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# Effects of Magnetic Water Irrigation on the Growth, N Uptake and Antioxidant Enzyme Activities of Cotton Seedlings

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**Abstract:** A hydroponic experiment was carried out to investigate the effects of magnetic water irrigation on the growth, nutritional status and antioxidant enzyme activity of cotton seedlings. Four levels of magnetic-treated water irrigation (0, 100, 300 and 500 mT) and three levels of salt stress (0, 100 and 200 mM NaCl) were applied. Salt stress adversely affected the dry weight, nutrient uptake and antioxidant enzyme activities of cotton seedlings. Magnetic-treated water irrigation significantly increased cotton seedling dry weight. Cotton seedling dry weight increased by 14%, 22% and 29% under the treatments of 100, 300 and 500 mT magnetic water irrigation, respectively, compared with the control, at a salt stress level of 100 mM NaCl. Moreover, magnetic water irrigation improved N uptake, but did not significantly affect P and K uptake. Magnetic water irrigation significantly increased the activity of superoxide dismutase (SOD), peroxidase (POD) and the proline content compared to the control (0 mT). Irrigation with magnetic water could be a promising technique in agriculture, especially under salt stress conditions. A suitable magnetic intensity of 300 mT is recommended.

**Key words:** Magnetic water, salt stress, cotton seedling, N uptake, enzyme activity.

## 1. Introduction

Soil salinization is a widespread phenomenon in arid and semi-arid areas and occurs primarily due to the inappropriate agricultural practices, such as flooding irrigation [1] and excessive fertilization [2]. According to the Food and Agriculture Organization (FAO), in 2000, saline areas have exceeded 950 million hectares at the global scale, accounting for approximately 10% of global land area [3].

The adverse effects of soil salinity on soil quality and crop productivity have been extensively studied. First, soil salinity severely deteriorates soil physiochemical properties by dispersing clay particles [4], destroying soil aggregates and deteriorating soil structure. Second, soil salinity adversely affects soil biological processes. Both soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) sharply decrease with increasing salt concentration [5]. The primary practice preventing soil salinization involves accelerating of the downward movement of salt and leaching salt ions from the cultivation layer via a given column of water irrigation; another method involves reducing the content of salt ions by burying pipes below the cultivation layer [6]. Those approaches can effectively reduce the topsoil salt content, but is financially costly and labor intense [7].

Recently, it was found that magnetic-treated water irrigation significantly accelerated soil water infiltration and promoted soil salt leaching [8]. As water molecules (polymers) passing through a given intensity of a magnetic field, the large aggregate water cluster becomes into smaller particles, making both water and nutrients more accessible to plants [9-11].

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Comparatively, small-sized magnetic-treated water clusters strongly facilitate water permeability and the downward movement of salt ions [9, 12]. As a result, soil salt ions, such as $Cl^-$, $Na^+$ and $HCO^{3-}$, are significantly leached from the cultivation layer by magnetic-water irrigation [8].

Moreover, irrigation with magnetic water can significantly improve crop growth [13] and nutrient uptake [14]. Alderfasi et al. [15] noted that magnetic water irrigation significantly increased both cereal crop growth and grain yield. For example, magnetic treatment significantly increased sunflower dry weight [16] and markedly increased the shoot development of maize plants [17]. Iqbal et al. [18] observed that magnetic treatment (10 mT; 40 h) substantially increased plant height, seed weight per spike and subsequent wheat yield. Furthermore, magnetic-treated water significantly contributed to nutrient uptake, assimilation and mobilization, thereby improving plant productivity [19]. On the other hand, magnetic treatment may not influence or even detrimentally impact crop growth or plant physiological characteristics [20]. Hirano et al. [21] found that 70 mT magnetic treatments clearly constrained the growth and photosynthesis of *Spirulina platensis*. Thus, different crop species may respond differently to magnetic treatment [22].

As one of the major cash crop, cotton production in Xinjiang approximately accounts for 60% of the total yield of China [23]. Thus, investing the effect of magnetic-treated water irrigation on cotton is needed. It was hypothesized that irrigation with a certain intensity of magnetic-treated water would facilitate crop water and nutrient uptake, improve crop antioxidant enzyme activity and alleviate the effects of salt stress on crop growth.

In this study, a hydroponic simulation experiment was performed with magnetic-treated water irrigation at four levels of magnetic field intensity (0, 100, 300 and 500 mT) and three levels of salt stress (0, 100 and 200 mM NaCl). The study aimed to explore the effects of magnetic-treated water irrigation on alleviating the salt stress on cotton growth and N, P, K uptake, ascertain the effect of magnetic-treated water irrigation on antioxidant enzyme activities in cotton plants and the interactive effects of magnetic water irrigation with salt stress, and identify the most effective magnetic intensity to improve cotton plant resistance to salt stress.

## 2. Materials and Methods

A hydroponic experiment was performed in the greenhouse at the Agricultural Experimental Research Station of Shihezi University during May-July 2011. The mean annual temperature was 7.5-8.2 °C, and the average rainfall and evaporation were 270 mm and 1,500 mm, respectively.

### *2.1 Experimental Design*

Magnetic-treated water irrigation at four levels of magnetic field intensity (0, 100, 300 and 500 mT) was applied with three levels of salt stress (0, 100 and 200 mM NaCl), with total 12 treatments considered. Each treatment was replicated three times. Cotton (*Gossypium hirsutum* L. cv. Xinluzao 36) seeds were sown on May, 2, 2011 in a 12 cm × 12 cm polyethylene pot and four plants were sown per pot. Vermiculite (1-2 cm) was added to each pot. Pots were placed in 45 cm × 33 cm × 20 cm plastic boxes, and each box contained nine pots. Deionized water was applied daily after the seedling emergence period. After both cotyledons were fully expanded, seedlings were transported to 1/4-strength Hoagland solution for 3 d, followed by half-strength Hoagland solution for another 3 d, and then full-strength Hoagland solution until harvest.

### *2.2 Salt Treatments*

Twelve days after cotton plant emergence, NaCl stress was applied to the seedlings. The seedlings were initially exposed to 50 mM NaCl solution in the growth medium with increments of 50 mM every 12 h until the salt concentration reached the final concentration of 100 or 200 mM NaCl. Nutrient solution and magnetic water were added via gravimetric method every other day to compensate water losses by transpiration. The solution pH was adjusted daily to 5.8-6.0 using 0.1 M NaOH or 0.1 M HCl.

### *2.3 Sampling and Measurements*

Cotton plants were harvested 30 d after salinity treatment (cultivated for 42 d). Sampled seedlings were then divided into two parts (aboveground and roots). Samples were washed with distilled water and then dried in an oven at 70 °C for 72 h, to measure the dry weight. All samples were ground, passed through a 1-mm sieve, and then digested with 98% $H_2SO_4$ (300 g/L) before nutrient element analysis. The N content was determined using the Kjeldahl method, the P content was measured by the yellow phosphovanadate complex method and the K content was determined by flame photometry following the methods described by Varley [24].

Thirty days after salt treatment, fresh leaves of cotton seedlings were collected to analyze enzymatic activity. Briefly, leaf tissues (0.400 g) were homogenized in an ice-cold phosphate-buffered solution (PBS, 50 mM; pH 7.8) containing 1 mM EDTA and 1% polyvinylpyrrolidone (PVP), followed by centrifugation at 10,000 rpm for 15 min at 4 °C. The supernatant was then used immediately to determine the activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and proline. All steps of enzyme extract preparations were carried out at 4 °C.

SOD activity was assayed using a photochemical method. The reaction mixture (3 mL) consisted of 50 mM 4 (2-hydroxyethyl) piperazine-1-ethanesulfonic acid potassium salt (HEPES-KOH; pH 7.8), 0.1 mM EDTA, 13 mM methionine, 75 μM nitro blue tetrazolium (NBT) and 20 μM riboflavin as well as 0.1 mL of enzyme extract. One unit of SOD activity was defined as the amount of enzyme required for 50% inhibition of the rate of NBT reduction measured at 560 nm. The SOD activity of plant extracts was expressed as units per mg of protein (U/mg protein).

CAT activity was measured using the ultraviolet absorbance method. The reaction mixture (3 mL) consisted of 50 mM Na-phosphate (pH 7.0) and 100 mM $H_2O_2$ as well as 0.2 mL of enzyme extract. The breakdown of $H_2O_2$ was monitored by the reduction in absorbance at 240 nm. CAT activity was expressed as the change in absorbance relative to protein content ($\Delta Abs_{240\ nm}$/mg protein).

POD activity was determined using the guaiacol method. The reaction mixture (3 mL) contained 50 mM PBS (pH 5.5) and 20 mM guaiacol as well as 1 mL of 30% (w/v) $H_2O_2$ and 0.1 mL of enzyme extract. The change in absorbance of brown guaiacol at 460 nm was recorded. POD specific activity was expressed as units (μmol of dianisidine oxidized/min) per mg of protein.

Proline content was estimated using the ninhydrin method. Frozen leaf tissue (0.40 g) was homogenized in 10 mL of 3% sulfosalicylic acid at 4 °C and then filtered. A mixture of 2 mL of filtrate, 3 mL of acid-ninhydrin and 2 mL of glacial acetic acid was incubated at 100 °C for 40 min. The reaction was terminated on ice and then extracted with 5 mL of toluene. The chromophore-containing toluene was separated from the hydrated phase. Absorbance of toluene at 520 nm was used the blank. Proline concentration was calculated based on a standard curve and was expressed as μg of proline/g of fresh weight (FW).

Roots were washed with deionized water and dried with filter paper. The roots were placed into a graduated cylinder filled with water to determine the root volume.

### *2.4 Statistical Analysis*

The data were presented as the means ± standard errors (SE) of the measurements and analyzed by one-way analysis of variance (ANOVA), followed by

Duncan's multiple comparison test using the SPSS 17.0 software package (SPSS, Chicago, IL, USA). Two-way ANOVA was performed using the vision of Prism 5.0 software for Windows (GraphPad Software, La Jolla, CA).

## 3. Results

### *3.1 Effects of Magnetic-Treated Water and Salt Stress on Cotton Seedlings Growth*

The responses of cotton seedling growth parameters, such as shoot and root dry weight and root volume to magnetic water irrigation, are presented in Fig. 1. On one hand, the shoot and root dry weight and root volume of cotton seedlings substantially decreased with increasing salt concentration. The average shoot dry weight decreased by 40% under 100 mM NaCl and by 60% under 200 mM NaCl, compared to 0 mM NaCl (Fig. 1a). Similar trends were observed regarding root dry weight and root volume. On the other hand, magnetic water irrigation reduced the detrimental effect of salt stress on seedling growth. For instance, the average root dry weight increased by

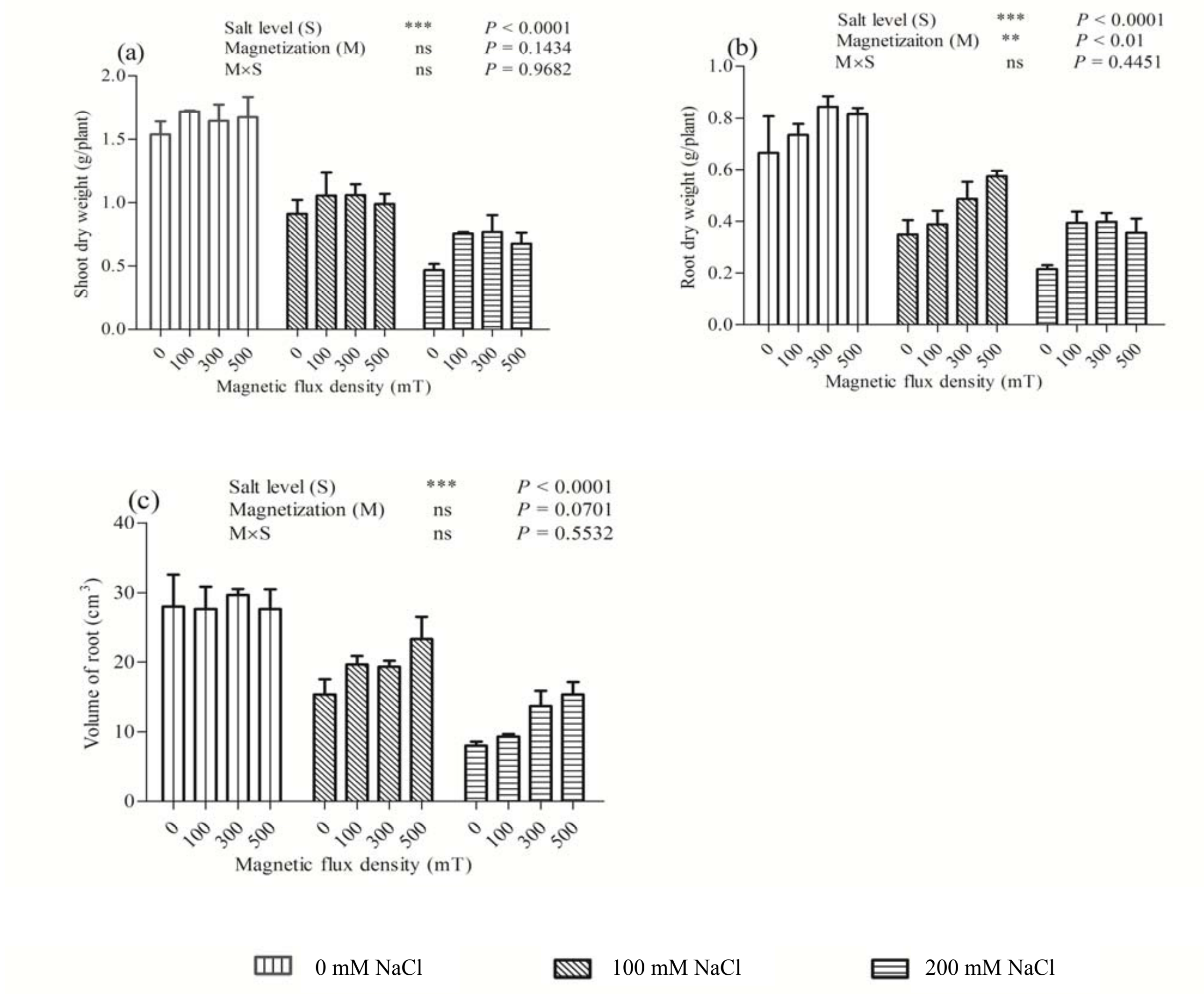


**Fig. 1 Effects of magnetic-treated water irrigation and salt stress on shoot dry weight (a), root dry weight (b) and root volume (c) of cotton seedlings.**

Data are mean ± SE ($n$ = 3). Bars represent the average SE of the means. * significant at $P < 0.05$, ** significant at $P < 0.01$ and *** significant at $P < 0.0001$ according to the Student's $t$ test.

11% under 100 mT, by 39% under 300 mT and by 64% under 500 mT, compared to 0 mT for 100 mM NaCl treatment (Fig. 1b). However, in the absence of salt stress, the effects of magnetic treatment were not considerable on the growth parameters. These results indicate that magnetic water significantly alleviated the effects of salt stress on cotton seedling growth.

### *3.2 Effects of Magnetic Water Irrigation and Salt Stress on N, P, K Uptake of Cotton Seedlings*

As shown in Table 1, the average total N uptake per seedling markedly decreased by 41% under 100 mM NaCl stress and by 56% under 200 mM NaCl stress, compared to the control treatment (0 mM NaCl). At the magnetic range of 0-300 mT, the amount of N uptake per cotton seedling increased with magnetic-treated water irrigation across all salt levels. However, compared with the 300 mT magnetic water irrigation treatment, the N status of cotton seedlings significantly decreased as the magnetic intensity reached 500 mT, suggesting that irrigation water treated with a reasonable intensity of magnetic field promotes N uptake. Magnetic water did not significantly influence P and K nutritional status of cotton seedlings.

### *3.3 Effects of Magnetic Water Irrigation on the Resistance Indicators of Cotton Seedlings*

The proline content significantly increased by 40% under 100 mM NaCl and by 99% under 200 mM NaCl treatments, compared with that under 0 mM NaCl treatment (Fig. 2a). The enzymatic activities of SOD, CAT and POD sharply decreased by salt stress (Figs. 2b, 2c and 2d). The average SOD activity decreased by 24% and 11% as cotton seedlings exposed to salt treatments of 0 mM NaCl and 100 mM NaCl compared with that of seedlings exposed to salt treatment of 200 mM NaCl, respectively. However, the SOD activities under magnetic water irrigation treatments of 100 mT, 300 mT and 500 mT were 0.84,

**Table 1 Effects of magnetic water irrigation and salt stress on the N, P, K uptake of cotton seedlings.**

| Treatment | | Amount of N uptake (mg/plant) | Amount of P uptake (mg/plant) | Amount of K uptake (mg/plant) |
|---|---|---|---|---|
| Salt level (mM) | Magnetic treatment (mT) | | | |
| 0 | 0 | $18.07 \pm 2.53^{bc}$ | $14.25 \pm 2.16^{a}$ | $35.4 \pm 1.14^{a}$ |
| | 100 | $21.09 \pm 1.31^{bc}$ | $16.11 \pm 0.15^{a}$ | $34.00 \pm 0.61^{a}$ |
| | 300 | $35.15 \pm 4.12^{a}$ | $14.39 \pm 2.38^{a}$ | $36.95 \pm 5.70^{a}$ |
| | 500 | $28.21 \pm 0.56^{ab}$ | $14.80 \pm 3.13^{a}$ | $40.12 \pm 6.52^{a}$ |
| | Mean | 25.63 | 14.89 | 36.62 |
| 100 | 0 | $9.64 \pm 3.01^{b}$ | $3.40 \pm 0.90^{a}$ | $19.76 \pm 4.79^{a}$ |
| | 100 | $15.80 \pm 1.72^{ab}$ | $3.91 \pm 0.29^{a}$ | $24.34 \pm 5.70^{a}$ |
| | 300 | $20.57 \pm 0.50^{a}$ | $3.95 \pm 0.96^{a}$ | $21.59 \pm 0.54^{a}$ |
| | 500 | $14.57 \pm 5.89^{ab}$ | $4.01 \pm 0.14^{a}$ | $21.14 \pm 5.98^{a}$ |
| | Mean | 15.15 | 3.82 | 21.71 |
| 200 | 0 | $5.45 \pm 1.24^{b}$ | $0.58 \pm 0.09^{b}$ | $8.88 \pm 1.14^{b}$ |
| | 100 | $14.64 \pm 2.77^{a}$ | $1.43 \pm 0.20^{a}$ | $16.88 \pm 1.84^{a}$ |
| | 300 | $13.96 \pm 1.59^{a}$ | $1.32 \pm 0.50^{a}$ | $13.06 \pm 1.29^{ab}$ |
| | 500 | $10.80 \pm 1.19^{a}$ | $1.07 \pm 0.13^{ab}$ | $13.21 \pm 4.82^{ab}$ |
| | Mean | 11.21 | 1.10 | 13.00 |
| Salt level | | *** | *** | *** |
| Magnetic | | *** | ns | ns |
| Interaction | | * | ns | ns |

Statistical data are expressed as the mean ± SE ($n = 3$). Different letters in the same column indicate significant differences at $P < 0.05$.
* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.0001$ according to the Student's *t*-test.

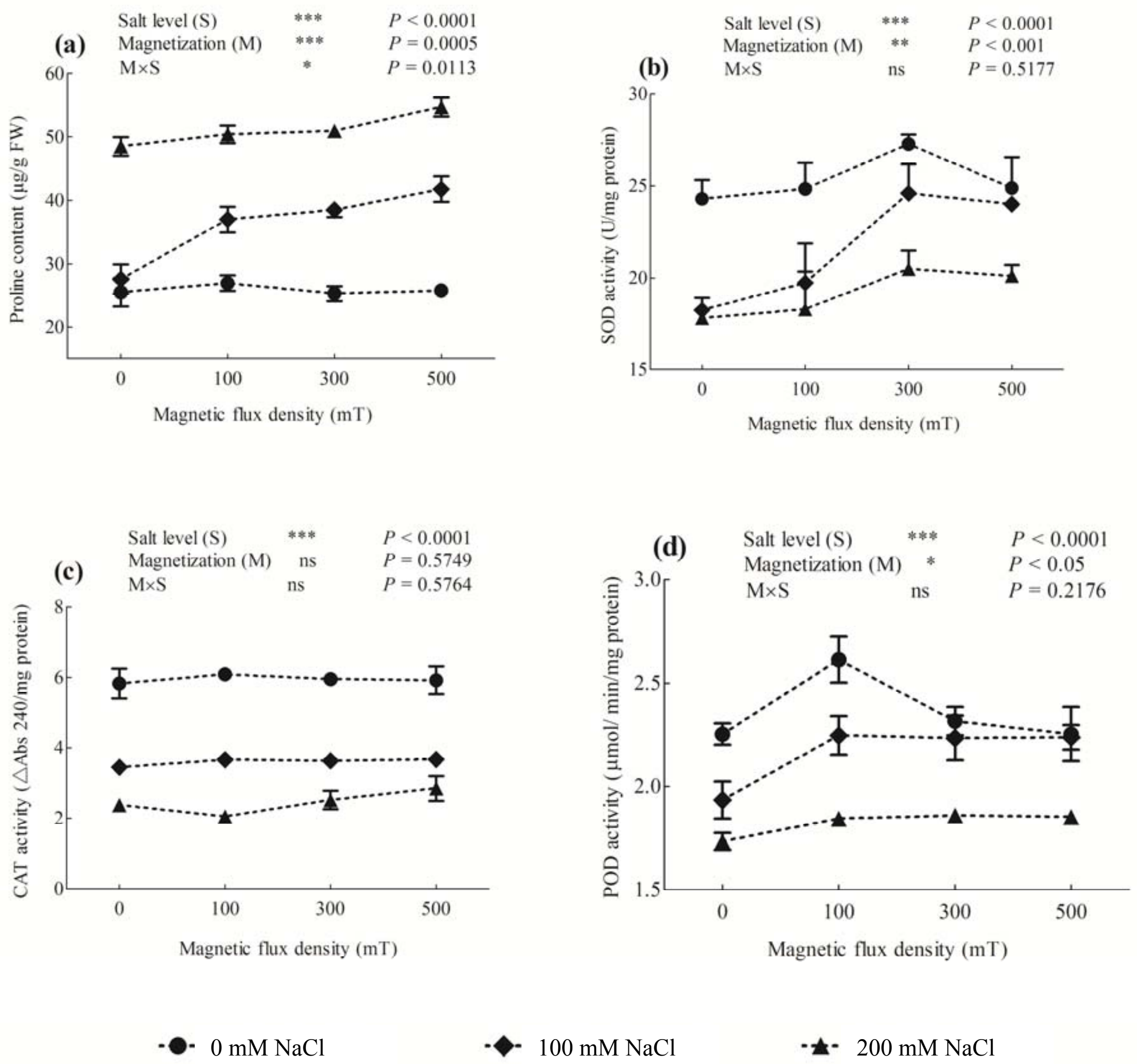


**Fig. 2 Effects of magnetic-treated irrigation and salt on the resistance indicators of cotton seedlings.**
Data are the mean ± SE ($n$ = 3). Bars represent the average SE of the means. * significant at $P < 0.05$, ** significant at $P < 0.01$ and *** significant at $P < 0.0001$ according to the Student's $t$ test.

4.0 and 2.88 U/mg protein higher than that under the 0 mT treatment, respectively. A similar trend was also observed for POD (Fig. 2d). In contrary, no significant advantage effect on CAT. In addition, no significant interactive effect between salt treatment and magnetic-treated water irrigation on SOD, POD and CAT activity was observed.

## 4. Discussion

Magnetic-treated water not only markedly increases crop nutrient uptake [19] and growth [25], but also significantly increases plant resistance to salt stress [26]. In this study, salt stress significantly ($P < 0.001$) reduced the shoot and root dry weight, root volume, nutrient uptake and antioxidant enzyme activity of cotton seedlings, whereas magnetic-treated water significantly alleviated the reduction of these values ($P < 0.05$).

### *4.1 Effects of Magnetic Water Irrigation on Growth and Nutrient Uptake*

It showed that irrigation with the magnetic intensity of 0-300 mT treated water increased the N uptake of cotton seedlings. The results in this study were consistent with those reported by Carbonell et al. [27] and Al-Khazan et al. [28], who found that irrigation with magnetic-treated water significantly increased plant uptake of both cations and immobile nutrients. There was no significant effect of magnetic-treated water on P and K uptake of cotton seedlings. In contrast, Maheshwari [19] and Grewal found that the magnetic treatment significantly increased the dry matter content of plant, and the N, P, K content. Those differences may be explained by Mostafazadeh-Fard et al. [8], who found the factors, such as the intensity of the magnetic field, duration of the magnetic exposure and the flow rate of water solution, influence the effect of magnetic-treated water on plant growth. Kato et al. [29] reported that the roots of *Daucus carota* and *Atropa belladonna* were more sensitive than the shoots to magnetic fields and root hairs were well developed at a magnetic field of 500 mT. The findings in this study showed that salt stress significantly decreased the dry weight of cotton seedlings, while magnetic water irrigation largely alleviated its detrimental effect on root dry weight. The findings agree with the results of Ozdemir et al. [30], who reported that electromagnetic-treated water (76 mT) increased the root dry weight of coleus seeds by 10% under no salt stress. Abdul-Qados and Hozayn [31] reported the similar results that magnetic treatment increased root growth and improved plant tolerance to salt stress.

### *4.2 Effect of Magnetic Treatment on Antioxidant Enzyme Activity of Cotton Seedlings*

It was found in this study that salt stress significantly ($P < 0.001$) reduced the activities of SOD, POD and CAT, while magnetic water treatments significantly ($P < 0.05$) increased the activities of SOD and POD. Salt stress adversely impacts plant growth and metabolism, mainly due to the accumulation of the $Na^+$ uptake and the increase of reactive oxygen species (ROS) in plant tissues, consequently, membrane damage, protein degradation and DNA mutation can occur [32]. To eliminate the oxidative damage to plant growth, plants up-regulate different types of enzymatic (SOD, CAT, POD) and non-enzymatic (proline) antioxidants to scavenge the excess ROS [33]. Badea et al. [34] reported that magnetic fields stimulated the activities of POD and SOD. Moreover, Moussa [35] reported that magnetic water stimulates the defense system in common bean plants. Numerous researches showed that magnetic water induced plant defense systems, which may also explain why magnetic water irrigation increases crop resistant-related enzyme activities (SOD and POD).

## 5. Conclusions

The results showed that salt stress significantly reduced the dry weight and root volume of cotton seedlings, whereas magnetic water irrigation significantly alleviated the detrimental effect caused by salt stress. Moreover, salt stress markedly decreased the uptake of N, P and K of cotton seedlings, while irrigation with the intensity of 0-300 mT magnetic-treated water significantly increased the N uptake, the enzymatic activities of POD and SOD and proline content of cotton seedlings. However, the 500 mT magnetic water treatment significantly reduced the N uptake of cotton seedlings, compared with 300 mT treatment. It is suggested that magnetic-treated water irrigation could not only benefit cotton growth, but also alleviate the inhibition effect caused by salt stress. Therefore, this practice should be extensively applied in irrigation agriculture system, especially in arid and semi-arid regions, because these areas easily suffer from soil salinization, and the intensity of 300 mT is the effective level for magnetic-treated water irrigation.

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