Magnetized Water Confers Drought Stress Tolerance in *Moringa* Biotype via Modulation of Growth, Gas Exchange, Lipid Peroxidation and Antioxidant Activity


1State Key Laboratory of Grassland Agro-Ecosystems, School of Life Sciences, Lanzhou University, Lanzhou, Gansu Province, China
2Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
3Department of Arid Land Agriculture, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, Jeddah, Saudi Arabia
4Department of Horticulture, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh
5Department of Biochemistry and Microbiology, School of Health and Life Sciences, North South University, Dhaka, Bangladesh

Received: 16 April 2019
Accepted: 27 June 2019

Abstract

The present study assesses the effect of drought stress on the *Moringa* biotype under magnetized water treatment (MWT). The *Moringa* biotype seedlings were subjected to drought stress with varying field capacities (FC) viz., control (100% FC), moderate drought stress (MS, 50% FC), and severe drought stress (SS, 20% FC). Magnetized water (MW) significantly increased plant height, leaflet number, internode distances, leaf area, dry weight of the leaf, shoot, root of the seedlings and markedly improved the assimilation, transpiration, stomatal conductance, water use efficiency and vapor pressure deficit under drought stress conditions. The maximum quantum efficiency of PSII (Fv/Fm) and maximum chlorophyll fluorescence (Fm) were increased and minimum chlorophyll fluorescence (F0) in the dark-adapted state was decreased under drought stress with MWT. Photosynthetic pigments (Chl a, Chl b, carotenoids) significantly decreased under drought stress, but MW significantly increased them. The MW application in *Moringa* biotype resulted in a decrease in total phenolic content (TPC) by 19% and 26% under MS and SS conditions, respectively. Malondialdehyde (MDA), hydrogen peroxide (H2O2) and accumulation of proline in leaf were decreased with the prolongation of drought with MW. MW could be

*e-mail: hasanmahadikau@gmail.com
**e-mail: fangxw@lzu.edu.cn
used for alleviating the drought stress in *Moringa* biotype seedlings and improve drought tolerance by modulating the physiological activities.

**Keywords:** magnetized water, malondialdehyde, hydrogen peroxide, phenolic compounds, maximum quantum efficiency of PSII

**Introduction**

Increasing greenhouse gas emissions have caused huge changes in climatic conditions and this phenomenon has led to increased global temperatures [1]. There are two key natural features, viz., drought and high temperature stresses, that can remarkably affect crop productivity [2]. Due to rising temperature and shortage of rainfall, most of the cultivated land becomes dry land or desert and it rapidly changes into semi-arid and arid areas [3-4]. A shortage of water hinders the development and potency of the plants, which results in low crop production [5-7]. Drought stress is one of the detrimental abiotic stresses affecting the leaf, shoot, root growth and development as well as depleting plant maturation [8, 9].

Drought stress has been mitigated by using different approaches, including the exogenous application of various chemical and biological agents as well as incorporating various physical methods. Research suggests that various chemical compounds, including proline, abscisic acid (ABA), salicylic acid, jasmonic acid, betain and spermidine, amino acid, hydrogen peroxide (H$_2$O$_2$), humic acid, nitric acid and antioxidants have been proven to be useful agents to mitigate drought stress [10-13]. In addition to these exogenous supplies of biochemical agents, recently the latest strategies and techniques – including robotics, GIS technology, sensors and magnetic water technology – were used as beneficial tools in order to alleviate drought stress among crop plants [14].

Magnetic water technology is regarded as one of the most eco-friendly and effective tools in modern agriculture practices. Magnetized water (MW) is usually generated by treating the normal irrigation water by magnetic field [15]. Studies have reported that magnetic water treatment changes water quality, including its molecular and other properties [16]. These changes occur due to changes in the nuclei of water molecules [16-19] and the increasing number of centers of crystallization and free gas amount [20], which in turn improves water quality. For example, MW exhibits weakened hydrogen bonds, reduced polymerization and improved water solubility as compared to pure water [21]. MW for irrigation can promote water productivity and nutrients by plants [22-23]. Recently, the potential of MW has been studied in order to increase germination, seedling growth and development – particularly when plants are under various abiotic stresses [21, 24]. Liu et al. (2019) [21] found that irrigating *Populus* seedlings with MW could reduce salt stress.

*Moringa* sp. belonging to the family Moringaceae is an important source of food and medicine [14]. In our previous studies, *Moringa oleifera* and *Moringa peregrina* have shown great potential during drought conditions when treated with MW [14, 25]. The *Moringa* biotype was used in this study, which have both *Moringa oleifera* and *Moringa peregrina* characteristics, and it was found in the Al Bahah Region of Saudi Arabia [26-27]. The studied *Moringa* biotype has leaflets round, oblanceolate or elliptical, which are analogous to leaflets of *M. oleifera* and *M. peregrina* [27]. The morphological and genetic studies of the *Moringa* biotype have been reported previously [26-27], but physiological and biochemical studies of this *Moringa* biotype under drought stresses are absent. Therefore, in the current study, we have investigated the effects of MW on this *Moringa* biotype under different levels of drought stress. We have compared the effects of magnetized water with the control (Normal irrigated tap water) on the morphological characteristics, including growth and various physiological as well as biochemical parameters, including leaf gas exchange, chlorophyll content, phenolic compounds and antioxidant capacity of *Moringa* biotype seedlings during drought stress conditions. Studying the biochemical and physiological markers of drought tolerance of this *Moringa* biotype could further help us in understanding the effect of MW in alleviating drought stress in plants.

**Materials and Methods**

**Plant Material and Experimental Design**

Seeds of the *Moringa* biotype were collected from the Al Bahah region (18.22° and 42.51°) in Saudi Arabia, as described by Robiansyahet et al. (2015) [26]. The experiment was carried out in a 9 m long and 4 m wide greenhouse located at KAU, Jeddah, Saudi Arabia and sandy loam soil mixed with peat moss and compost (1:1:1) was used during the experiment. The seeds of the *Moringa* biotype were sown on the same day in the pots and arranged in randomized completely block design (RCBD) with three replications. Plants were cultivated in the greenhouse with a day:night mean temperature of 25:20°C and a 16h day/8h night photoperiod. The relative humidity was 50-70%.

**Drought Stress Treatments**

Sixty-day-old plants were subjected to drought treatments including 100% FC, 50% FC, and 20% FC,
Magnetized Water Confers Drought... and the FC was maintained by weighing the pot every day. The leaf samples were collected during harvest time.

Magnetic Treatments

The water was treated with a magnetic device by following the methods of Selim and El-Nady (2011) and Liu et al. (2019) [21, 28]. A magnetic treatment device was used for treating water with a permanent magnet (U050 mg, 0.5 in., output 4-6 m3 h−1) and a magnetic induction of approximately 300 Gs, produced by Magnetic Technologies L.C.C. (Russia, United Arab Emirates branch). The potted seedlings were irrigated with drought treatments in the presence or absence of magnetic treatment. The pots were arranged into six experimental groups:

i. (Control +NW): the plants were grown with normal water treatment with 100% FC.

ii. (Control +MW): the plants were grown with magnetic water treatment with 100% FC.

iii. (MS+NW): the plants were cultivated with normal water treatment that was subjected to moderate drought stress (MS, 50% FC).

iv. (MS+MW): the plants were cultivated with magnetic water treatment that was subjected to moderate drought stress (MS, 50% FC).

v. (SS+NW): the plants were cultivated with normal water treatment that was subjected to severe drought stress (SS, 20% FC).

vi. SS+MW): the plants were cultivated with magnetic water treatment that was subjected to severe drought stress (SS, 20% FC).

Growth Conditions

Four growth-related parameters such as plant height, leaf number, leaflet number, and internode distances were measured at one-week intervals until harvest. Harvesting was done by cutting plants from the soil surface and the fresh weight was determined after the separation of stems and leaves. Two leaves of each plant were collected and put into sealed vials, frozen in liquid N2 and stored in a freezer (-80ºC). Roots were cleaned by removing the soil through gentle washing by tap water and then dried using thick tissue. The same root was weighed and put into a paper bag and oven-dried at 65°C for 72h to measure the dry weight.

Leaf Area (LA) and Relative Water Content (RWC)

LA of Moringa biotype was measured by a leaf area meter supplied by LICOR-3000A, USA. Fresh weight (FW) of the leaf samples were collected followed by keeping the leaf disc in a petri dish in deionized water for 8 h in the dark to calculate the RWC. Turgid weight (TW) was measured after removing excess water in the surface and the dry weight data were collected after oven drying the samples for 48 hours at 80°C.

To calculate RWC, the following formula was used:

\[
\text{Relative water content, } RWC(\%) = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100
\]

Leaf Gas Exchange

Assimilation rate \((A, \text{CO}_2 \text{m}^2 \text{s}^{-1})\), stomatal conductance \((g_s, \text{mol CO}_2 \text{m}^{-1} \text{s}^{-1})\), transpiration rate \((E, \text{mmol CO}_2 \text{m}^{-2} \text{s}^{-1})\), vapor pressure deficit \((VPD, \text{kPa})\) and water use efficiency \((\text{WUE, } \mu\text{molCO}_2\text{mmol}^{-1}\text{H}_2\text{O})\) were measured as leaf gas exchange parameters using a CIRAS III photosynthesis system. The young and healthy leaf samples were taken into consideration for measurement and the data were collected in-between 12:00 and 14:00h in the afternoon. An average of three randomly selected leaves data were considered for analysis.

Fv/Fm

Fv/Fm was collected at an interval basis on the same leaves used for gas exchange measurements. All the leaf samples were measured after dark adaption for 30 mins with leaf cuvette using a CIRAS III photosynthesis system.

Photosynthetic Pigments

Photosynthetic pigments were extracted following the method explained by Lichtenthaler and Wellburn (1983) [29]. Mortar and pestle were used to crush 0.5 g homogenized leaf samples and 10 ml of acetone (80% v/v) added, followed by centrifuging at 5,000×g for 10 min. A UV-visible spectrophotometer (UV-1900) was used to find the absorbance of 663, 645 and 470 nm respectively.

Determining Ion Content

One ml of sulphuric acid (H₂SO₄) was added to each digestion tube containing plant sample (powder form) and then transfer to the sand heater. Completion of digestion takes 15-20 min by changing into dark color. After cooling the samples, 1 ml mixture of H₂SO₄ and perchloric acid (HClO₄ in equal ratio) was added and heated for 30-40 min again until the transparent color appeared. Then 100 ml distilled water was added to each sample tube. Optima ICP-OECS machine was used to determine the ion contents such as Na⁺, K⁺, Ca²⁺, Mg²⁺, Cu²⁺, Fe²⁺, Mn²⁺, P⁵⁺, and Ni²⁺. This method was described by Humpheries (1956) [30].

Methanolic Extract Preparation

Methanolic extract was prepared by centrifuging 2.0 g of leaves of Moringa biotype 150 rpm along with
20 ml methanol (80%) for 12 h, followed by filtration by filter paper.

**Estimating Total Phenolic Content (TPC)**

TPC was measured following the procedures explained by Velioglu et al. (1998) [31]. 50 µL of methanolic extract was mixed with Folin-Ciocalteu reagent (100 µ) and methanol (850µ). The mixture was kept for 5 min at ambient condition. 500 µL of 20% NaCO₃ was added again to this mixture and we allowed the reaction to occur for another 30 mins. OD was measured at 750 nm and TPC was quantified using a calibration curve that was made by measuring the absorbance of known concentrations of gallic acid. Gallic acid equivalent/g tissues were used to express the results.

**Estimating Total Flavonoid Content (TFC)**

TFC was determined by using a modified colorimetric method described by Zhishen et al. (1999) [32]. 250 µL of ME (standard solution) was mixed with 1.25 ml distilled water and 75 µL of 5% sodium nitrite solution and kept for 6 min for reaction. 150 µL of 10% AlCl₃, 0.5 ml of 1 M NaOH and 275 µL distilled water were also added to the mixture after 5 mins. Flavonoids were quantified using a calibration curve obtained by measuring the OD of catechin of known concentrations, and absorbance was calculated at 510 nm. The results were expressed as milligram (mg) of catechin equivalent/g tissues.

**Proline Assay**

Free proline content was measured following the procedures described by Bates et al. (1973) [33] with minor modifications. Fresh leaf tissues (0.5 g) were taken and homogenized in 10 mL of 3% sulfosalicylic acid in ice. The sample was centrifuged for 15 min at 11,500 g followed by a collection of 2 mL of the filtrate. Then 2 mL acid ninhydrin and 2mL glacial acetic acid was added to the extract and allowed for reaction. The mixture was incubated at 100°C for an hour. After cooling the mixture, 4 mL of toluene was added. The absorbance was taken at 520 nm. From the standard curve, the amount of proline was determined and expressed as µg/g FW.

**DPPH Radical Scavenging Assay**

Free radical scavenging activity of methanol extract (ME, equivalent to 20, 40, and 60µl) was determined by using 1-diphenyl-2-picrylhydrazyl (DPPH) described by Ao et al. (2008) [34]. 0.1 ml ME was added to 0.9 ml newly prepared 0.1mM of DPPH methanol solution. The same amount of methanol was used as control. The reacted mixture was incubated for half an hour in a dark room under normal temperatures. The OD was measured at 517nm and the following formula was used to calculate the radical scavenging activity (%):

\[
\text{DPPH radical scavenging (\%)} = \left( \frac{\text{OD Control} - \text{OD Sample}}{\text{OD Control}} \right) \times 100
\]

**Measurement of Lipid Peroxidation**

Malondialdehyde (MDA) concentrations act as an indicator for the level of lipid peroxidation and measurement was done by following the methods described by Heath and Packer (1968) [35]. Thiobarbituric acid (TBA) was used as the reactive material for measuring the MDA. 0.5 g leaf samples were homogenized in 3 mL 5% (w/v) trichloroacetic acid (TCA). The homogenate was allowed for centrifugation at 11,500×g for 10min. 1 mL supernatant was collected and mixed with 4 mL TBA reagent, which consisted of 0.5% TBA dissolved in 20% TCA. A hot
Magnetized Water Confers Drought...

1629

Water bath was used to heat the reacting mixture up to 95°C for 30 min, followed by rapid cooling in an ice bath. The cooled mixture was centrifuged at 11,500×g for 15 mins, which produced colored supernatant whose optical density was measured at 532 nm. In addition, a correction was made for non-specific absorbance at 600 nm. MDA concentration was expressed as nanomoles / gram fresh weight using extinction coefficient as 155 mM⁻¹ cm⁻¹.

Measuring H₂O₂

To measure the hydrogen peroxide (H₂O₂), a homogenate was prepared from 0.5 g leaf sample followed by the addition of 3 mL of 50 mM potassium-phosphate buffer (pH 6.5) at 4°C. At 11,500xg speed and 15 min time, the homogenate was centrifuged, and 3 ml supernatant was collected, which was then allowed for the reaction with 1 mL of 0.1% titanium tetrachloride in 20% H₂SO₄. The mixture was centrifuged again at 11,500xg for 15 min and kept at room temperature for 10 min. The absorbance was quantified at 410 nm to determine the concentration of H₂O₂ and expressed as nanomoles/gram fresh weight.

Statistical Analysis

Minitab statistical software vs. 17 was used to perform basic statistical analysis and an ANOVA test at 5% (P≤0.05) level of significance. Mean differences were among the groups tested by Fisher’s LSD test.

Results

Growth Parameters

Drought-induced stress retarded the plant height, internode distances and leaflet number. We observed that MWT had a statistically significant effect in plant height of Moringa biotype measured at 5 DAT to 30 DAT (Fig. 1) compared to the normal water treatment (NWT). Application of MWT to the drought stress seedlings significantly restored the plant growth compared to the seedlings treated with NWT alone. In addition, morphological components such as root, shoot, and fresh and dry leaf weight were found to be mostly influenced by the MS and SS drought levels under NWT irrigation and MWT (Table 1).

Table 1. Interaction effect between Moringa biotype vs. water on fresh weight (FW) and dry weight (DW) of root, stem, leaf, and root number.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Root FW</th>
<th>Root DW</th>
<th>Shoot FW</th>
<th>Shoot DW</th>
<th>Leaf FW</th>
<th>Leaf DW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control+ NW</td>
<td>18.4±1ab</td>
<td>3.2±0.1ab</td>
<td>1.63±0.3bc</td>
<td>0.44±0.03b</td>
<td>6.8±0.7bc</td>
<td>1.7±0.08b</td>
</tr>
<tr>
<td>Control+ MW</td>
<td>19.2±1.75a</td>
<td>3.3±0.17a</td>
<td>2.16±0.2a</td>
<td>0.54±0.05a</td>
<td>8.1±0.7a</td>
<td>2.06±0.25a</td>
</tr>
<tr>
<td>MS+NW</td>
<td>17.2±0.45bc</td>
<td>3.01±0.1bc</td>
<td>1.33±0.15c</td>
<td>0.36±0.02c</td>
<td>6.1±0.9c</td>
<td>1.5±0.02bc</td>
</tr>
<tr>
<td>MS+MW</td>
<td>18.06±0.6ab</td>
<td>3.24±0.1a</td>
<td>1.8±0.1b</td>
<td>0.4±0.02bc</td>
<td>7.5±0.3ab</td>
<td>1.7±0.02b</td>
</tr>
<tr>
<td>SS+NW</td>
<td>16.2±0.65c</td>
<td>2.9±0.1c</td>
<td>1.04±0.04d</td>
<td>0.24±0.01e</td>
<td>6.4±0.4c</td>
<td>1.41±0.04c</td>
</tr>
<tr>
<td>SS+MW</td>
<td>17.1±1bc</td>
<td>2.97±0.1c</td>
<td>1.5±0.3bc</td>
<td>0.3±0.01d</td>
<td>7.5±0.4ab</td>
<td>1.66±0.02b</td>
</tr>
</tbody>
</table>

Dissimilar letters within mean and between columns are significantly different at p≤0.05 level of significance by applying FISHER’s LSD Test.
Table 2. Interaction effect between *Moringa* biotype vs. water on the chlorophyll content under different levels of drought stress (mg g⁻¹FW).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Chl a</th>
<th>Chl b</th>
<th>Chl (a+b)</th>
<th>Carotenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control+NW</td>
<td>1.3±0.03c</td>
<td>0.78±0.02b</td>
<td>2.09±0.05c</td>
<td>1.41±0.04c</td>
</tr>
<tr>
<td>Control+MW</td>
<td>1.54±0.03a</td>
<td>0.87±0.01a</td>
<td>2.41±0.04a</td>
<td>1.63±0.03a</td>
</tr>
<tr>
<td>MS+NW</td>
<td>1.23±0.03d</td>
<td>0.67±0.04c</td>
<td>1.9±0.01e</td>
<td>1.38±0.01d</td>
</tr>
<tr>
<td>MS+MW</td>
<td>1.41±0.03b</td>
<td>0.79±0.02b</td>
<td>2.2±0.01b</td>
<td>1.52±0.03b</td>
</tr>
<tr>
<td>SS+NW</td>
<td>1.1±0.01c</td>
<td>0.6±0.04d</td>
<td>1.7±0.05f</td>
<td>1.23±0.03e</td>
</tr>
<tr>
<td>SS+MW</td>
<td>1.26±0.02cd</td>
<td>0.72±0.02c</td>
<td>1.98±0.06d</td>
<td>1.44±0.01e</td>
</tr>
</tbody>
</table>

Dissimilar letters within mean and between columns are significantly different at $p \leq 0.05$ level of significance by applying FISHER’s LSD Test.

Fig. 3. Effect of magnetized water (MW) on the assimilation, $A$; a), transpiration, $E$; b), and stomatal conductance, $gs$; c), water use efficiency, WUE; d), and vapour pressure deficit, VPD; e) of the biotype under drought stress at 5DAT to 30DAT (days after treatment); vertical bars represent the standard deviation and different letters above and below the series denote statistically significant ($P \leq 0.05$) differences among treatments.
Leaf Area (LA) and Relative Water Content (RWC)

Drought stress significantly reduced the leaf area in *Moringa* biotype seedlings by 3% and 8% respectively, exposed to MS and SS level as compared to control under NWT (Fig. 2a). The *Moringa* biotype seedlings exposed to drought stress (MS, SS) showed 1% and 4% reductions in RWC compared to control seedlings under NWT (Fig. 2b).

Table 3. Interaction effect between *Moringa* biotype vs. water on ion content.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Ca²⁺ (mg/L)</th>
<th>Mg²⁺ (mg/L)</th>
<th>Mn²⁺ (mg/L)</th>
<th>Pi⁺⁺mg/L</th>
<th>Zn²⁺(mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Root</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control+NW</td>
<td>27±1.8c</td>
<td>12.4±1.17cd</td>
<td>0.18±0.02a</td>
<td>14.3±0.57d</td>
<td>0.8±0.1a</td>
</tr>
<tr>
<td>Control+MW</td>
<td>31.9±1.9a</td>
<td>15.4±1.2a</td>
<td>0.18±0.01a</td>
<td>24.3±0.57a</td>
<td>0.84±0.06a</td>
</tr>
<tr>
<td>MS+NW</td>
<td>25.7±1.8d</td>
<td>11.2±1.2d</td>
<td>0.17±0.01b</td>
<td>13±1.1de</td>
<td>0.74±0.02b</td>
</tr>
<tr>
<td>MS+MW</td>
<td>29.9±1.85b</td>
<td>14.3±1.2b</td>
<td>0.18±0.02a</td>
<td>17.86±0.5c</td>
<td>0.81±0.03a</td>
</tr>
<tr>
<td>SS+NW</td>
<td>25.7±1.8d</td>
<td>9.6±1.2c</td>
<td>0.14±0.025d</td>
<td>11.3±0.57c</td>
<td>0.65±0.02d</td>
</tr>
<tr>
<td>SS+MW</td>
<td>29.9±1.85b</td>
<td>13.2±1.2c</td>
<td>0.16±0.02c</td>
<td>21±1b</td>
<td>0.72±0.01c</td>
</tr>
<tr>
<td><strong>Shoot</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control+NW</td>
<td>60.4±2d</td>
<td>16.01±1.1c</td>
<td>0.2±0.06cd</td>
<td>16.6±0.57c</td>
<td>1.3±0.02a</td>
</tr>
<tr>
<td>Control+MW</td>
<td>79.5±2a</td>
<td>27±1.14a</td>
<td>0.5±0.06a</td>
<td>27±1a</td>
<td>0.8±0.03e</td>
</tr>
<tr>
<td>MS+NW</td>
<td>56.3±2.05e</td>
<td>12±1.14d</td>
<td>0.16±0.05d</td>
<td>15.66±0.5cd</td>
<td>1.12±0.03b</td>
</tr>
<tr>
<td>MS+MW</td>
<td>72.66±2.05b</td>
<td>25±1.14ab</td>
<td>0.34±0.06b</td>
<td>26.33±1.5a</td>
<td>0.87±0.02d</td>
</tr>
<tr>
<td>SS+NW</td>
<td>54.43±1.6e</td>
<td>10.01±1.14d</td>
<td>0.15±0.05d</td>
<td>14±1d</td>
<td>1.0±0.005c</td>
</tr>
<tr>
<td>SS+MW</td>
<td>68.3±2c</td>
<td>23.1±1.1b</td>
<td>0.28±0.06bc</td>
<td>23.66±1.5b</td>
<td>0.8±0.02e</td>
</tr>
<tr>
<td><strong>Leaf</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control+NW</td>
<td>74.5±2.008d</td>
<td>21±1.7c</td>
<td>0.38±0.08b</td>
<td>22.3±0.57b</td>
<td>0.94±0.02b</td>
</tr>
<tr>
<td>Control+MW</td>
<td>95.6±2.1a</td>
<td>28.9±1.65a</td>
<td>0.6±0.07a</td>
<td>26±1a</td>
<td>0.97±0.01a</td>
</tr>
<tr>
<td>MS+NW</td>
<td>63.3±2.15e</td>
<td>16.23±1.65d</td>
<td>0.34±0.09c</td>
<td>21±1bc</td>
<td>0.91±0.01c</td>
</tr>
<tr>
<td>MS+MW</td>
<td>85.6±2.12b</td>
<td>26.03±1.7ab</td>
<td>0.36±0.08bc</td>
<td>27±1a</td>
<td>0.96±0.01a</td>
</tr>
<tr>
<td>SS+NW</td>
<td>60.13±2.15c</td>
<td>12.1±1.7e</td>
<td>0.3±0.08d</td>
<td>20±2c</td>
<td>0.81±0.01d</td>
</tr>
<tr>
<td>SS+MW</td>
<td>80.6±2.1c</td>
<td>23.6±1.4bc</td>
<td>0.32±0.08cd</td>
<td>23.3±2b</td>
<td>0.9±0.01c</td>
</tr>
</tbody>
</table>

Dissimilar letters within mean and between columns are significantly different at p≤0.05 level of significance by applying FISHER’S LSD Test.
Leaf Gas Exchange

Under 100% FC (control level), assimilation (A), transpiration (E), and stomatal conductance (gs) were high in Moringa biotype at 5DAT to 30 DAT (Fig. 3a-c). At 30 DAT, drought stress caused a significant decrease in transpiration by 27%; 44% exposed to MS and SS level under NWT. In addition, MWT seedlings showed a significantly higher transpiration rate than normal water-treated seedlings. Treating the Moringa biotype seedlings with drought considerably decreased the stomatal conductance compared with the control seedlings, whereas MW increased the stomatal conductance compared with the seedlings treated with drought alone. WUE and VPD were significantly affected by the different treatments. Different time of drought stress also was significantly affected by the MW (Fig. 3d,e).

Chlorophyll Content

The chlorophyll content (Chl a, Chl b, Chl (a+b), carotenoids) increased under MWT (Table 2). Seedlings treated with MW and exposed to SS level showed 10%, 14%, 11%, and 12% decreased in Chl a, Chl b, Chl (a+b), and carotenoids content as compared to control seedlings. Chl a, Chl b, Chl (a+b), and carotenoids were decreased by 27%, 31%, 29%, and 22% respectively, and exposed to SS under normal water treatment (NWT) as compared to control seedlings (Table 2).

Chlorophyll Fluorescence

The minimum chlorophyll fluorescence (Fo) was significantly increased in drought treatment for Moringa biotype. A significant reduction of maximum chlorophyll fluorescence (Fm) was decreased in dark-adapted leaves under MS and SS levels. Fv/Fm ratio of the dark-adapted leaves was decreased in Moringa biotype seedlings by 2%, 4% exposed to MS, SS level under NWT (Fig. 4). The Fv/Fm ratio significantly increased in the drought-stressed seedlings with MWT application.

Determining Ion Content

The ion content (Ca²⁺, Mg²⁺, Mn²⁺, P⁵⁺, Zn²⁺) in the roots, shoots and leaves decreased under drought stress (Table 3). The stress induced by drought decreased the Ca²⁺, Mg²⁺, Mn²⁺, P⁵⁺, and Zn²⁺ in the shoots of Moringa biotype by 15%, 41%, 12%, 14%, and 23% respectively, exposed to SS under normal water treatment as compared to control seedlings. However, MW improved the nutrient content in the Moringa biotype seedlings. The Na⁺/K⁺ ratio was increased in the roots, shoot and

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total phenolic Content (TP) (mg/gallic acid/g FW)</th>
<th>Total Flavonoids Content (TF) (mg/ catechin/g FW)</th>
<th>Proline content (µg/g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control+NW</td>
<td>7.94±0.03d</td>
<td>3.94±0.04ef</td>
<td>824.3±2.52d</td>
</tr>
<tr>
<td>Control+MW</td>
<td>8.07±0.01de</td>
<td>4.04±0.03e</td>
<td>818±2.65e</td>
</tr>
<tr>
<td>MS+NW</td>
<td>9.23±0.04c</td>
<td>4.27±0.06d</td>
<td>905±5c</td>
</tr>
<tr>
<td>MS+MW</td>
<td>7.49±0.01f</td>
<td>3.57±0.06gh</td>
<td>707.3±2.5f</td>
</tr>
<tr>
<td>SS+NW</td>
<td>12.28±0.05a</td>
<td>5.21±0.21a</td>
<td>1280.6±3.7a</td>
</tr>
<tr>
<td>SS+MW</td>
<td>9.13±0.11c</td>
<td>4.61±0.03c</td>
<td>1009±5.5b</td>
</tr>
</tbody>
</table>

Means within each column followed by the same letter are not significantly different at level P<0.05
Magnetized Water Confers Drought...  

1633

leaf in the drought-stressed seedlings and decreased with MW application (Fig. 5).

Estimating Total Phenol Content (TPC)

In the present study, we observed that magnetic water treatment (MWT) had a significant effect on TP in the Moringa biotype (Table 4). The Moringa biotype seedlings exposed to MS and SS levels exhibited 14% and 35% increased TP under NWT (Table 4).

Estimating Total Flavonoid Content (TFC)

The total flavonoid contents were significantly increased under drought stress conditions. Under normal water treatment (NWT), the Moringa biotype seedlings exposed to MS and SS levels exhibited 8% and 24% increases in TF (Table 4).

Proline Assay

The proline content was significantly increased in Moringa biotype under drought stress (Table 4). Under drought stress (MS, SS), MWT resulted in decreased proline by 8%, 7%, and 20%, 13% respectively.

DPPH Assay

DPPH was increased in Moringa biotype seedlings by 12% and 24% exposed to MS, SS level under NWT (Table 5). The Moringa biotype seedlings exposed to drought stress (MS, SS) showed 3% and 4% declines in DPPH under MWT.

Lipid Peroxidation

The Moringa biotype seedlings exposed to drought stress (MS, SS) showed 15% and 27% increases in MDA content in the normal water treatment (NWT). A significant decrease in MDA content was observed in the Moringa biotype seedlings exposed to MW (Table 5).

H$_2$O$_2$ Content

A significant increase in H$_2$O$_2$ content was observed under drought stress and NWT. The Moringa biotype seedlings exposed to drought stress (MS, SS) showed 12% and 14% decreases in H$_2$O$_2$ under MWT (Table 5).

Discussion

Drought stress affects the physiological and phonological characteristics of plants [36-37]. In our study, plant height, internode distances, and leaflet number were declined under MS and SS levels (Fig. 1), and the notable recovery was found under MWT during drought stress due to the improvement of cell division and cell expansion. MWT might have enhanced the photosynthetic reaction rate, which in turn increased LA, FW, and DW (Table 1). These results corresponded with the findings of Souza et al. (2006) [38], who claimed that DW of seedlings was highly increased under magnetic water treatment in comparison to control. In corn plants, MW reduced the adverse effects on the growth [39] caused by drought. Moringa biotype seedlings that underwent drought stress showed low RWC (Fig. 2), which proved that drought stress creates an osmotic stress condition and causes imbalance of water [37]. Magnetic water helped to restore the water loss by enhancing the RWC in the seedlings affected by drought stress.

In our study, we have found that MW increases the assimilation (A), transpiration rate (E), stomatal conductance (gs), WUE, and VPD in Moringa biotype during MS and SS levels of drought. Similar results were explained by Anand et al. 2012 [40] in Zea mays (Fig. 3). The chlorophyll a and photochemical quenching and non-photochemical quenching were improved by the MWT, which helped improve the photosynthetic reaction rate in Moringa biotype.

Fluorescence parameter Fv/Fm was used to determine the stress in plants [41]. Fv/Fm was decreased in Moringa biotype, which is caused by harmful effects of drought (Fig. 4). The reduction of Fv/Fm and Fm could be due to structural obliteration of the PSII complex. Fv/Fm ratio, Fo and Fm were recovered by the MWT in Moringa biotype. Perhaps MWT helps to restore the photochemical efficiency of photosystem II in Moringa biotype. Chlorophyll fluorescence yield was found to be higher in soybean plants [42] under MWT, supported our result.

We observed that Chl a, Chl b Chl (a+b) and carotenoids content declined under drought stress, and chlorophyll content increased in the leaves of Moringa

<table>
<thead>
<tr>
<th>Treatments</th>
<th>MDA Content (nmol/g FW)</th>
<th>H$_2$O$_2$ Content (nmol/g FW)</th>
<th>DPPH (%Inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control+NW</td>
<td>21.48±0.19cd</td>
<td>5.81±0.01c</td>
<td>49.7±0.06ef</td>
</tr>
<tr>
<td>Control+MW</td>
<td>22.43±0.5c</td>
<td>5.75±0.01c</td>
<td>50.68±0.07e</td>
</tr>
<tr>
<td>MS+NW</td>
<td>25.3±0.19b</td>
<td>7.37±0.06b</td>
<td>56.5±0.05c</td>
</tr>
<tr>
<td>MS+MW</td>
<td>19.59±0.12c</td>
<td>6.49±0.09d</td>
<td>54.4±0.07d</td>
</tr>
<tr>
<td>SS+NW</td>
<td>29.87±0.4a</td>
<td>9.04±0.002a</td>
<td>65.5±0.3a</td>
</tr>
<tr>
<td>SS+MW</td>
<td>25.14±0.19b</td>
<td>7.7±0.05b</td>
<td>62.64±1.07b</td>
</tr>
</tbody>
</table>

Means within each column followed by the same letter are not significantly different at level $P\leq0.05$.
biotype seedlings during MWT (Table 2). Previous studies showed similar results, where MW increased the chlorophyll content in soybean and maize leaves [43-46].

In our studies, we noticed that during drought stress conditions caused by water shortage and the amount of Ca$^{2+}$, Mg$^{2+}$, Mn$^{2+}$, P$^{5+}$, and Zn$^{2+}$ was decreased (Table 3). Previous studies supported our result, where MW helps to increase the ion content [47]. The sodium ion concentration was found to be higher in the roots and shoots of the Moringa biotype (Fig. 5). Na$^+$/K ratio was increased due to the higher concentration of Na$^+$ in Moringa biotype seedlings (Fig. 5). This may be due to the displacement of calcium ion by sodium ion (Na$^+$), causing the lower content of Ca$^{2+}$. The similar result was found in rice seedlings under salt stress [48].

Phenolic and flavonoid content was affected under water deficit conditions [49-50]. Moringa biotype had higher phenolic compounds under drought stress environment (Table 4). The soluble carbohydrates accumulation in the plant cells were attributed to the phenolic compounds due to decreasing the transportation of soluble sugars [51-52]. The magnetic field rearranged the pyramid shape of water structure and it became the new hexagonal structure that can easily pass in cell membrane [24]. Possibly, magnetized water accelerates soluble sugar transportation in cell membranes by decreasing the accumulation of phenolic compounds under a drought environment. The proline content was increased in Moringa biotype under the different levels of drought stress. Increasing proline content in plants under drought is the sign of stress initiation [53] (Table 4). MW lowering the proline content in Moringa biotype may be due to the decrease of osmotic stress. The higher antioxidant capacity was found in Moringa biotype under drought stress, and it was minimized by the MWT (Table 5). It has been reported that magnetic field (MF) affects the antioxidant system in the plants [54] that supported our outcome. Malonaldehyde (MDA) and H$_2$O$_2$ content were significantly increased in the Moringa biotype under drought conditions (Table 5). MW helped lower the MDA and H$_2$O$_2$ contents under drought conditions as compared to NW-treated seedlings (Table 5).

Conclusions

In our study, drought stress markedly impaired growth, leaf gas exchange and chlorophyll content of Moringa biotype, and MW was effective in recovering the drought tolerance by maintaining the growth and physiology. Thus, we recommend that the application of MW under a water deficit environment that could be useful for mitigating drought stress.

Abbreviations

DPPH, 1-diphenyl-2-pircyldihyrazyl; DW, Dry weight; FC, Field capacity; FW, Fresh weight; H$_2$O$_2$, Hydrogen peroxide; LA, Leaf Area; MDA, Malondialdehyde; MS, Moderate drought stress; MW, Magnetized water; MWT, Magnetic water treatment; MF, Magnetic field; NWT, Normal water treatment; OD, Optical density; RWC, Relative water content; SS, Severe drought stress; TCA, Trichloroacetic acid; TPC, Total phenolic content; TFC, Total flavonoid content; TBA, Thiobarbituric acid; VPD, Vapor pressure deficit; WUE, Water use efficiency; F$_{m}$/F$_{0}$, Minimum chlorophyll fluorescence; Fm, Maximum chlorophyll fluorescence

Acknowledgements

The authors are thankful to the Dean of Scientific Research (DSR), King Abdulaziz University, Saudi Arabia for providing financial support.

Conflict of Interest

The authors declare no conflict of interest.

References


