stresses are known to induce H$_2$O$_2$ and other toxic oxygen species production in cellular compartments and result in acceleration of leaf senescence through lipid peroxidation and other oxidative damage. It also changes the redox status of surrounding cells where it initiates an antioxidative response by acting as a signal of oxidative stress (Sairam and Srivastava, 2000). Some authors suggested that H$_2$O$_2$ plays a dual role in plants: at low concentration, it acts as a messenger involved in signaling and in triggering tolerance against various abiotic stresses, but at high concentrations H$_2$O$_2$ causes oxidative stress which leads to a loss

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https://doi.org/10.1016/j.heliyon.2019.e01886
Received 15 February 2019; Received in revised form 19 May 2019; Accepted 30 May 2019
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of protein function, membrane integrity, and to programmed cell death (Asada, 1996). Hemalatha et al. (2017) reported that seed priming with H₂O₂ could be recommended for mitigating the effect of salt stress even under higher salt concentrations. However, the obtained results of Zlatica et al. (2018) revealed that the effects of H₂O₂ priming depended on soybean line and treatment, some lines responded favorably to immersion, while in others priming had an inhibitory effect, causing a significant decrease in germination.

Magnetic treatment of seeds became very popular in the agricultural sector. Pre-treatment of seedling with magnetic field is gaining more application with significant advantages such as magnetic treatment improves first stages of growth in higher plants and increases stress enzyme (Nykane et al., 2019). Pre-sowing exposure of seeds of different crops to static magnetic field (SMF) called ‘magnetopriming’, is a non-destructive dry seed priming treatment that has been reported to increase percentage of germination, rate of germination and seedling vigor of many crops. There are few reports on the metabolic changes occurring during germination in the seed in response to magnetopriming under non-stressed environment (Shine et al., 2011; Bhardwaj et al., 2012). The effect of magnetic biostimulation of seeds using stationary MF was presented by Thomas et al. (2013); Kataria et al. (2015) and Kataria et al. (2017). Electric and/or magnetic treatments are assumed to enhance seed vigour by influencing the biochemical processes that involve free radicals and by stimulating the activity of proteins and enzymes (Karimi et al., 2017).

Our study here aims to improve plant production under sea water by using halopriming and magnetopriming. Thus the experiment studied the responses of S. marianum to H₂O₂ with different concentrations and MF with different duration time under tap water and 10% sea water irrigation in terms of growth and physiological attributes compared with their controls (water and sea water).

2. Materials and methods

2.1. Seeds collection

The seeds of S. marianum seeds were collected in the fall seasons from different habitats in the Mediterranean region of Egypt. The study area lies between Alexandria and Elhammam (Fig. 1) which belongs to the semi-arid climate with mild winter and warm summer, the annual rain fall is 150 mm mostly in the winter season (UNESCO, 1977).

2.2. Preparation of seeds and growth experiments

The seeds of S. marianum were sterilized and uniform sized and shaped seeds were divided into two groups, the first group were primed with different concentrations of H₂O₂ (0, 80, 160, 240 μM) for 8h as reported by Wahid et al. (2007). While the second group was soaked in distilled water for 8h then, exposed to MF 0.18T (Magnetic susceptibility device, Teslameter LEYBOLD DIDACTIC GMBH QKD2955777, Fig. 2) with different durations (0, 10, 20, 30 min). Priming seeds were grown in plastic pots (15 cm in diameter, 20 cm in length) filled with 1kg soil (1:2 clay to sand soil), 10 seeds were planted separately each treatment had six replicates. The pots were arranged randomly under greenhouse condition in the Faculty of Education, Alexandria University. Pots were irrigated with different concentration of seawater (0, 10, 20, 30 and 50% and then 15%) from the running surface of Mediterranean Sea (Electrical conductivity 50 Ms/cm). The pots were kept at 60% water holding capacity for the soil type. When the plants became well established (after 50 days) (Fig. 3), they were carefully freed from the soil by gentle motion in tap water, then the plants were washed with distilled water. Irrigated pots with more than 10 % sea water were excluded because the germination was very low and the seedlings were dead after a few days from germination.

2.3. Growth parameters and photosynthetic pigments

Fifteen individuals were selected from each treatment for determination of shoot, root lengths and the biomass. The length of shoot/root ratio was calculated for each treatment. Leaf area (A) was estimated using the following equation (Cain and Castro, 1959): 

\[ A = 0.667 \times L \times W, \]

Where L is the leaf length, W is the leaf width, and 0.667 is a correction factor used to convert the rectangular product of leaf length and width into the area of the leaf. Photosynthetic pigments chlorophyll a (chl a), chlorophyll b (chl b) and carotenoids (carot) were determined by N, N-dimethylformamide (DMF) method according to Inskeep and Bloom (1985). Chl a/b, Carot/total % and Carot/chl (a+b) % ratios were calculated, for the fresh sample.

2.4. Metabolic compounds

Aqueous extract was prepared following Migahid and El-Khazan (2002) protocol for measurements of soluble protein, amino acids and proline by the methods described in Bradford (1976), Ya and Tunekazu (1966) and Bates et al. (1973) respectively. The extract of the total

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**Fig. 1.** The study area at Mediterranean coastal region of Egypt which lies between Alexandria and Elhammam (denoted by thick black arrow).
phenolic and flavonoid compounds were prepared from fresh samples (0.5 g) were refluxed with 5 ml absolute methanol at 50 °C for 2 h, then the extract was filtered by Whatman No. 4 filter paper and the filtrates were completed up to 5 ml with absolute methanol. Measurements of phenolic and flavonoid content were carried out spectrophotometrically according to Kaur and Kapoor (2002) and Kanatt et al. (2007) at 650 nm and 510 nm respectively.

2.5. Statistical analysis

Analysis of variance (ANOVA) was applied to assess the significant variations of the plant in response to different treatments (salinity of seawater, H2O2 concentrations, and MF durations). Statistical evaluation concerning all parameters was performed by using Minitab software (Minitab 12 for windows). Significant results were presented at two significant levels (highly significant at p ≤ 0.01 and significant at p < 0.05). While Tukey’s HSD test was applied within treatments in metabolic compounds at 0.05% level.

3. Results

3.1. Growth parameters

3.1.1. Plant biomass

Table 1 shows a highly significant decrease in the total fresh weights for S. marianum after sea water treatment. The reduction in fresh weight was more obvious for shoot than root. Both shoot and root’s fresh weights of S. marianum increased significantly in response to H2O2 pretreatment as compared with the control. The largest increase of the shoot fresh weight (19%) after irrigation with tap water and (42%) after irrigation with 10 % sea water were attained at 160 μM H2O2. The opposite trend appeared in the MF treatments of the shoot fresh weight especially after tap water and 10% seawater irrigation for which there was no statistically significant reduction at 30 min MF (20.1% and 15.2% respectively) compared to control.

The shoot and root dry weights of S. marianum were decreased significantly in response to the seawater treatment, the reduction in the shoot was more obvious than that in the root (Table 1). The response of shoot dry weights to H2O2 and MF priming were a non-significant increase, whereas the highest increase of shoot dry weight after tap water and seawater irrigation were attained at 160 μM H2O2 (22.2% and 16.6% respectively). However, there was no obvious change in the root dry weight in response to H2O2. The highest increase in shoot dry weight which produced from the priming of S. marianum seeds with MF after irrigation with tap water was attained at 10 min MF (11.1%), and that treated with sea water irrigation was attained at 20 min MF (33.3%) as.

![Fig. 2. Magnetic susceptibility device (0.18 T).](image1)

![Fig. 3. Potted plants of S. marianum in green house of 50-days old priming seeds with H2O2 (0, 80, 160 and 240 μM) after irrigation with tap water and 10% sea water, while the priming with magnetic field (0, 10, 20 and 30 min duration) after both irrigation with tap water and 10% sea water arranged respectively.](image2)
compared with control.

3.1.2. Shoot and root lengths

There is a highly significant decrease in shoot and root lengths for S. marianum due to seawater treatment (Table 2). The pretreated S. marianum seeds with H2O2 exhibited a highly significant increase of shoot and root lengths. The highest increase in shoot length which irrigated with tap water and 10% sea water was attained at 80 μM H2O2 (16.5%). The highest increase compared to control in the root length after irrigation with tap water was obtained at 160 μM H2O2 (16.18%), whereas the highest increase at 10% seawater (40.5%) was recorded at 240 μM H2O2. It is well noted that the pretreated S. marianum seeds with MF exhibited longer shoot and root lengths than the control (Table 2). The highest increase of shoot length which irrigated with tap water was attained at 30 min MF (4.2%), while after seawater it was 20% at 20 min MF. On the other hand, the highest root length after tap water irrigation attained at 20 min MF (23.7%), while at 10 % sea water the highest root length (35%) was at 10 min. The shoot/root length ratio showed significant increase in the individuals under tap water and highly significant decrease in those irrigated with 10 % sea water in the pretreated with H2O2, while it was reduced with non-significant in case of MF priming.

3.1.3. Leaf area

The leaf area of plant decreased with highly significant p-values in response to seawater treatment (Table 3). However, the leaf area was increased with highly significant in response to H2O2 priming under irrigation with tap water and seawater. The highest value (0.57%) after irrigation with tap water was attained at 80 μM H2O2, while after seawater irrigation it was attained 70.25% at 160 μM H2O2 compared to control. There was a significant increase of the leaf area in which pretreated seeds with MF. The highest increase (1.6%) in the leaf area after tap water irrigation was recorded at 30 min MF, whereas it was recorded 6.9% after seawater irrigation at 10 min MF.

3.2. Photosynthetic pigments

The content of pigments in the plant at different treatments was recorded in the Table 5. The present study showed no great variation in the content of the total pigment in response to seawater and pretreated seeds treatments. The carotenoid of S. marianum increased significantly in response to seawater and pretreated seeds, while chlorophyll a (Chl a) showed a slight increase as compared to control in response to H2O2 priming. The highest increase in chl a content was at 240 μM H2O2 after both tap water and seawater irrigation (2.9% and 6.8% respectively). Priming with 160 μM H2O2 indicated that chlorophyll b (chl b) showed the highest increase after irrigation with tap water (1.9%). Meanwhile, the highest increase after 10% sea water irrigation was recorded at 240 μM H2O2 (3.4%). In case of carotenoid the highest increase was recorded at 160 μM H2O2 and 240 μM H2O2 treatments (1.4%) after tap water irrigation, while 80 μM H2O2 and 240 μM H2O2 obtained the highest value (5%) after 10% seawater irrigation. Moreover, the chl a content in S. marianum increased significantly in

| Treatment | Silybum marianum |
|-----------|------------------|
|           | length of shoot | length of root | Shoot/root |
| Control (H2O2 & magnet) | 8.06 ± 1.38 | 3.61 ± 1.38 | 6.18 ± 1.76 | 5.13 ± 1.97 | 1.30 | 0.70 |
| 80 μM H2O2 | 9.39 ± 1.23 | 4.21 ± 0.75 | 6.82 ± 2.66 | 6.29 ± 2.61 | 1.38 | 0.67 |
| 160 μM H2O2 | 8.10 ± 0.80 | 3.91 ± 0.95 | 7.18 ± 2.39 | 6.75 ± 2.34 | 1.13 | 0.58 |
| 240 μM H2O2 | 8.64 ± 1.26 | 4.01 ± 0.88 | 5.94 ± 1.86 | 7.21 ± 2.02 | 1.45 | 0.56 |
| F-values | 745.54** | 0.12 | 83.32** | 3.31** | 4.31** |
| Sea water conc. | | | | | |
| H2O2 treatment | 5.34** | 0.41 | 7.24** | 5.55** | 3.31** |
| 10 min magnet | 8.11 ± 1.42 | 4.08 ± 0.68 | 7.38 ± 1.65 | 6.93 ± 2.41 | 1.10 | 0.59 |
| 20 min magnet | 8.18 ± 1.35 | 4.33 ± 1.27 | 7.65 ± 2.59 | 6.77 ± 1.58 | 1.07 | 0.75 |
| 30 min magnet | 8.40 ± 1.38 | 4.07 ± 0.84 | 7.52 ± 2.32 | 5.80 ± 1.47 | 1.12 | 0.69 |
| F-values | 432.90** | 20.93** | 61.05** | |
| Sea water conc. | | | | | |
| magnetic treatment | 0.42 | 3.11* | 0.28 | 0.57 | 0.57 | 0.39 |

*Significant at (p < 0.05), ** highly significant at (p ≤ 0.01).
response to MF priming under irrigation with tap water. The highest increase (5.8%) compared with control was recorded at 10 min MF, while at 20 min MF, chl a content attained the highest increase (6.8%) after seawater irrigation. The same trend was exhibited in chl b. Results revealed a slight increase in chl b due to the priming with MF. The highest increase in chl b (2.9%) was attained at 10 min MF after tap water irrigation, whereas after seawater treatment was recorded at 20 min MF (2.4%). The carotenoid content increased with highly significant in response to MF priming under tap water. The highest value was recorded at 10 min MF in irrigated with tap water (38.8%), while after 10% sea water was attained at 30 min MF (27.7%).

3.3. Metabolic compounds

3.3.1. Proteins, amino acids, proline

Fig. 4 and Table 4 show that the soluble protein of S. marianum showed a highly significant decrease in response to seawater after H2O2 primings. However, this decrease was insignificant after priming with MF. On the other hand, the soluble protein was significantly increased after priming with H2O2 in individuals irrigated with tap water while the increase of soluble protein after irrigation with 10% sea water was non-significant. Whereas the highest increases of soluble protein (30.5%, 4.16%) were recorded at 80 μM H2O2 and 160 μM H2O2 after tap water and 10% sea water irrigation respectively compared with the control. The increase in soluble protein in response to MF was non-significant in both plants irrigated with tap water and 10% sea water. The maximum increase in soluble protein content of S. marianum was recorded at the priming with 10 min MF after irrigation with tap water (22.03%), after seawater irrigation it attained 35.4% at 30 min MF.

The content of free amino acid of S. marianum was decreased in response to seawater and pretreated seeds with H2O2 and MF (Fig. 4). However, this decrease was insignificant after H2O2 treatment and highly significant after MF (Table 4). The content of free amino acids of S. marianum individuals increased non-significantly with H2O2 priming after both irrigation with tap water and 10% seawater treatment. The highest content in this species was recorded at 80 μM H2O2 after tap water irrigation and at 160 μM H2O2 after 10% sea water irrigation (50.7% and 16.4% respectively). On the other hand, the amino acids content exhibited highly significant increase in response to priming with MF after irrigation with sea water, the highest amino acids content after tap water irrigation was attained at 30 min MF (23.9% of control), while the highest amino acid content after irrigation with 10 % sea water was attained at 20 min MF (5.9%).

The content of free proline in the studied species exhibited highly significant increases after 20 min MF irrigation and 10% seawater treatment, whereas the highest increases of proline (30.5%, 4.15%) were recorded at 160 μM H2O2 and 20 min MF after tap water and 10% sea water irrigation respectively compared with the control. The increase in proline in response to MF was non-significant in both plants irrigated with tap water and 10% sea water. The maximum increase in proline content of S. marianum was recorded at the priming with 10 min MF after irrigation with tap water (22.03%), after seawater irrigation it attained 35.4% at 30 min MF.

Fig. 4. Variation of proteins (P), amino acids (AA) and proline (Pr) (mg/g f. w.) in priming seeds of Silybum marianum with H2O2 and magnetic field (0.18 T). Means with identical letters within graphs do not differ significantly at the 0.05% level of probability based on Tukey's HSD test. 0% sea water □ (capital letters) and 10% seawater ■ (small letters) were separately grouped.
significant accumulation in response to 10% sea water in priming with H2O2 and different durations of MF (Fig. 4 and Table 4). The highest accumulation of free proline was recorded at 240 μM H2O2 and 80 μM H2O2 (750%, 524% respectively), while the highest accumulation of free proline after MF was attained at 30 min and 20 min (5500%, 913.5% respectively) compared with control. Soluble proline increased non-significantly in response to H2O2 priming after tap water and 10% seawater irrigation. The highest proline content after tap water irrigation was attained at 10 min MF (39.2%). While the highest phenolic compound content was recorded at 240 μM H2O2 (117.7%).

### 3.3.2. Phenolic and flavonoids compounds

The total phenolic content in S. marianum after H2O2 and MF priming showed significant increases in response to sea water treatment (Table 4 and Fig. 5). The highest content of phenolic compound was recorded at 160 μM H2O2 (23.6%) in plant individuals irrigated with tap water, while the highest phenolic content compound was recorded at 240 μM H2O2 (62.4%) in plant individuals irrigated with 10% sea water. The highest phenolic compound in the treated seeds with MF was recorded at 30 min MF (47.8%). Significant increase of proline in pretreated seeds with MF was recorded at 10% seawater irrigation. The highest proline content at seawater irrigation was attained at 30 min MF (143%). However, in tap water irrigation there was a non-significant reduction (50%) at all duration of MF compared with control.

### Table 4

| Treatment | Protein | Amino acids | Proline | Phenolic | Flavonoids |
|-----------|---------|-------------|---------|----------|-----------|
|           | 0%      | 10%         | 0%      | 10%      | 0%        | 10%      |
| F-value   | Sea water conc. | 14.93**   | 7.26*   | 4.07     | 0.26     |
|           | H2O2 treatment | 3.59      | 0.81    | 1.74     | 0.86     |
|           | 0%      | 10%         | 35.28** | 200.89** | 8.60     |
| F-value   | Sea water conc. | 3.33      | 2.84    | 6.95*    | 6.81     |
|           | 0%      | 10%         | 6.98*   | 17.56**  | 40.08** |
|           | 0%      | 10%         | 140.05**| 120.57** |

*Significant at (p < 0.05), ** highly significant at (p ≤ 0.01).

Flavonoid content increase was more than double time after 10% of sea water irrigation was obtained at 20 min MF when compared with the control.

### 4. Discussion

It is well documented that salinity reduces the germination as well as seedling growth in crop plants and seed priming ameliorates salinity during early seedling growth (Afzal et al., 2006). The present study evaluates the priming effects for S. marianum seeds with different concentrations of H2O2 and different durations of MF which grown under irrigation with tap water and 10% sea water. In the present study, the salinity of seawater caused a highly significant reduction in the growth parameters of S. marianum compared with control. The fresh and dry weights of root and shoot of S. marianum decreased progressively due to salinity as compared to control. These results are in agreement with those reported by Memon et al. (2010) and Hessamoddin et al. (2015) that showed a negative effect of salinity on plant growth of several plant species.

In our study there was a highly significant reduction in shoot and root lengths of S. marianum due to seawater stress under different priming techniques. The rate of root growth inhibition is more prominent compared to shoot inhibition. The reductions in the shoot and root length with more reduction in root growth than shoot growth due to salt stress are similar to those El-Katony et al. (2019) and Devi et al. (2008). High salinity may inhibit root and shoot elongation due to slowing down the water uptake by the plant (Werner and Finkelstein, 1995), which may be another reason for this decrease. Kaymakanova and Stoeva (2008) reported that salinity had adverse effects not only on the biomass, but also on other morphological parameters such as plant height, number of leaves, root length and shoot/root ratio. The shoot/root ratio in the present study of S. marianum showed highly significant decrease due to seawater treatment. Under prevailing experimental conditions, salinity significantly reduced leaf area of S. marianum these observations were
pretreated faster germination of maize seeds when exposed to MF of 125 or 250 mT. Similar observations have been reported by Chen et al. (2007) who found that exposing Vigna unguiculata plants at the age of 14 days−75 mM of NaCl reduced soluble protein content in the plant. These results were confirmed by Cheruth et al. (2008) with their study on Catharanthus roseus seedling and Khorasvnejad et al. (2009) on Hordeum vulgare. There is a marked increase in soluble protein for the studied species in response to H2O2 as compared with the control. H2O2 induced small heat shock proteins (HSP26) in tomato and rice (Chevallier, 2001). Thus, H2O2 may play an important role in signal transduction for abiotic stress tolerance, although it is toxic at high concentrations.

Shine et al. (2017) exhibited that MF treatment influences the physiological and biochemical process in the seeds and thereby contributes to better vigor and improved crop stand. In the present study, there is an obvious increase in soluble protein as a response to MF but this increase was non-significant under both irrigations with tap water and 10% sea water. The effect of MF on protein synthesis has been studied in some experiments. It has been observed that the protein values were slightly higher in MF-exposed seedlings, in comparison with the controls (Placentini et al., 2001). Novitsky et al. (2001) demonstrated that low-intensity MF application increased protein in onion plant. In fact, the content of free amino acids in the studied species decreased in response to seawater under H2O2 and MF priming but the reduction under H2O2 was non-significant. Also, the reduction and/or the increase in the content of free amino acid in response to H2O2 priming were insignificant for the studied species. Under the prevailing experimental condition, the amino acids content increased significantly in response to MF in S. marianum.

Proline accumulation is a widespread response of plants to environmental stresses (Anjum, 2008), which is shown to be involved in defense of plants against salinity and osmotic stress (Karimi et al., 2017). The present study revealed that soluble proline in the studied species accumulated with highly significant in response to 10% sea water stress under pretreated with H2O2 and MF durations. It was suggested that proline accumulation may be caused by increased proteolysis or by decreased protein synthesis. The higher concentration of proline under salt stress is favorable to plants as proline participate to osmotic potential of leaf and thus to osmotic adjustment. Besides the role of osmolyte, proline can also confer enzyme protection and increase membrane stability under various condition. Proline accumulation may also help in nonenzymic free radical detoxifications (Khan et al., 2002). Results showed that seeds pretreated with H2O2 enhanced free proline content; similar results were reported by He et al. (2009), they showed that seed priming with H2O2 significantly enhanced free proline content. The slight increase in proline, an osmoprotectant in H2O2 (0.2 mM) stressed senescing rice leaves may be attributed to the free radical scavenging function of proline as reported elsewhere (Matysik et al., 2002). The MF priming seeds of S. marianum enhanced free proline content as compared with control, similar observations have been reported by Dhawi and Al-Khayri (2008), who found that at the lowest intensity, 10 mT, proline concentration increased in response to longer exposure durations reaching a maximum at 240 min. Plants produce a large variety of secondary products that contain a phenol group; abiotic stresses may also modulate the level of secondary metabolites such as phenolics (Dixon and Paiva, 1995). In the present study, the content of the total phenolic compounds increased in studied species in response to the salinity of sea water but this increase was non-significant which could be a defense mechanism and a biochemical adaptation to environmental stress (Dixon and Paiva, 1995). These compounds are thought to protect the plant against salt-induced stress signal in low concentrations (Desikan et al., 2004). The present results indicate that priming seeds with H2O2 revealed the increase in the root and shoot fresh and dry weight, organs length and leaf area of S. marianum as compared with the control especially under the irrigation with 10% sea water. This result indicated that the role H2O2 in deleterious effects of salt stress on the plant growth may be due to the activation of antioxidants (Wahid et al., 2007). The capacity of the antioxidant defense system is often increased under stress conditions (Taibi et al., 2016).

Effective tool for alleviation salinity stress on germination of barley crops. Magneto-priming could be promising and more effective in alleviating the deleterious effects of salinity on seed germination and seedling and germination growth H2O2 is a strong oxidizing agent that injures cells and damages photosynthesis at high concentration when produced internally or applied externally (Sairam et al., 2002). However, it acts as stress signal in low concentrations (Desikan et al., 2004). The present results indicate that priming seeds with H2O2 revealed the increase in the root and shoot fresh and dry weight, organs length and leaf area of S. marianum as compared with the control especially under the irrigation with 10% sea water. This result indicated that the role H2O2 in deleterious effects of salt stress on the plant growth may be due to the activation of antioxidants (Wahid et al., 2007). The capacity of the antioxidant defense system is often increased under stress conditions (Taibi et al., 2016).

MF is an inescapable environmental factor for plants on the earth. However, its impact on plant growth is not well understood. The intensity of MF used in this study was 0.18 T based on Majd et al. (2009), they found that suitable MF-priming 0.18 T could speed up seedling development and increase biomass. The present study exhibited that the growth parameters allow us to conclude that the magnetic treatment improves the growth of the studied species under irrigation with tap water as well as seawater treatments. Whereas the shoot and root weights, length and leaf area in the studied species were enhanced in response to MF treatment. Magneto-priming could be promising and effective tool for alleviation salinity stress on germination of barley crops (Hozayn et al., 2018). The results of Hessamoddin et al. (2015) on S. marianum indicated that different strengths of the magnetic field and different time durations are important factors which can influence the plant growth. Karimi et al. (2017) found that magnetic priming for 6 hours was suggested for enhancing germination and growth of sweet corn under salt stress. Podlesny et al. (2005) showed a positive effect of magnetic stimulation of seeds on the increase of pea hypocotyl and root length.

Results of the present study revealed that seed priming with MF of the studied species increase in leaf area (not-significant). Yinan et al. (2005) reported that cucumber seedlings with MF-priming grew much better than the untreated, and above ground biomass and leaf area were significantly increased. The ratio between shoot/root lengths exhibited no significant change due to priming with MF for studied species. On the other hand, Ananta and Shantha (2008) noted that there is a significant increase in germination, seedling vigor and shoot/root growth in maize and chick pea seeds exposed to static MF. Florêz et al. (2007) reported faster germination of maize seeds when exposed to MF of 125 or 250 mT for varying periods of time.

The photosynthetic pigments are some of the most important internal factors, which in certain cases can limit the photosynthesis rate. The response of plant pigments (chlorophyll a, chlorophyll b and carotenoids) to seawater stress in the present study exhibited a slight increase in pretreated S. marianum with H2O2. Under prevailing experimental conditions the ratio of chlorophyll a/b at pretreated plants of S. marianum did not significantly change. Increase in chlorophyll content with sea water irrigation agrees with results reported by Misra et al. (1997). They indicated that stressing rice seedlings of Oryza sativa with sodium chloride increased significantly the chlorophyll content of seedlings (15 days old). Also, it was mentioned by Jamil et al. (2007a and b) that increased concentrations of sodium chloride increased the total chlorophyll content of Beta vulgaris leaves, and that was a significant increase. The MF priming enhanced total pigmentation production in the studied species. Dhawi and Al-Khayri (2008) found that low intensity of MF or short period of exposure, increased chlorophyll content, whereas high-intensity MF and long exposure to MF reduced the concentration. A similar pattern of response, MF can cause an inconsistency in the function of antioxidant enzymes in Nicotiana tabacum (Sahibjamei et al., 2007).

In the present study, the soluble protein significantly decreased in response to seawater in S. marianum especially for individuals treated with H2O2. Similar observations have been reported by Chen et al. (2007) who found that exposing Vigna unguiculata plants at the age of 14 days−75 mM of NaCl reduced soluble protein content in the plant. These results were confirmed by Cheruth et al. (2008) with their study on Catharanthus roseus seedling and Khorasvnejad et al. (2009) on Hordeum vulgare. There is a marked increase in soluble protein for the studied species in response to H2O2 as compared with the control. H2O2 induced small heat shock proteins (HSP26) in tomato and rice (Chevallier, 2001). Thus, H2O2 may play an important role in signal transduction for abiotic stress tolerance, although it is toxic at high concentrations.

Plants produce a large variety of secondary products that contain a phenol group; abiotic stresses may also modulate the level of secondary metabolites such as phenolics (Dixon and Paiva, 1995). In the present study, the content of the total phenolic compounds increased in studied species in response to the salinity of sea water but this increase was non-significant which could be a defense mechanism and a biochemical adaptation to environmental stress (Dixon and Paiva, 1995). These compounds are thought to protect the plant against salt-induced
oxidative stress (Oh et al., 2009). Phenolics are electron donors and thus could mitigate the effect of oxidative stress as an excellent substrate for antioxidant enzymes such as peroxidases (Posmyk et al., 2009). Reports on the effect of salinity on phenolic contents are limited. Total polyphenol content increased in Mentha pulegium leaves (Oueslati et al., 2010) and varied during fruit ripening in pepper (Navarro et al., 2006) exposed to salt stress. Bourou et al. (2012) found that salinity influenced significantly the total phenol contents of Nigella sativa organs, the content increased in the shoots while it decreased in the roots. In this study, the content of the total phenolic compounds in S. marianum did not significantly change in response to H2O2 and MF. Results of the present study demonstrated that the non-significant increase in total flavonoids in S. marianum in response to seawater. The result expressed by Ali and Abbas (2003), they recorded that flavonoids content increase significantly in response to salt stress in Hordeum vulgare. But flavonoids content showed a significant increase in response to the treatment with H2O2 and MF.

In conclusion, our study demonstrated that sea water stress caused a highly significant reduction in the growth parameters and stimulation in proline and phenolic compounds. In fact, the present study showed that the irrigation with tap water exhibited longer shoots and well-developed leaves after H2O2 and MF pretreatment than seawater. Moreover, the plant individuals which produced from pretreated seeds with H2O2 and MF were characterized by longer shoot and root length than non-treated seeds. Priming seeds of S. marianum with H2O2 and MF in different duration may alleviate the oxidative damage, leading to improvements in physiological attributes for the plant growth under sea water stress but with different effects. This opens an unusual perspective on plant responses that should be tested under combination of H2O2 and MF.

Declarations

Author contribution statement

MM Migahid: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

RM Elghobashy: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

LM Bidak, AW Amin: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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