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Magnetized Water Alleviates Drought Damages by Reducing Oxidative Stress and Proline Accumulation in Mung Bean (*Vigna radiata* L. Wilczek)

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ABSTRACT
Drought is the most important abiotic stress limiting crop growth and productivity in many regions of the world. This study was carried out to investigate the effect of magnetized water on alleviation of drought stress in mung bean plant. Results showed that water stress raised membrane lipid peroxidation via increasing malondialdehyde (MDA) content as well as some antioxidant enzymes activity such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) and proline level. Nonetheless, magnetized water alleviated drought injuries by means of reducing lipid peroxidation through decreasing MDA content and further increasing in antioxidant enzymes activity and proline level. Results signify that irrigation with magnetized water as a simple, safe and practical technique could help reducing the adverse effects of water stress and might have a key role in mung bean tolerance to drought by decreasing oxidative damage via further activities of antioxidant enzymes and more proline accumulation.
Keywords: *Antioxidant enzymes, Magnetic irrigation, Malondialdehyde, Proline, Drought*

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ABBREVIATIONS*: APX, ascorbate peroxidase; ASH, ascorbic acid; CAT, catalase; DHAR, Dehydroascorbate reductase; GSH, glutathione; GPX, glutathione peroxidase; GR, glutathione reductase; GST, glutathione-S-transferase; GOPX, guaicol peroxidase;* H_2O_2*, hydrogen peroxide;* $OH^{\bullet-}$*, hydroxyl radical; MDA, malondialdehyde; MDHAR, monodehydroascorbate reductase; POD, peroxidase; ROS, reactive oxygen species;* $^1O_2$*, singlet oxygen;* $O_2^{\bullet-}$*, superoxide; SOD, superoxide dismutase;*

INTRODUCTION

Drought is one of the most important environmental factors that limit crop growth and yield. A common effect of drought stress is oxidative damage. Exposure to drought results in a loss of balance between the production of reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\bullet-}$) and singlet oxygen ($^1O_2$) and their scavenging (1). If not effectively and rapidly removed from plants, excessive levels of ROS can damage a wide range of cellular macromolecules such as lipids, proteins and DNA and ultimately cause cell death. To protect the subcellular components from ROS accumulation, plants respond with an induction of enzymatic antioxidants such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) and gluthatione reductase (GR), as well as antioxidant metabolites such as glutathione and ascorbate (2). It has been reported that membrane of plant cells are subject to rapid damage with increase in water stress. This leakage of membrane is caused by an uncontrolled enhancement of free radical, which cause lipid peroxidation. Damage to fatty acids of membrane could produce small hydrocarbon fragments including malondialdehyde (MDA) (3). MDA is the final product of plant cell membrane lipid peroxidation and is one important sign of membrane system injury (4).

In addition to an enzymatic scavenging system, accumulation of proline is one of the important adaptive strategies which plants use to cope with environmental stresses, particularly low water stress. Proline is also closely related with plant drought stress as free proline can accumulate significantly in crops and other plants (5-7). As an osmoprotectant in plants subjected to drought conditions, proline can accumulate to high concentrations in plant cells without disrupting cellular structure or metabolism.

Therefore, proline accumulation plays an important role in osmotic adjustment, detoxification of ROS, and membrane integrity in plants under stress conditions (8, 9). Proline acts as a free radical scavenger and may be more important in overcoming stress than in acting as a simple osmolyte (10).

Water is the most important factor for plant growth. The water treated by the magnetic field or pass through a magnetic device called magnetized water. Magnetic treatment of water has been reported to change some of the physical and chemical properties of water, mainly hydrogen bonding, polarity, surface tension, conductivity, pH and solubility of salts. These changes in water properties may be capable of affecting the growth of plants. The results of Grewal and Maheshwari (11) showed magnetic treatment of irrigation water and magnetic treatment of seeds had the potential to improve the early seedling growth and nutrient contents of seedlings. Utilization of magnetized water improved quantity and quality of common bean crop. Irrigation of common bean plants with magnetic water increased significantly the growth characteristics, potassium, GA3, kinetin, nucleic acids (RNA and DNA), photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoid), photosynthetic activity and translocation efficiency of photoassimilates as compared with control plants (12). It was detected that the magnetic field stimulated the shoot development and led to the increase of the germinating energy, germination, fresh weight and shoot length of maize (13).

Mung bean (*Vigna radiata* L.) is an important short duration grain legume crop with wide adaptability, low input requirements and the ability to improve the soil by fixing atmospheric nitrogen. Mung bean is well suited to a large number of cropping systems and constitutes an important source of high quality protein in the cereal based diets of many people in Asia (14).

It is hypothesized that magnetized water can diminish the harmful effects of water stress and oxidative injury. The present study therefore was carried out to investigate the impact of magnetic irrigation on drought tolerance of mung bean.

MATERIALS AND METHODS

Experimental design and plant material

In order to investigate the effect of magnetized water on drought tolerance of mung bean a pot experiment was done in Yadegar-e-Imam Khomeini (RAH) Branch, Islamic Azad University, Tehran, Iran at 2013. Longitude, latitude and altitude are 51° 28´ E, 35° 35´ N and 1000 m, respectively. This region is located in an arid climate where the summer is hot and dry and the winter is cool and dry. This experiment was conducted as a factorial completely randomized design with four replications. The first factor consisted of two irrigation levels including irrigation after 50 mm evaporation from a class A evaporation pan as normal and irrigation after 100 mm evaporation from a class A evaporation pan as water stress conditions. The second factor consisted of two types of water: ordinary water and magnetized water. Half of the pots were irrigated with ordinary water, while the other 8 pots were irrigated with the ordinary water after magnetization through passing in magnetic device (two magnet with length 15 cm, width and diameter 5 cm) which were connected to the water pipe. Water properties before and after magnetization are presented in Table 1. Mung bean seeds (cv. Partow) without visible defect, insect damage and malformation were surface sterilized using 5% sodium hypochlorite solution for 5 min and then rinsed 3 times with sterile distilled water. Then seeds were planted in 16 plastic pots (50 cm in diameter and depth) containing an equal mixture of peat, decomposed manure and farm soil. Sowing date was 1 June 2013 and then pots were placed in farm conditions. In each pot 15 seeds were sown in 3 cm depth of the soil and at the 3 leaf stage after thinning; 6 seedlings remained. At the flowering stage, several fully expanded leaves randomly selected and immediately frozen in liquid nitrogen and stored at -80˚C for biochemical analysis.

Assay of MDA

Lipid peroxidation was estimated in terms of MDA content according to the method of Heath and Packer (15). Leaf samples (1 g) were homogenized in 10 ml of trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000 rpm for 5 min. 4 ml (0.5%) of thiobarbituric acid in 20% trichloroacetic acid was added to 1 ml aliquot of the supernatant. Mixture was heated at 95 ˚C for 30 min and then cooled rapidly in an ice bath. After centrifugation at 10,000 rpm for 10 min, the absorbance was recorded at 532 nm. The value for non-specific absorption at 600 nm was subtracted. The MDA content was calculated using its absorption coefficient of 155 mM^{-1} cm^{-1} and expressed as μmol MDA g^{-1} fresh weight.

Assay of antioxidant enzymes activity

For enzyme extractions, leaf samples (0.5 g) were homogenized with 0.05 M sodium phosphate buffer (pH 7.8) containing 1 mM EDTA×Na_2 and 2% (w/v) polyvinylpolypyrrolidone (PVPP). Homogenates were centrifuged at 14,000 rpm for 40 min at 4 ˚C. The supernatants were used for the determination of protein content and activities of SOD, CAT and APX enzymes. Total soluble protein contents were determined according to Bradford (16).

SOD activity was assayed by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) according to the methods of Beyer and Fridovich (17). 5 ml reaction mixture containing 5 mM hydroxyethyl piperazine ethane sulfonic acid (HEPES) (pH 7.6), 0.1 mM EDTA, 50 mM Na_2CO_3 (pH 10.0), 13 mM methionine, 0.025% (v/v) Triton X-100, 63 µmol (NBT), 1.3 µmol riboflavin and an enzyme extract was illuminated for 15 min (360 µmol m^{-2} s^{-1}) and a control set was not illuminated to correct for background absorbance. A unit of SOD was defined as the amount of enzyme required to cause 50% inhibition of the reaction of NBT at 560 nm.

CAT activity was assayed by monitoring the decomposition of H_2O_2 at 240 nm by the procedure of Aebi (18). The reaction mixture contained 50 mM phosphate buffer (pH 7.0.), 0.1% (v/v) Triton X-100, 10.5 mM H_2O_2 and 0.05 ml leaf extract. The reaction carried out at 25˚C for 3 min was started with the H_2O_2 addition.

APX activity was determined according to Nakano and Asada (19) by the decrease in absorbance of ascorbate at 290 nm. The assay mixture contained phosphate buffer (50 mM, pH 7.0), 0.1 mM EDTA, 0.5 mM ascorbate, 0.1 mM H_2O_2 and enzyme extract. APX activity was calculated by using the extinction coefficient 2.8 mM^{-1} cm^{-1}. One unit of enzyme is the amount necessary to decompose 1 µmol of substrate per min at 25 ˚C.

Assay of proline

For proline estimation following the method of Bates et al. (20), 0.5 g of dried powdered leaves was homogenized in 10 ml 3% aqueous sulfosalicylic acid and the homogenate filtered. 2 ml acid ninhydrin (prepared by warming 1.2 g of ninhydrin in 30 ml glacial acetic acid) was added to 2 ml filtrate in a digestion tube and placed in a boiling water bath for 90 min. The reaction was terminated in an ice bath. 4 ml toluene was added to the reaction mixture and agitated vigorously for 30 min. The chromophore containing toluene was aspirated from the aqueous phase and the absorbance read at 520 nm.

Statistical analysis

Finally all data were analyzed by MSTAT-C statistical software and the means were compared by Duncan's Multiple Range Test (DMRT) at the 5% probability level.

RESULTS AND DISCUSSION

Abiotic stresses such as drought can disturb plant growth and development, leading to crop losses. For food security and increasing quantity and quality of agricultural production, more efforts are needed to develop multiple effective strategies to improve crop tolerance to water stress. In this study, we investigated the effects of magnetized water on mung bean biochemical responses to drought. Magnetic irrigation is an effective method which can significantly affects growth and production of some plants. However mechanisms of its action in improving water stress tolerance are not fully explained.

MDA content

In the present study, the lipid peroxidation level measured by the concentration of MDA. Water stress markedly increased MDA content by 64% as compared with normal conditions. Our research also revealed that the magnetized water significantly decreased MDA content under water stress conditions by 35% as compared to ordinary water, while effect of magnetized water on MDA content under normal conditions was not significant (Figure 1). Functions of plasma membrane are adversely affected by environmental stresses that can be measured as level of membrane lipid peroxidation. MDA (a product of membrane lipid peroxidation) content could reflect the degree of membrane lipid peroxidation. In this research, MDA content was increased significantly under water stress conditions. The membrane damage is caused either by the generation of ROS or by the direct degradation of polyunsaturated fatty acids (21). However, this oxidative damage was ameliorated by magnetized water because the MDA level decreased in this treatment. This indicates that magnetized water has a key role in ROS scavenging and reduction of oxidative stress in mung bean subjected to water stress.

Antioxidant enzymes activity

All antioxidant enzyme activities were increased drastically during water stress. Under water stress conditions SOD, CAT and APX activities were raised by 67%, 32% and 43% respectively. We also observed that under water stress conditions the magnetized water treatment caused more activity of antioxidant enzymes. This treatment increased SOD, CAT and APX activities by 23%, 18% and 19% under water stress conditions as compared to ordinary water but under normal conditions the effect of magnetized water on SOD, CAT and APX activities was not significant (Figures 2, 3, 4).

Table 1. Water properties before and after magnetization

Water properties	Ordinary water	Magnetized water
EC (µS/cm)	1210	1197
pH	7.68	7.80
NO_3 (ppm)	1.1	1.1
PO_4 (ppm)	19	18
K (ppm)	31	29
SO_4 (ppm)	211	202
Ca (ppm)	118	110
Mg (ppm)	79	74
Hardness ($CaCo_3$) (ppm)	435	410

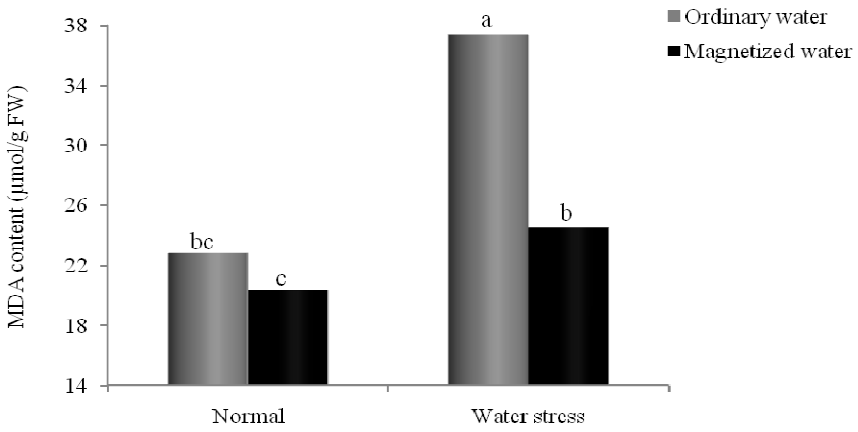

Figure 1. Effect of magnetized water on MDA content under normal and water stress conditions. Different letters indicated significant differences at P ≤ 0.05 level using DMRT.

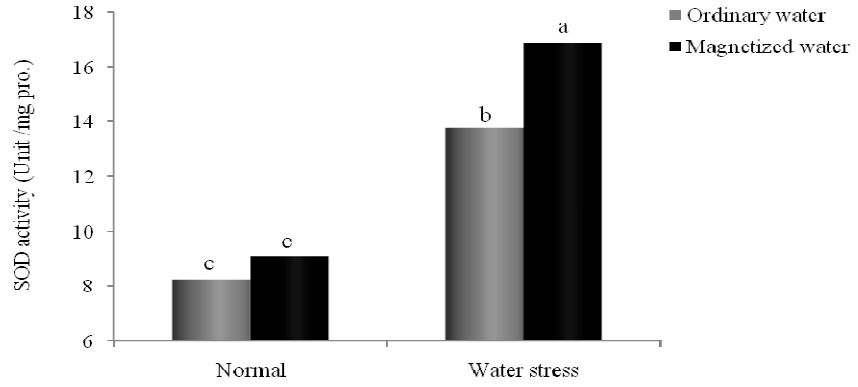

Figure 2. Effect of magnetized water on SOD activity under normal and water stress conditions. Different letters indicated significant differences at P ≤ 0.05 level using DMRT.

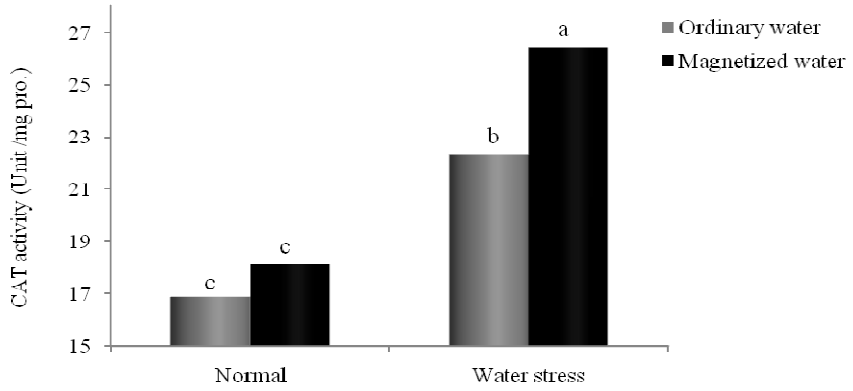

Figure 3. Effect of magnetized water on CAT activity under normal and water stress conditions. Different letters indicated significant differences at P ≤ 0.05 level using DMRT.

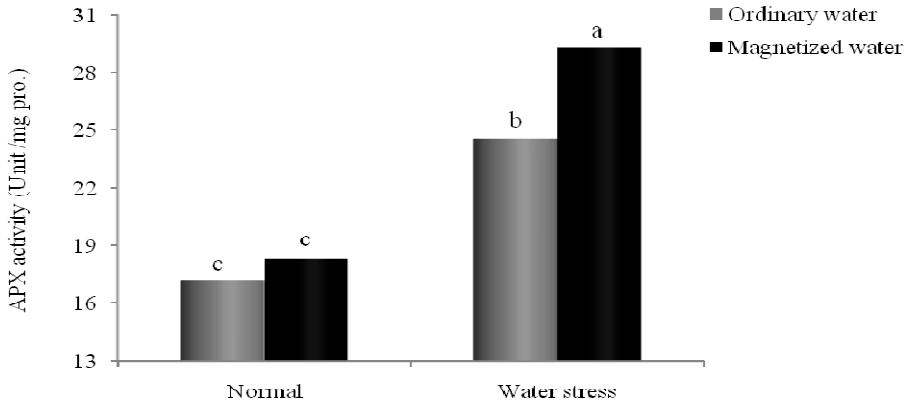

Figure 4. Effect of magnetized water on APX activity under normal and water stress conditions. Different letters indicated significant differences at *P* ≤ 0.05 level using DMRT.

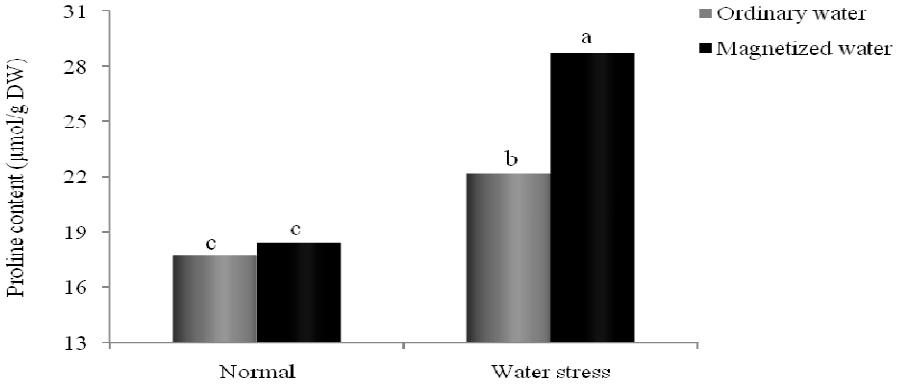

Figure 5. Effect of magnetized water on proline content under normal and water stress conditions. Different letters indicated significant differences at P ≤ 0.05 level using DMRT.

Various abiotic stresses such as drought lead to the overproduction of ROS e.g., $^1O_2$, $O_2^{\bullet-}$, H_2O_2 and $OH^{\bullet}$ in plants which are highly reactive and toxic and cause damage to proteins, lipids, carbohydrates and DNA which ultimately results in oxidative stress. To protect themselves against these toxic oxygen intermediates, plant cells and its organelles like chloroplast, mitochondria and peroxisomes employ antioxidant defense systems. Plants possess very efficient enzymatic (SOD, CAT, APX, GR, MDHAR, DHAR, GPX, GOPX and GST) and non-enzymatic (ASH, GSH, phenolic compounds, alkaloids, non-protein amino acids and α-tocopherols) antioxidant defense systems which work in concert to control the cascades of uncontrolled oxidation and protect plant cells from oxidative damage by scavenging of ROS (22).

Metalloenzyme SOD is the most effective intracellular enzymatic antioxidant which is ubiquitous in all aerobic organisms and in all subcellular compartments prone to ROS mediated oxidative stress. It is well established that various environmental stresses often lead to the increased generation of ROS, where, SOD has been proposed to be important in plant stress tolerance and provide the first line of defense against the toxic effects of elevated levels of ROS. The SODs remove $O_2^{\bullet-}$ by catalyzing its dismutation, one $O_2^{\bullet-}$ being reduced to H_2O_2 and another oxidized to O_2. It removes $O_2^{\bullet-}$ and hence decreases the risk of $OH^{\bullet}$ formation via the metal catalyzed Haber-Weiss-type reaction (22).

CATs are tetrameric heme containing enzymes with the potential to directly dismutate H_2O_2 into H_2O and O_2 and are indispensable for ROS detoxification during stressed conditions (23). CAT has one of the highest turnover rates for all enzymes: one molecule of CAT can convert about 6 million molecules of H_2O_2 to H_2O and O_2 per minute. CAT is important in the removal of H_2O_2 generated in peroxisomes by oxidases involved in B-oxidation of fatty acids, photorespiration and purine catabolism (22).

APX is thought to play the most essential role in scavenging ROS and protecting cells in higher plants, algae, euglena and other organisms. APX is involved in scavenging of H_2O_2 in water-water and ASH-GSH cycles and utilizes ASH as the electron donor (22).

The results showed that water stress raised the activities of antioxidant enzymes such as SOD, CAT and APX. On the other hand magnetized water further enhanced these enzymes activities. From the results, it is clear that the magnetized water have induced SOD, CAT and APX activities and increased total antioxidant capacity in mung bean plants under drought conditions, which may be related to the induction of antioxidant enzymatic responses that protect the plant from oxidative damage. These results for increasing antioxidant enzymes activity as affected by magnetized water are in good concurrence with that of El sayed and El sayed (24), Pintilie et al. (25) and Moussa (12).

Proline content

According to our study leaf free proline content was increased significantly under water stress by 25% as compared to normal conditions. On the other hand magnetized water treatment induced more accumulation of the free proline under water stress conditions by 28% as compared with ordinary water. However magnetized water had no significant effect on free proline accumulation under normal conditions (Figure 5). We observed that water stress led to an increase in proline concentration in mung bean leaves and much more proline accumulated when magnetized water was used. Proline is a non-protein amino acid that forms in most tissues subjected to water stress and together with sugar, it is readily metabolized upon recovery from drought (26).

Accumulation of proline is an important indicator of drought stress tolerance in higher plants (27). The accumulation of proline is believed to facilitate osmotic adjustment by which the internal osmotic potential of plants is lowered and may then contribute to drought tolerance. Proline accumulation under drought stress may be that it contributes a protective role as scavenges of ROS, resulted in improved adaptation ability and growth of plants under drought conditions (28). In addition to acting as an osmo-protectant, proline also serves as a sink for energy to regulate redox potentials, as a hydroxyl radical scavenger (29), as a solute that protects macromolecules against denaturation and as a means of reducing acidity in the cell (30). However, Vendruscolo *et al*. (31) stated that proline might confer drought stress tolerance to wheat plants by increasing the antioxidant system rather than as an osmotic adjustment. Furthermore, in the present study the proline contents increasing in mung bean plants irrigated with magnetized water under drought conditions may be also ascribed to enhancement of antioxidant enzymes activity and osmotic adjustment. Samarzadeh Vajdehfar *et al*. (32) and El Sayed and El Sayed (24) have also reported that magnetized water increased proline accumulation in sunflower and broad bean plants.

CONCLUSIONS

The present study indicated that water stress increased oxidative damage, membrane lipid peroxidation as well as antioxidant enzymes (SOD, CAT and APX) activity and proline level in mung bean leaves, on the other hand irrigation with magnetized water as a simple and safe method induced protection against drought stress via maintenance of membrane integrity by decline in MDA content and more increase in

antioxidant enzymes (SOD, CAT and APX) activity as well as proline accumulation. It suggests that irrigation with magnetized water as a simple, safe and economic technique can help to decrease the adverse effects of water stress in mung bean.

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