Canadian Journal of Forest Research
Revue canadienne de recherche forestière

Effects of magnetized water treatment on growth characteristics and ion absorption, transportation and distribution in *Populus × euramericana* 'Neva' under NaCl stress

Journal:	<i>Canadian Journal of Forest Research</i>
Manuscript ID	cjfr-2016-0460.R2
Manuscript Type:	Article
Date Submitted by the Author:	19-Jan-2017
Complete List of Authors:	liu, xiumei; Shandong Agricultural University ma, fengyun; Shandong Agricultural University, zhu, hong; Shandong Agricultural University ma, xuesong; Shandong Agricultural University, college of forestry guo, jianhao; Shandong Agricultural University wan , xiao; Shandong Agricultural University wang, lu; Shandong Agricultural University wang , huatian; Shandong Agricultural University wang, yanping ; Shandong Agricultural University, College of Forestry; Shandong Agricultural University
Keyword:	ionic homeostasis, ion content, ion flux, magnetization, <i>Populus × euramericana</i> 'Neva'
Is the invited manuscript for consideration in a Special Issue? :	

SCHOLARONE™
Manuscripts

Effects of magnetized water treatment on growth characteristics and ion absorption, transportation and distribution in *Populus × euramericana* ‘Neva’ under NaCl stress

Xiumei Liu^{1,2}, Fengyun Ma^{1,2}, Hong Zhu^{1,2}, Xuesong Ma^{1,2}, Jianyao Guo^{1,2}, Xiao Wan^{1,2}, Lu Wang^{1,2}, Huatian Wang^{1,2*} and Yanping Wang^{1,2}

¹Key Laboratory of Silviculture of Shandong Province, 271018, Taian, Shandong, China

²Forestry College of Shandong Agricultural University, 271018, Taian, Shandong, China

Abstract

The effects of magnetized water irrigation on the growth and ionic movements of one-year-old potted seedlings of *Populus × euramericana* ‘Neva’. A magnetic treatment device was used to treat the plants. The content of K⁺, Na⁺, Ca²⁺ and Mg²⁺ in leaves and roots were analyzed by atomic absorption spectrophotometry (AAS), and fluxes of K⁺, Na⁺, Ca²⁺, Mg²⁺ and H⁺ in mesophyll cells and in meristematic zones were measured using a non-invasive micro-test technique (NIMT) after 30 days of treatment. After 90 days, the plants were harvested, and their growth indices and root morphology were measured. The results showed that (1) compared with non-magnetic treatments (NMT), the magnetic treatments (MT) led to higher K⁺ and Mg²⁺ content and lower Ca²⁺ content in roots and leaves, while the Na⁺ content was lower, and the K⁺/Na⁺ ratio was higher; (2) MT enhanced Na⁺ efflux, increased H⁺ influx, and decreased K⁺ and Mg²⁺ efflux compared with NMT; (3) MT resulted in greater height, diameter and leaf area of the plants and increased the length, surface area and number of root tips compared to NMT; (4) Stomatal conductance (Gs), net photosynthetic rate (Pn), intercellular CO₂ concentration (Ci) and water use efficiency (WUE) were increased in MT, whereas both transpiration rate (Tr) and stomatal limiting value (Ls) were decreased compared with NMT. The results indicate that the use of magnetized water can promote plant quality and regulate the ion absorption, transpiration and distribution. Thus, MT is conducive to the re-establishment of ionic homeostatic mechanisms via ion-selective absorption and transportation under salt stress.

Key words: ionic homeostasis, ion content, ion flux, magnetization, *Populus × euramericana* ‘Neva’

*Corresponding author. Tel.: +86-05388242986; Fax: +86-05388249164.

E-mail address: wanght@sdau.edu.cn

Introduction

Soil salinization resulting from water shortages and irrational irrigation has become an important limiting factor for agricultural production, ecological security and sustainable economic development (Jesus et al., 2015; Zhu et al., 2013). Some success has been achieved in salinization control through water conservation, hydraulic engineering and physical, chemical and biological methods, as well as through other comprehensive measures (Song and Wang, 2015), although issues related to the shortage of fresh water resources and the slow progress of desalination still exist. Therefore, methods for reasonably exploiting fresh groundwater and desalinating saline groundwater to satisfy the needs of water resources management are of great importance. Over the last 20 years, remarkable results have been obtained from the use of magnetization technology in brackish water irrigation, especially for high-salinity groundwater, for soil desalination in salinized areas (Mohamed and Ebead, 2013). Magnetized water irrigation not only increases the rate of salt dissolution, migration and leaching from the soil but also significantly improves the adaptability of plants to saline environments and promotes the growth and development of plants (Basant and Harsharn, 2009). Studies have shown that irrigation with magnetized water can significantly reduce the content of SO_4^{2-} and Cl^- in the soil in saline-alkali areas, achieving a net desalination rate of 20-30% (Bu et al., 2010). It also enhances the biomass productivity, yield and quality of crops. The mechanisms used by plants to adapt to saline environments under magnetized brackish water irrigation have been discussed in a limited number of previous studies. However, there is no clear understanding of the mechanisms behind these effects and the changes in ionic movement that are caused by magnetic treatment (MT).

The excessive accumulation of a single type of salt ion can influence nutrient transport and distribution and cause poisoning from salt hydronium and nutrient imbalances. Due to these effects, competitive absorption of Na^+ , K^+ , Ca^{2+} and Mg^{2+} occurs (Brini and Masmoudi, 2012). Ionic absorption and compartmentalization in different tissues of plants are important components of salt tolerance mechanisms. By absorbing inorganic ions from their surroundings, plants adjust their rates of osmosis, decrease cell osmotic potential and maintain water absorption (Marques et al., 2013). Ions such as K^+ , Na^+ , Ca^{2+} and Mg^{2+} are major inorganic ions that play roles in plant osmotic adjustment under conditions of NaCl stress. Excessive accumulation of Na^+ can lead to salt toxicity in plants and affect the equilibrium absorption of K^+ , Ca^{2+} and Mg^{2+} (Turner et al., 2013). Therefore, maintaining ion homeostasis in plants and plant cells under NaCl stress can alleviate ion toxicity (Teakle and Teyrman, 2010). As a second messenger, Ca^{2+} plays a vital role in biological processes in plants, particularly in signal transduction related to stress resistance under conditions of salt stress (Hirschi et al., 1996). Ca^{2+} specifically interacts with a channel protein located on the outer surface of the plasma membrane to regulate the K^+ channel switch, ultimately stimulating the accumulation of K^+ by inhibiting the accumulation of Na^+ (Nemchinov et al., 2008). Studies of salt stress showed that Na^+ and K^+/H^+ transporters controlled by the *NHX*

gene family can maintain osmotic balance of the cytoplasm by discharging and compartmentalizing Na^+ and K^+ ions efficiently to maintain lower levels of Na^+ and K^+ in the cells. The change in cytosol can regulate the cytoplasmic pH and the concentration of Na^+ and K^+ and maintain a high ratio of K^+/Na^+ in the cytoplasm (Li et al., 2010). Additionally, it has been found that through the expression of H^+ -ATPase in the plasma membrane and H^+ -ATPase and H^+ -PPase in the vacuolar membrane, plants generate sufficient H^+ proton motive force to transport substances across these membranes, which helps to maintain the stability of the cytoplasmic pH and facilitate the selective absorption of different ions (Mansour, 2013). Furthermore, ion transport occurs through the inverse cation concentration gradient catalyzed by ATP-dependent and proton electrochemical potential-dependent H-ATPases (Silva and Gerós, 2009), thereby allowing plants to maintain relatively stable intracellular K^+/Na^+ ion homeostasis (Mansour et al., 2015). Moreover, plants can regulate their ion uptake and transport under NaCl stress (Rauf et al., 2014).

When exposed to a magnetic field of sufficient strength, the hydrogen bonds (H bonds) in water in the liquid state are broken by Lorentz forces, and large aggregates of water molecules break up to form smaller aggregates or even single water molecules, leading to various changes in the characteristics of liquid water such as reduced surface tension, increased specific surface area, enhanced osmotic pressure and improved solvent activity (Hozayn et al., 2011). These changes improve ion hydration, which regulates the activity of ion channels and improves ion transport and water, mineral ion and nutrient absorption by the plant (Al-Khazan et al., 2011). All of these changes help alleviate the poisoning caused by high concentrations of NaCl and maintain the newly established ion homeostasis (Belyavskaya, 2001). The increased ion hydration accelerates soil desalinization and alters the adsorption status of anions and cations in the soil colloids (Liu et al., 2014a), which improves the condition of saline-alkali soils (Selim, 2008).

It is known that plants need mineral salts and microelements from the soil and the water that is usually used to irrigate plants is insufficient to supply a considerable amount of nutritional elements to plants. When plants begin to grow and require larger amounts of nutrients, however, further consumption of these nutrients from soil is very rare. The deficit of nutrients in the soil is the main reason for decreased growth rates of plants and low crop yields. For this reason, magnetic water should be used for irrigation. The studies of Oleshko et al. (1981) and Tkatchenko (1997) proposed the use of an inexpensive magnetic device to improve soil properties and water quality. Tackashinko (1997) showed that magnetized water removed 50% to 80% of the Cl in soil compared to a removal of 30% by irrigation with normal water. Similarly, Zhu et al. reported that desalination of a saline soil using magnetized water was 29% and 33% greater in the first and second leachings, respectively, than when untreated water was used. Phirke et al. (1996) found that 46% of *Glycine soja* L. Merr., 32% of *Gossypium hirsutum* L. and 35% of *Triticum aestivum* L. plants showed increased yield when exposed to an optimal magnetic field (MF). A significant increase in the fresh weight of

both shoots and roots was detected in sunflower, and the germination rate, root fresh and dry weight and total fresh and dry weight of the plants increased significantly compared to the controls when the plants were exposed to a vertical MF (Fischer et al., 2004). The growth and yield of butterhead lettuce (*Lactuca sativa* var. 'Salina') were evaluated when the variety was cultivated in hydroponic culture after the seeds were exposed to a static MF (Poinapen et al., 2005). In addition, exposure to an MF increased the yield and yield parameters of maize (*Zea mays* L.), lentil (*Cicer arietinum* L.), salvia (*Salvia officinalis* L.), rice (*Oryza sativa* L.), tomato (*Lycopersicon esculentum* L.), strawberry (*Fragaria × ananassa* Duchesne 'Camarosa') and radish (*Raphanus sativus* L.) (Râcuciu et al., 2008; Shabrangi and Majd, 2009; Martínez et al., 2008; Flórez et al., 2004; De Souza et al., 2006; Eşitken, 2003; Krawiec et al., 2013). Thus, it is important to continue determining the biological effects of exposure of plants to MF.

Magnetized water can be obtained by passing water through permanent magnets or electromagnets installed in a feed pipeline; the permanent magnets or the electromagnets are installed around the incoming water pipe. When electricity is passed into a wire connected to the magnets, a magnetized field is created around the pipe. To date, a variety of devices with different structures and shapes has been used to produce magnetized water, but the mechanisms of action of these devices are almost identical. When a fluid passes through an MF, its structure and some of its physical characteristics, such as density, salt solution capacity and the deposition ratio of solid particles, are changed (Higashitani et al. 1993). The changes caused by the MF depend on many factors, including the strength of the magnetic field, the direction in which it is applied, the duration of magnetic exposure, the flow rate of the solution, the additives present in the system and the pH (Chibowski et al. 2005).

The poplar is the most prominent fast-growing timber species; however, most species or varieties (clones) of poplar exhibit poor salt tolerance, a characteristic that restricts its cultivation in areas with soil salinization. Effective soil desalination methods coupled with improvement of the salt tolerance of poplar species are the main strategies that are used to expand the cultivation areas of poplar trees. In this study, *Populus × euramericana* 'Neva' was selected and continuously irrigated with magnetized water. The ion concentration in the plant leaves and roots, as well as the ion movement, flux and ion concentration on both sides of the cell membrane of the mesophyll cells and the root apical meristem region of the plant were measured to study the impact of magnetized water irrigation on *P. × euramericana* 'Neva'. The aim of the study was to determine the effect of magnetized water treatment on the compartmentalization of Na^+ and ion transport inside the plant cells as well as the mechanism of ion transport of salt-sensitive poplar plants under NaCl stress.

Materials and Methods

Plant materials and treatments

Hardwood cuttings (12 cm in length, 1.52 cm in diameter) of *P. × euramericana* ‘Neva’ (“Neva” hereafter) were obtained from the integrated experimental base of the Forestry College of Shandong Agricultural University (E117°08′, N36°11′). The cuttings were taken from the middle sections of the stems of one-year-old poplar plants. In late March 2014, the cuttings were planted in ceramic pots 25 cm in diameter and 20 cm in height. The culture matrix was vermiculite. There were four cuttings per pot and 15 pots for each plant type studied, and a single factor randomized block design was applied.

The plants used in the experiments were maintained in a glasshouse with natural light, day and night temperatures of 20-25°C, a relative humidity of 60%-70% and a 12-hour photoperiod with 800-1000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ of photosynthetically active radiation and irrigated with tap water. In mid-May, plants with similar growth (20 cm in height with six leaves) were selected and cultivated in $1/2$ -strength modified Hoagland solution (Hao et al., 2008) for four weeks. Finally, the plants were irrigated with a modified Hoagland solution containing NaCl.

At the beginning of June, the plants were divided into four groups. The groups were irrigated with Hoagland solution containing 0 g/L or 4 g/L NaCl in the presence or absence of magnetization. The groups were as follows: plants that were irrigated with $1/2$ -strength modified Hoagland solution containing 4 g/L NaCl (M4); plants that were irrigated with $1/2$ -strength modified Hoagland containing 4 g/L NaCl (T4); plants that were irrigated with magnetized $1/2$ -strength modified Hoagland solution (M0); and plants that were irrigated with $1/2$ -strength modified Hoagland solution (T0). The pots were irrigated with magnetized brackish water that flowed through a magnetized device (U050 mg, 0.5 inch, output 4-6 m^3/hr) produced by Magnetic Technologies L.C.C. (Russia, United Arab Emirates branch). The pots were irrigated every five days.

Ionic content measurement

Thirty days after treatment, the plant content of K^+ , Na^+ , Ca^{2+} , and Mg^{2+} was measured using oven-dried, pulverized samples (samples from multiple plants were mixed together for analysis) as follows (modified from Shi et al. (2010)): leaves from the middle section of the plants and the fine roots of the plants were baked at 105°C for 30 minutes and then placed in an oven at 80°C until constant weight was achieved. The dried samples were then pulverized and sieved through a 60-mesh sieve. Samples (0.1 g) were weighed, placed in digestion vessels containing 5 ml of concentrated H_2SO_4 (Aladdin, CAS: 7664-93-9) and digested in a temperature-controlled digestion system (KDX-60). Concentrated H_2O_2 (Aladdin, CAS: 7722-84-1) was added to increase the rate of digestion to a transparent state. Finally, the solution was diluted to a standard volume with deionized water. The contents of K^+ , Na^+ , Ca^{2+} and Mg^{2+} in the leaves and roots were determined using atomic absorption spectrophotometry (AAS, TAS-990MFG) at the determination wavelengths of 766.5, 589.0, 422.7 and 285.2 nm, respectively.

Ionic content was calculated according to the formula $K/Na/Ca/Mg (\%) = (C \times D \times V) / (W \times 10000)$, where C is the ionic concentration from the standard curve ($\mu\text{g/ml}$), D is the dilution ratio, V is the volume of testing liquid and W is the dry weight of the tissue (leaves and roots).

Ionic flux measurement

The net fluxes of K^+ , Na^+ , Ca^{2+} , Mg^{2+} and H^+ were measured at the YoungerUSA (Xuyue Beijing) NIMT service center using Non-invasive Micro-test Technology (NMT100 Series, YoungerUSA LLC, Amherst, MA01002, USA) and the iFluxes/imFluxes 1.0 (YoungerUSA, LLC, Amherst, MA 01002, USA) software. Pre-pulled, silanized glass micropipettes ($\Phi 5 \pm 1 \mu\text{m}$, XY-DJ-01, YoungerUSA) were first filled with a backfilling solution (K^+ : 100 mM KCl; Na^+ : 250 mM NaCl; Ca^{2+} : 100 mM $CaCl_2$; Mg^{2+} : 500 mM $MgCl_2$; H^+ : 15 mM NaCl + 40 mM KH_2PO_4 , pH 7.0) for the mesophyll cells and root meristematic zone (the tested area was approximately 480 μm from the root tip). The micropipettes were front-filled with 15-180 μm (Na^+ , Ca^{2+} , Mg^{2+} , and H^+ : 15-50 μm ; K^+ : 180 μm) columns of selective liquid ion-exchange cocktails (K^+ LIX: XY-SJ-K, Na^+ LIX: XY-SJ-Na, Ca^{2+} LIX: XY-SJ-Ca, Mg^{2+} LIX: XY-SJ-Mg, H^+ LIX: XY-SJ-H, YoungerUSA). An Ag/AgCl wire electrode holder (XY-DJGD, YoungerUSA) was inserted into the back of the electrode to make electrical contact with the electrolyte solution. A YG003-Y05 electrode (YoungerUSA) was used as the reference electrode. Ion-selective electrodes of the target ions were calibrated prior to flux measurements. Only electrodes with a Nernstian slope $> 53 \text{ mV/decade}$ (Na^+ , K^+ , H^+) or $> 22 \text{ mV/decade}$ (Ca^{2+} , Mg^{2+}) were used in this study. The ionic fluxes were calculated based on Fick's law of diffusion: $J = -D_0 \cdot (dc/dx)$, where J is the ion flux (unit: $\text{picomole cm}^{-2} \text{ s}^{-1}$), dc is the ionic concentration gradient, dx is the distance over which the microelectrode moved repeatedly from one point to another perpendicular to the surface of the sample at a frequency of ca. 0.3 Hz (this distance was usually between 5 and 35 μm) and D_0 is its diffusion constant. The direction of the flux was derived from Fick's law of diffusion.

The barehanded sections of leaves and root tips were washed three times with redistilled water. The leaves and roots were immobilized at the bottom of a chamber containing 5-10 ml of measuring buffer (0.1 mM KCl, 0.1 mM $MgCl_2$, 0.1 mM NaCl, 0.1 mM $CaCl_2$, 0.3 mM MES, pH 5.7) for 20 minutes; they were then tested by moving the ion-selective microelectrode between two positions close to the plant mesophyll cells and the meristematic zone over a distance of 20 μm . Continuous recording was performed for 10 minutes at each measurement point. The steady-state cation flux rates were expressed as the mean of the measured points of eight repeats; the error bars indicate the SD.

Determination of the growth characteristics of the seedlings

In the middle of September prior to harvest, the individual heights, and ground diameters of the plants were measured using Vernier calipers and tape, and the data were recorded. After harvest, the root length, the root surface area and the number of root tips were determined using a WinRHIZO PRO2007 root analysis system. A portable leaf area meter (CI-202) was used to analyze the leaf area.

Data processing and analysis

The data are reported as the mean $\pm$ standard error (SE) of three replicates. An analysis of variance was performed using a single-factor analysis of variance (SAS, v. 9.0). Treatment means were analyzed with Duncan's multiple range test at $p < 0.05$. Ionic fluxes were calculated using JCal V3.3 (a free MS Excel spreadsheet, youngerusa.com or xuyue.net).

Results

Effects of MT on the K^+ and Na^+ content of leaves and roots

Under NaCl stress, the antagonism between Na^+ and K^+ (competitive absorption) inhibits K^+ absorption in Neva and disrupts the metabolic balance within the cells. The high-affinity K^+ uptake system and K^+ ion channels promote the absorption of K^+ , permitting the cells to maintain a high ratio of K^+/Na^+ . As shown in Table 1, under 4 g/L NaCl stress, the Na^+ content of the leaves and roots increased by 211.7%-215.1% and by 6.6%-13.0% in the leaves and roots, respectively, of M4 and T4 plants compared to the controls (M0, T0); the Na^+ content of the leaves of the salt-treated plants was significantly higher than that of the controls ($p < 0.05$). Compared to their respective controls, the K^+ content of the leaves and roots of the plants in the NaCl-treated groups (M4, T4) decreased by 8.2%-12.2% and by 9.2%-14.2%, respectively; the decrease in the K^+ content of the leaves was statistically significant ($p < 0.05$). Compared with the plants in the non-magnetic treatment (NMT) groups (T0, T4), the Na^+ content of the leaves and roots of the plants in the MT groups decreased by 10.7%-11.7% and by 2.2%-7.8%, respectively, and the K^+ content increased by 2.3%-6.9% and 2.4%-8.5%, respectively. The overall Na^+ content of the roots of the plants in all treatment groups (T4, M4, T0, M0) was slightly but significantly higher than that of the leaves ($p < 0.05$), whereas the K^+ content of the roots was lower than that of the leaves. In the M4 and T4 plants, the K^+/Na^+ ratio in the leaves and roots decreased by 3.6%-20.0% compared to the controls (M0, T0), and there were significant differences in the K^+/Na^+ ratio in the leaves ($p < 0.05$). The magnetic treatments produced higher K^+/Na^+ ratios than the TWs, and these ratios were significantly higher in the leaves than in the roots ($p < 0.05$). The decrease in the amount of Na^+ in the leaves likely reduced toxicity to the leaves and caused the roots to be the main Na^+ accumulation region. This process produced a marked inhibition of the K^+ intake, resulting in a dynamic trend of high K^+ and low Na^+ in the M4 and M0

groups, which resulted in a higher K^+ content and a lower Na^+ content than in the T4 and T0 groups. In general, the aboveground parts of the plants, such as the leaves, maintained higher levels of K^+/Na^+ than the roots, and the K^+/Na^+ ratios were higher in the plants that received MT than in the plants that did not receive such treatment.

Effects of MT on the Ca^{2+} and Mg^{2+} ion content of leaves and roots

Ca^{2+} is involved in complex interactions and biological activities. Exogenous Ca^{2+} has been shown to block the accumulation of Na^+ and reduce the toxic effect of Na^+ on plants (Han et al., 2005), although the plant must maintain low cytosolic Ca^{2+} concentrations for normal growth. The Ca^{2+} content of both the leaves and roots of plants under salt stress (M4, T4) showed an increasing trend, with increases of 323.5%-337.1% and 1.6%-2.2%, respectively, compared to the controls (M0, T0). Additionally, the Ca^{2+} content of the leaves was greater than that of the roots (Table 2, $p < 0.05$); the Ca^{2+} content of plants in the M4 and M0 groups was maintained at a relatively low level, which might stimulate K^+ accumulation and increase the K^+/Na^+ ratio. Compared with the plants that received NMT (T4, Table 2), the Mg^{2+} content of plants that received MT (M4) increased by 0.8%-12.5%, especially in the roots, with statistically significant increases of 4.3%-12.5% ($p < 0.05$). Overall, the Ca^{2+} content increased due to salt stress, and long exposure to high concentrations of Ca^{2+} resulted in toxicity to cells. At the same time, the lower Mg^{2+} content was beneficial to plant root activity.

Effects of MT on cell fluxes in the root meristem region and mesophyll

Effects on Na^+ flux in cells of the root meristem region and mesophyll

NaCl stress resulted in strong Na^+ efflux during the recording, as shown in Fig. 1, compared with the controls (M0, T0), which showed a Na^+ efflux of 160-440 $\text{pmol cm}^{-2} \text{s}^{-1}$ in the mesophyll cells and white fine roots. The NaCl-treated plants (M4, T4) displayed strong Na^+ efflux in mesophyll cells (2600-2700 $\text{pmol cm}^{-2} \text{s}^{-1}$); this efflux was slightly stronger than that observed in the roots (2500-2600 $\text{pmol cm}^{-2} \text{s}^{-1}$) (Fig. 1A, C), indicating that salt stress induced higher levels of Na^+ efflux in the mesophyll cells than in the root meristem cells (Fig. 1D). The differences in Na^+ efflux between the plants that received treatment and the control plants were significant ($p < 0.05$). The Na^+ efflux of 360-2700 $\text{pmol cm}^{-2} \text{s}^{-1}$ observed in the plants of the M4 and M0 groups was greater than the Na^+ efflux of 160-2600 $\text{pmol cm}^{-2} \text{s}^{-1}$ observed in the plants of the T4 and T0 groups; therefore, the magnetization induced a higher and more stable Na^+ efflux than the Na^+ effluxes previously observed in the salt-tolerant species *P. euphratica* (200 $\text{pmol cm}^{-2} \text{s}^{-1}$) and the salt-sensitive species *P. popularis* (50 $\text{pmol cm}^{-2} \text{s}^{-1}$) (Sunet al., 2009a). Salt stress induced high levels of Na^+ efflux in the apical meristem region of Neva roots, and the Na^+ efflux in mesophyll cells (Fig. 1B) was significantly higher than that in the root meristem region (Fig. 1D).

311 **Fig. 1.** Changes in net Na^+ flux in mesophyll cells (A) and in the apical meristem region (B)
312 in plants that received MT and NMT under NaCl stress. The mean $\pm SE$ ($n = 3$) of the values
313 obtained for mesophyll cells (C) and for the apical meristem region (D) are shown. Values
314 followed by different lowercase letters represent statistically significant differences at the
315 0.05 probability level.

316

317 ***Effects on K^+ flux in cells of the root meristem region and mesophyll***

318 NaCl-induced stable K^+ efflux in mesophyll cells and fluctuating K^+ efflux in the root
319 tip meristem region. Both regions showed a K^+ efflux (Fig. 2A, C) of 1500-2500 $\text{pmol cm}^{-2} \text{s}^{-1}$
320 in plants under NaCl stress (M4, T4) and of 100-220 $\text{pmol cm}^{-2} \text{s}^{-1}$ in the two controls (M0, T0,
321 Fig. 2B, D), indicating that the K^+ efflux in plants under NaCl stress was significantly higher
322 than in the controls ($p < 0.05$). In the plants that received MT (M0, M4), the K^+ efflux was
323 relatively lower and more stable, showing values of 100-1600 $\text{pmol cm}^{-2} \text{s}^{-1}$. At the same time,
324 the K^+ efflux of plants that received NMT (130-2500 $\text{pmol cm}^{-2} \text{s}^{-1}$) was slightly higher and
325 more volatile than that of the plants that received MT.

326 **Fig. 2.** Changes in net K^+ flux in mesophyll cells (A) and in the apical meristem region (B)
327 after MT and NMT of plants under NaCl stress. The mean $\pm SE$ ($n = 3$) of the values obtained
328 for mesophyll cells (C) and the apical meristem region (D) are shown. Values followed by
329 different lowercase letters represent statistically significant differences at the 0.05 probability
330 level.

331

332 ***Effects on Ca^{2+} flux in cells of the root meristem region and mesophyll***

333 Ca^{2+} showed an efflux of 21 $\text{pmol cm}^{-2} \text{s}^{-1}$ and 50 $\text{pmol cm}^{-2} \text{s}^{-1}$ in mesophyll cells
334 with (M4) and without (T4) magnetized water irrigation, respectively, under salt stress; the
335 Ca^{2+} efflux in the MT plants (M4) was significantly lower than that in the T4 plants ($p <$
336 0.05). As the time point at which the measurement was taken was increased, the Ca^{2+} flux
337 gradually changed from an efflux to an influx (Fig. 3A). However, in the controls (M0, T0),
338 Ca^{2+} showed an influx, and MT of the plants in the M0 group helped maintain high Ca^{2+}
339 influx in those plants (-45 $\text{pmol cm}^{-2} \text{s}^{-1}$), significantly higher than that of the plants that
340 received NMT (T0, -170 $\text{pmol cm}^{-2} \text{s}^{-1}$; Fig. 3A, B).

341 The rate of flux of Ca^{2+} in the apical meristem region of the roots showed an opposite
342 trend to that of the mesophyll cells under salt stress, with an influx of -150 $\text{pmol cm}^{-2} \text{s}^{-1}$ in
343 M4 and an influx of -240 $\text{pmol cm}^{-2} \text{s}^{-1}$ in T4. The measured Ca^{2+} influx showed significant
344 differences ($p < 0.05$) between plants that received MT and the plants that received NMT.
345 Whereas the M0 control showed a Ca^{2+} efflux of 18 $\text{pmol cm}^{-2} \text{s}^{-1}$ and the T0 control showed
346 a Ca^{2+} efflux of 63 $\text{pmol cm}^{-2} \text{s}^{-1}$, the Ca^{2+} efflux was significantly higher in M0 than in the
347 magnetically treated T0 plants ($p < 0.05$) (Fig. 3B, D).

348 **Fig. 3.** Changes in net Ca^{2+} flux in mesophyll cells (A) and in the apical meristem region (B)
349 in plants subjected to MT and NMT under NaCl stress. The mean $\pm SE$ ($n = 3$) of the values

obtained for mesophyll cells (C) and the apical meristem region (D) is shown. Values followed by different lowercase letters represent statistically significant differences at the 0.05 probability level.

Effects on Mg^{2+} and H^+ fluxes in cells of the root meristem region and mesophyll

TW plants (T4) showed higher levels of Mg^{2+} efflux (approximately 2600 pmol cm⁻² s⁻¹) in the mesophyll cells than the plants that received MT (M4, approximately 2100 pmol cm⁻² s⁻¹). The Mg^{2+} ion flux in plants that were not subjected to NaCl stress (M0, T0) showed the opposite trend; the M0 plants, which were irrigated with magnetized 1/2-strength modified Hoagland solution (M0) showed a Mg^{2+} influx of -990 pmol cm⁻² s⁻¹, slightly higher than the influx of -900 pmol cm⁻² s⁻¹ in the T0 plants irrigated with 1/2-strength modified Hoagland solution, and the differences were all statistically significant (Fig. 4B) ($p < 0.05$). A lower efflux and a higher influx of Mg^{2+} were induced by the MT; as a result, a relatively high Mg^{2+} concentration was maintained in the intracellular parts of the plants.

The H^+ influx in the apical meristem region of the root increased significantly in the plants that were subjected to NaCl stress (Fig. 4C), and the H^+ influx of plants irrigated with magnetized 1/2-strength modified Hoagland solution plus 4 g/L NaCl (M4, -10 pmol cm⁻² s⁻¹) was significantly higher than that of the T4 plants (-6 pmol cm⁻² s⁻¹, $p < 0.05$). However, both groups of plants subjected to MT maintained higher levels of H^+ influx than plants that received NMT ($p < 0.05$); the ion flow movement observed in the control plants was similar to that of plants under salt stress.

Fig. 4. Changes in net Mg^{2+} flux in mesophyll cells (A) and net H^+ flux in the apical meristem region (B) of plants that received MT and NMT under conditions of NaCl stress. The mean $\pm$ SE ($n = 3$) of the values obtained for Mg^{2+} flux in mesophyll cells (C) and net H^+ flux in the apical meristem region (D) is shown. Values followed by different lowercase letters represent statistically significant differences at the 0.05 probability level.

Effects of MT on plant growth and root morphology

After 90 days of treatment of the plants in the experimental groups, four main morphological indices were measured and analyzed (Table 3). Compared with the T0 and M0 groups, the plant height, basal diameter, leaf area and root cap ratio of the plants of the T4 and M4 groups were decreased by 10.7%-55.8%. The differences in plant height and basal diameter were statistically significant ($p < 0.05$), indicating the inhibitory effect of salt stress on the growth of the plants. As indicated by height, diameter and leaf area, the plants in the M0 and M4 groups showed better growth than the plants in the T0 and T4 groups; MT resulted in improvements of 11.1%-59.7% in these parameters, and the values were significantly different from those for plants that received NMT ($p < 0.05$).

Root morphology is one of the main indices used to evaluate the absorptive function of roots, and it can affect the ability of plants to absorb nutrients and moisture from the soil.

Root length, root surface area and number of root tips displayed a significant decrease in the T4 and M4 groups ($p < 0.05$) compared to the T0 and M0 groups; the decreases ranged from 19.7% to 51.5% (Table 3). Root length, surface area, and number of tips were significantly increased by 11.9%-41.7% in M0 and M4 compared to T0 and T4 ($p < 0.05$).

Effects of MT on gas exchange parameters in leaves

Photosynthesis is not only an important physiological metabolic activity of plants but also a vital criterion that can be used to evaluate the strength, growth and hardness of plants. The results presented in Table 4 show that salt stress led to significantly lower values of net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO₂ concentration (Ci), transpiration rate (Tr) and water use efficiency (WUE) ($p < 0.05$); these values decreased by 1.5%-51.1% compared to those of plants in the T0 and M0 groups. The limiting value of stomata (Ls) showed the opposite trend, differing significantly in the plants that received saline treatment compared to the controls. Compared with T0 and M0, Pn, Gs, Tr, Ci and WUE all decreased by 5.6%-24.8% in T4 and M4, whereas Ls increased by 12.2%-26.7%. Variance analysis showed that all of the gas exchange parameters except Tr showed significant differences ($p < 0.05$) in saline-treated plants compared to the controls. Compared with T0 and T4, Gs, Pn and WUE were increased by 5.3%-29.3% in M0 and M4, whereas Tr and Ls were reduced by 0.7%-45.4%.

Discussion

Differences in the morphological characteristics of Neva plants irrigated with magnetized brackish water

Growth inhibition is the most sensitive physiological response of plants to salt stress (Munns, 2002). Neva plants subjected to long-term salt stress showed decreased height, ground diameter, leaf area and root cap ratio compared to the controls (Table 3). The edges of the leaves withered, and the leaf color became lighter under salt stress, similar to the responses of *P. popularis* under salt stress (Sunet al., 2009b). The roots showed a similar trend in that the salt stress inhibited the development of the root morphology. The effects of salt stress were less severe in Neva plants irrigated with magnetized water than in plants irrigated with non-magnetized water, and the growth of the aboveground portions of the plants was more robust in the plants that received magnetized water. Snow pea (*Pisum sativum* L. var. macrocarpon) and Kabuli chickpea (*Cicer arietinum* L.) seeds irrigated with magnetized water grew taller and heavier than those irrigated with tap water (Hozayn and Abdul-Qados, 2010). Our results are consistent with those results. The effect of MT on plant height, ground diameter, leaf area and root cap ratio was also investigated. Although the chlorophyll content was not determined, the photosynthetic rate ($9.18\text{-}12.2 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) was found to increase in the plants that received MT through the determination of

photosynthetic characteristics ($8.7\text{--}10.1 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). Atak et al. (2003) found an increase in chlorophyll content after exposure of plants to an MF and demonstrated increases in all photosynthetic pigments through the improvement of cytokine synthesis, which was induced by exposure of the plant to an MF. The amount of photosynthetic pigment present in leaves is closely related to the net photosynthetic rate and to leaf color. The exact relationship between the net photosynthetic rate and the amount of photosynthetic pigment remains to be explored.

Magnetization could influence the cellular metabolism and mitotic activity of meristematic cells in plants. In this study, when the roots of Neva plants were irrigated with magnetized brackish water, a positive effect on the regeneration of plant tissues was observed. The effects of magnetization were investigated; the total root length, root surface area and the number of root tips were found to increase, along with more vigorous growth of newly developed fine roots. Similar results indicating that magnetization induced root generation and formation were reported by Yaycili and Alikamanoglu (2005). The increased root length and surface area helped the plants extract moisture and nutrients from the soil, which could promote plant growth.

Externally applied magnetization has previously been shown to have a positive effect on plant growth, accelerating plant development and increasing plant height and leaf area (Yaycili and Alikamanoglu, 2005). These advantageous effects were also observed in our study. The noticeable enhancement of seedling growth by MT might be ascribed to the long-lasting effects of MFs on nutrient mobility in Neva, which might lead to increased production of hormones. Gibberellic acid equivalents (GAs), indole-3-acetic acid and trans-zeatin hormones were found to increase in sunflower plants exposed to MFs (Yaycili and Alikamanoglu, 2005). The breakage of H bonds in water, which was enhanced for a period of time after magnetization (Toledo et al., 2008), increased the surface tension and solute activity at the water surface, decreased the intracellular pH and increased conductivity in the cells (Chang and Weng, 2008). These changes reduced the rate of generation of reactive oxygen species and led to increases in the diffusion and absorption of nutrients and in water use efficiency. They also maintained the normal life activities of plants irrigated with magnetized brackish water.

Magnetization regulated the ionic absorption and transport of K^+ , Na^+ , Ca^{2+} and Mg^{2+} in Neva under NaCl stress

Higher plants can maintain the stability of the cytoplasmic microenvironment by adjusting the amount and type of inorganic ions present in the cytoplasm, maintaining a high K^+/Na^+ ratio and using cellular ion homeostasis to adapt to the saline environment. Increased Na^+ uptake could inhibit the absorption and transport of K^+ , Ca^{2+} and Mg^{2+} by plants. In this study, NaCl treatment resulted in higher intracellular concentrations of Na^+ and Ca^{2+} , lower intracellular concentrations of K^+ and Mg^{2+} , and a lower K^+/Na^+ ratio in both the leaves and

roots of the plants. The increase in intracellular Na^+ and Ca^{2+} that occurs under long-term NaCl stress, especially excessive Na^+ accumulation, affects the selective absorption of ions and causes ionic imbalances and hyperosmotic stress in plants (Waditee et al., 2002). Neva that received magnetized brackish water irrigation maintained lower intracellular concentrations of Na^+ and Ca^{2+} , higher intracellular concentrations of K^+ and Mg^{2+} , and a higher K^+/Na^+ ratio, and these differences were more marked in the aboveground parts of the plant than in the roots, indicating that the transport of K^+ and Mg^{2+} from the roots to the aboveground tissues was promoted by magnetization. Excess Na^+ was transported to the roots, restricting the upward transport of Na^+ and weakening the negative effect on the leaves caused by Na^+ accumulation. Similar results were obtained by Liu et al. (2014b). The increase in intracellular Ca^{2+} in the roots induced by magnetization caused a reduction in the transport coefficient of Ca^{2+} from the roots to the leaves (1.10-1.42) compared to that in the plants that received NMT (1.12-1.49). This result differs from the findings of Yang et al. (2013). High concentrations of Ca^{2+} might directly or indirectly activate target enzymes such as Ca^{2+} -CaM dependent protein kinases, which could stimulate the activity of the Ca^{2+} transporters located in the cytoplasmic and vacuolar membranes and thereby regulate the intracellular ratio of Ca^{2+} to other ions (Bush, 1995). A rapid increase in Ca^{2+} levels in the cytosol can cause the induction of systems that maintain Ca^{2+} at baseline concentrations through the Ca^{2+} transport system in response to environmental changes (Hetherington and Brownlee, 2004). In tobacco and tomato, transcription of the Ca^{2+} -ATPase gene increased after NaCl treatment (Sze et al., 2000). Intracellular Ca^{2+} showed a transient increase after MT, although plants were able to maintain a lower Ca^{2+} concentration through the generation of Ca^{2+} transporters in the ground state due to the activation of SOS signaling pathways (Zhu, 2001).

In our study, the plants that received MT maintained a higher level of Na^+ efflux and a lower level of K^+ efflux on the scale of the entire plant because the magnetization resulted in increased capacity of the plants to reduce the loss of K^+ . This result is consistent with observations in *P. euphratica* (Sun et al., 2009b), barley (Chen et al., 2007) and wheat (Cuinet et al., 2008). This result might have been caused by potassium depolarization, which has been shown to activate K^+ channels (KORCs) and decrease the effect of substituting Na^+ for K^+ under magnetization (Zhu, 2001). This process led to enhanced K^+ absorption by the plant, reduced the effects of depolarization of the cytoplasmic membrane and led to a decrease in the K^+ efflux and an increase in the Na^+ efflux. This shows that magnetization confers on the poplar plant the ability to maintain K^+/Na^+ homeostasis in its leaves and roots by greater Na^+ extrusion and reduced K^+ efflux under conditions of NaCl stress (Lu et al., 2012).

The Ca^{2+} flux analysis indicated that a reduced level of Ca^{2+} efflux occurred in Neva mesophyll cells under salt stress compared with the controls; Ca^{2+} also showed a low level of influx in the apical meristem region of the roots. In addition, a low level of Ca^{2+} influx was maintained in the mesophyll cells, indicating that NaCl stress caused a transient increase in the cytosolic free Ca^{2+} in the roots. In yeast, Ca^{2+} has been shown to activate signals of salt

stress and to regulate ionic homeostasis and salt tolerance (Mendoza et al., 1994). In Neva, Ca^{2+} influx across the plasma membrane occurred during long-term hypertonic stress; moreover, an intracellular Ca^{2+} surge could activate protein phosphatase 2B (PP2B), the key intermediate component of salt stress signals controlling ionic homeostasis. In addition, an intracellular Ca^{2+} surge might induce the transcription of the P-H⁺-ATPase gene *ENAI* (Zhu, 2001), thereby strengthening the cross-membrane excretion of Na^+ in plants under magnetized water irrigation. Studies of the signal transduction pathways related to the control of ionic homeostasis and salt tolerance in *Arabidopsis* demonstrated that when activated by Ca^{2+} , the SOS signaling pathway regulates the homeostasis of Na^+ and K^+ (Zhu, 2001). Salt stress was shown to increase the expression of *SOS1* to stimulate the excretion of Na^+ under the regulation of *SOS3/SOS2* (Guo et al., 2001) and re-establish a new ionic homeostasis.

Chickpeas subjected to magnetized water irrigation showed increased absorption of K^+ , Ca^{2+} , Mg^{2+} and the trace elements Fe^{2+} and Mn^{2+} , thus improving the yield and quality of the crops. In this study, we obtained results similar to those of Harsharn and Basant (2011); in Neva, after 30 days of NaCl stress, the Mg^{2+} content of the roots and leaves was higher in the plants that received MT than in the plants that did not, especially in the roots (Table 2). In addition to an increase in Mg^{2+} , Ca^{2+} showed a downward trend in the plants that received MT. This might be because both Mg^{2+} and Ca^{2+} are bivalent cations and therefore compete with each other during ion absorption. The promotion of Mg^{2+} absorption, which was induced by the MT through reduction of Ca^{2+} uptake, helped alleviate the toxic effects of hydronium ions produced by the long-term presence of high Ca^{2+} concentrations. In a study of photosynthesis in *Ziziphus jujuba* in which the plants were irrigated with magnetized drip water, the photosynthetic rate of the jujube plants subjected to MT increased (Wang et al., 2010). Therefore, we inferred that the increased content of Mg^{2+} may be conducive to maintaining a high net photosynthetic rate in Neva plants irrigated with magnetized brackish water.

An analysis of the Mg^{2+} efflux in mesophyll cells under salt stress was performed. The plants that received MT showed lower Mg^{2+} efflux than those that did not, indicating that MT promoted the absorption and utilization of Mg^{2+} by the plants. Ca^{2+} tended to be transported from outside the cytoplasm into the chloroplasts, which stabilized the ultrastructure of the mesophyll cells and chloroplasts (Zai et al., 2005) and maintained the absorption capacity of the plants under salt stress. In the Neva plants that were irrigated with magnetized water, the synthesis of Mg^{2+} -containing chlorophyll was enhanced due to the increased levels of Ca^{2+} , which enhanced photosynthesis and root activity and maintained the integrity and stability of the membrane, thereby reducing the permeability of the cytoplasmic membrane and facilitating the plant's selective absorption of ions.

Magnetization was favorable for maintaining Na^+/H^+ antiporter activity, enhancing the adaptability of Neva to NaCl stress

Neva is susceptible to salt toxicity during the process of poplar cultivation. In our study, the mesophyll cells and apical meristem regions of the roots of plants that received MT exhibited more pronounced Na^+ efflux under conditions of long-term NaCl stress, consistent with the findings of Sun et al. (2009a) and Shabala (2000). Both treated and untreated plants also manifested a net H^+ influx corresponding to the Na^+ efflux under long-term salinity. NaCl-induced H^+ influx has previously been observed in the roots of *Arabidopsis* and *Populus* (Shabala, 2000). Based on these results, we concluded that the Na^+ efflux in roots mainly resulted from the activity of a Na^+/H^+ antiporter in the plasma membrane. A higher rate of Na^+/H^+ exchange was induced by magnetization, implying that the Na^+/H^+ antiporter system located in the plasma membrane of the plants that received MT was more effective at Na^+ extrusion than that of the plants that received NMT, especially during long-term saline treatment. Consistent with this inference, the Na^+ efflux in the roots of magnetically treated plants decreased correspondingly when the H^+ influx was limited (Lu et al., 2012). The correlation between Na^+ efflux and H^+ influx indicated that Na^+ extrusion in plants undergoing MT was highly dependent on the activity of H^+ -ATPase in the plasma membrane, which pumps protons to promote secondary active Na^+/H^+ antiport at the plasma membrane (Blumwald et al., 2000).

In this study, we found that the total Na^+ content of the leaves and roots of plants in the M4 group was lower than that of plants in the T4 group, and we concluded that the hydration capacity of the NaCl solution was enhanced when exposed to an MF, which could improve the migration rate and leaching of excessive amounts of Na^+ (Khoshravesh et al., 2011). The total Na^+ content of the roots of the treated plants was higher than that of the leaves, and the roots of the plants that received MT showed a relatively low Na^+ efflux compared to the leaves, whereas the total Na^+ content showed the opposite trend in the plants that received NMT; however, these plants still maintained good Na^+ efflux. From the increase in the Na^+ content and the decrease in the Na^+ efflux in roots, we deduced that a significant amount of Na^+ was compartmentalized into the vacuoles in the roots such that a limited amount of Na^+ entered the cytosol, thus restricting the transport from underground tissues to the aboveground parts of the plant. It is likely that MT enhanced Na^+ compartmentalization through the activity of a Na^+/H^+ antiporter in the vacuolar membrane (Pardo and Quintero, 2002). The function of the Na^+/H^+ antiporter was shown to be dependent on the electrochemical proton gradient across the membrane for H^+ transport by H^+ -ATPase or H^+ -PPase across the vacuole membrane (Shi et al., 2002). Through this process, excess Na^+ was transported out of the cytoplasm, and low levels of cytosolic Na^+ were maintained, thereby enhancing the ability of Neva to adapt to saline environments and improving the salt tolerance of the plant.

Conclusion

The above results showing that irrigation with magnetized water induced alterations of tissue and cellular ion fluxes under NaCl stress suggest that Na^+ is the dominant ion for salt tolerance in Neva. The observed differences between plants that were irrigated with magnetized water and those that were not were mainly attributed to the Na^+ capacity in the roots, which restricted Na^+ transport to the aboveground tissues, especially to the leaves. That is, excess Na^+ was taken up by the roots and was slowly transported to the aboveground plant tissues during long-term saline stress. Plants that did not receive magnetic treatment showed an opposite result; in those plants, more Na^+ was transported from the roots to the leaves. Moreover, this may also imply that the increased salt tolerance conferred by magnetic treatment was mainly related to the compartmentation of Na^+ at the cellular and/or tissue levels and the migration rate of Na^+ in the culture matrix. The results presented here describe a potentially productive mechanism for salt tolerance in Neva and should be highly emphasized and further investigated in future work.

Supporting Information

Fig. S1 Effects of irrigation with magnetized brackish water on the ion-selective transport of K^+ , Mg^{2+} and Ca^{2+} in Neva leaves and roots. The letters a, b and c denote the ion-selective transport capacities $S_{\text{K,Na}}$ (a), $S_{\text{Mg,Na}}$ (b) and $S_{\text{Ca,Na}}$ (c), respectively.

Author Contributions

Xiu-Mei Liu and Hua-Tian Wang conceived and designed the study. Jian-yao Guo, Xue-song Ma, Xiao Wan, Lu Wang and Hong Zhu performed the experiments. Xiu-Mei Liu and Yan-Ping Wang analyzed the data, and Xiu-Mei Liu, Hua-Tian Wang and Feng-Yun Ma wrote the manuscript.

Acknowledgements

The authors are grateful for the funding provided by the National Program of the International Introduction of Advanced Science and Technology in Forestry of China (948 Program, Grant No. 2011-4-60), the National Basic Research Program of China (973 Program, Grant No. 2012CB416904/zgc), the National Natural Science Funds of China (Grant No. 31270670, 31070550) and the Agricultural Major Application Technology Innovation Program of Shandong Province (Financial and Agricultural Indicators, 2016, No. 36). We would like to thank Yuan Xiaohong, Yang Meng, Zhang Menglu and Liu Yunqi of Xuyue (Beijing) Science and Technology Co., Ltd. for their technical support for the Non-invasive Micro-test Technique (NIMT).

References

Al-Khazan, M., Abdullatif, B.M., Al-Assaf, N. 2011. Effects of magnetically treated water on water status, chlorophyll pigments and some elements content of jojoba (*Simmondsia*

- 621 *chinensis* L.) at different growth stages. African Journal Environmental Science and
 622 Technology. 5: 722–731.
- 623 Atak, C., Emiroğlu, Ö., Aklımanoğlu, S., Rzakoulieva, A. 2003. Stimulation of regeneration by
 624 magnetized field in soybean (*Glycine max* L. Merrill) tissue cultures. J Cell Mol Biol.
 625 2: 113–119.
- 626 Basant, L.M., Harsharn, S.G. 2009. Magnetized treatment of irrigation water: its effects on
 627 vegetable crop yield and water productivity. Agricultural Water Management. 96(8):
 628 1229–1236. doi:10.1016/j.agwat.2009.03.016.
- 629 Belyavskaya, N.A. 2001. Ultrastructure and calcium balance in meristem cells of pea roots
 630 exposed to extremely low magnetic fields. Adv Space Res. 28: 645–650.
 631 doi:10.1016/S0273-1177(01)00373-8.
- 632 Blumwald, E., Aharon, G.S., Apse, M.P. 2000. Sodium transport in plant cells. Biochim
 633 Biophys Acta, 1465: 140–151. doi:10.1016/S0005-2736(00)00135-8.
- 634 Brini, F., Masmoudi, K. 2012. Ion transporters and abiotic stress tolerance in plants. ISRN
 635 Mol Biol. 2012: 1–13. doi:10.5402/2012/927436.
- 636 Bu, D.S., Feng, W.G., Cai, L.H., Zhou, L. 2010. Effects of magnetization water on
 637 desalinization in cotton farmland of under-film dripping irrigation in Xinjiang
 638 Province. Transactions of the CSAE. 26 (supp.2): 163–166.
 639 doi:10.3969/j.issn.1002-6819.2010.z2.031.
- 640 Bush, D.S. 1995. Calcium regulation in plant cells and its role in signaling. Annu Rev Plant
 641 Physiol Plant Mol Biol. 46: 95–122. doi:10.1146/annurev.pp.46.060195.000523.
- 642 Chang, K., Weng, C. 2008. An investigation into the structure of aqueous NaCl electrolyte
 643 solutions under magnetized fields. Comput Mater Sci. 43: 1048–1055.
 644 doi:10.1016/j.commatsci.2008.02.020.
- 645 Chen, Z., Pottosin, I.I., Cuin, T.A., Fuglsang, A.T., Tester, M., Jha, D., Zepeda-Jazo, I., Zhou,
 646 M., Palmgren, M.G., Newman, I.A., Shabala, S. 2007. Root plasma membrane
 647 transporters controlling K^+/Na^+ homeostasis in salt-stressed barley. Plant Physiol. 145:
 648 1714–1725. doi:10.1104/pp.107.110262.
- 649 Chibowski, E., Szcześ, A., Hołysz, L. 2005. Influence of sodium dodecyl sulfate and static
 650 magnetic field on the properties of freshly precipitated calcium carbonate. ACS
 651 Publications. 21 (18): 8114–8122. doi:http://dx.doi.org/10.1021/la050575f.
- 652 Cuin, T.A., Betts, S.A., Chalmandrier, R., Shabala, S. 2008. A root's ability to retain K^+
 653 correlates with salt tolerance in wheat. J Exp Bot. 59(10): 2697–2706.
 654 doi:10.1093/jxb/ern128.
- 655 De Souza, A., Garcia, D., Sueiro, L., Gilart, F., Porras, E., Licea, L. 2006. Presowing magnetic
 656 treatments of tomato seeds increase the growth and yield of plants.
 657 Bioelectromagnetics. 27: 247–257. doi:10.1002/bem.20206.
- 658 Eşitken, A. 2003. Effect of magnetic fields on yield and growth in strawberry 'Camarosa'. J
 659 Hortic Sci Biotechnol. 78(2): 145–147.

- 660 Fischer, G., Tausz, M., Köck, M., Grill, D. 2004. Effects of weak 16 $\frac{2}{3}$ Hz magnetic fields on
661 growth parameters of young sunflower and wheat seedlings. *Bioelectromagnetics*. 25:
662 638–641. doi:10.1002/bem.20058.
- 663 Flórez, M., Carbonell, M.V., Martínez, E. 2004. Early sprouting and first stages of growth of
664 rice seeds exposed to a magnetic field. *Electro Magnetobiol Med*. 23: 167–176.
665 doi:10.1159/000049353.
- 666 Guo, Y., Halfter, U., Ishitani, M., Zhu, J.K. 2001. Molecular characterization of function
667 aldodomains in the protein kinase *SOS2* that is required for plant salt tolerance. *Plant Cell*.
668 13(6): 1383–1400. doi:10.1105/TPC.010021.
- 669 Han, R., Bañuelos, M., Senn, M.E., Barreo-Gil, J., Rodríguez-Navarro, A. 2005. *HKT1*
670 mediates sodium uniport in roots. Pitfalls in the expression of *HKT1* in yeast. *Plant*
671 *Physiol*. 19(3): 1495–1506. doi:10.1104/pp.105.067553.
- 672 Huang, H., Chen, L., Liu, X.Q., Liu, C., Cao, W.Q., Lu, Y., Hong, F.H. 2008. Absorption and
673 transfer of light and photo reduction activities of spinach chloroplasts under calcium
674 deficiency: promotion by cerium. *Biol Trace Elem Res*. 122(2): 157–167.
675 doi:10.1007/s12011-007-8068-5.
- 676 Harsharn, S.G., Basant, L.M. 2011. Magnetic treatment of irrigation water and snow pea and
677 chickpea seeds enhances early growth and nutrient contents of seedlings.
678 *Bioelectromagnetics*. 32(1): 58–65. doi:10.1002/bem.20615.
- 679 Hetherington, A.M., Brownlee, C. 2004. The generation of Ca²⁺ signals in plants. *Annu. Rev.*
680 *Plant Biol*. 55: 401–427. doi:10.1146/annurev.arplant.55.031903.141624.
- 681 Hirschi, K.D., Zhen, R.G., Cunningham, K.W., Rea, P.A., Fink, G.R. 1996. *CAX1*: an H⁺/Ca²⁺
682 antiporter from Arabidopsis. *Proc Natl Acad Sci*. 93(16): 8782–8786.
683 doi:10.1073/pnas.93.16.8782.
- 684 Higashitani, K., Kage, A., Katamura, S., Imai, K., Hatade, S. 1993. Effects of a magnetic field
685 on the formation of CaCO₃ particles. *Colloid and Interface Science*. 156(1): 90–
686 95. doi:10.1006/jcis.
- 687 Hozayn, M., Abdel-Monem, A.A., Abdul-Qados, A.M.S., Abd El-Hameed, H.M. 2011.
688 Response of some food crops for irrigation with magnetized water under greenhouse
689 condition. *Aust J Basic Appl Science*. 5: 29–36.
- 690 Hozayn, M., Abdul-Qados, A.M.S. 2010. Irrigation with magnetized water enhances growth,
691 chemical constituent and yield of chickpea (*Cicer arietinum* L.). *Agric Biol J North*
692 *Am*. 1: 671–676.
- 693 Jesus, J., Castro, F., Niemelä, A., Borges, M., Danko, A.S. 2015. Evaluation of the impact of
694 different soil salinization processes on organic and Mineral soils. *Water Air Soil Pollut*.
695 226: 3–12. doi:10.1007/s11270-015-2373-y.
- 696 Khoshravesh, M., Mostafazadeh-Fard, B., Mousavi, S.F., Kiani, A.R. 2011. Effects of
697 magnetized water on the distribution pattern of soil water with respect to time in trickle
698 irrigation. *Soil Use Manag*. 27(4): 515–522. doi:10.1111/j.1475-2743.2011.00358.x

- 699 Krawiec, M., Komarzynski, K., Palonka, S., Kaplan, M., Baryla, P., Kiczorowski, P. 2013.
 700 Does the magnetic field improve the quality of radish seeds? *Acta Sci Pol Hortorum*
 701 *Cultus*. 12(6): 93–102.
- 702 Li, Y.H., Zhang, Y.Z., Feng, F.J., Dong, L., Cheng, L.L., Ma, F.W., Shi, S.G. 2010.
 703 Overexpression of a *Malus* vacuolar Na^+/H^+ antiporter gene (*MdNHX1*) in apple
 704 rootstock M.26 and its influence on salt tolerance. *Plant Cell Tissue Organ Cult.*
 705 102(3): 237–345. doi:10.1007/s11240-010-9738-0.
- 706 Liu, W., Yuan, X., Zhang, Y., Xuan, Y., Yan, Y. 2014a. Effects of salt stress and exogenous
 707 Ca^{2+} on Na^+ compartmentalization, ion pump activities of tonoplast and plasma
 708 membrane in *Nitraria tangutorum* Bobr. Leaves. *Acta Physiol Plant.* 36(8): 2183–
 709 2193. doi:10.1007/s11738-014-1595-8.
- 710 Liu, Z.X., Zhang, H.X., Yang, X.Y., Liu, T., Di, W.B. 2014b. Growth, and cationic absorption,
 711 transportation and allocation of *Elaeagnus angustifolia* seedlings under NaCl stress.
 712 *Acta Ecol Sin.* 34(2): 326–336. doi:10.11707/j.1001-7488.20140106.
- 713 Lu, Y.J., Li, N.Y., Sun, J., Hou, P.C., Jing, X.S., Zhu, H.P., Deng, S.D., Han, Y.S., Huang,
 714 X.X., Ma, X.J., Zhao, N., Zhang, Y.H., Shen, X., Chen, S.L. 2013. Exogenous
 715 hydrogen peroxide, nitric oxide and calcium mediate root ion influx in two
 716 non-secreter mangrove species subjected to NaCl stress. *Tree Physiol.* 33(1): 81–95.
 717 doi:10.1093/treephys/tps119.
- 718 Mansour, M.M.F. 2013. Plasma membrane permeability as an indicator of salt tolerance in
 719 plants. *Biol Plant.* 57(1): 1–10. doi:10.1007/s10535-012-0144-9.
- 720 Mansour, M.M.F., Salama, K.H.A., Allam, H.Y.H. 2015. Role of the plasma membrane in
 721 saline conditions: lipids and proteins. *Bot Rev.* 81: 416–451. doi:
 722 10.1007/s12229-015-9156-4.
- 723 Marques, E.C., Freitas, P.A.F., Alencar, N.L.M., Prisco, J.T., Gomes-Filho, E. 2013. Increased
 724 Na^+ and Cl^- accumulation induced by NaCl salinity inhibits cotyledonary reserve
 725 mobilization and alters the source-sink relationship in establishing dwarf cashew
 726 seedlings. *Acta Physiol Plant.* 35(7): 2171–2182. doi:10.1007/s11738-013-1254-5.
- 727 Martínez, E., Carbonell, M.V., Flórez, M., Amaya, J.M. 2008. Effect of static magnetic field
 728 exposure of salvia seeds on germination characteristics (*Salvia officinalis* L.). In:
 729 Technical and Technological Progress in Agriculture, 13th International Conference,
 730 Institute of Agricultural Engineering LUA, Raudondvaris, 25–26, September 2008, pp.
 731 226–232.
- 732 Mendoza, I., Rubio, F., Rodriguez-Navarro, A., Pardo, J.M. 1994. The protein phosphatase
 733 calcineurin is essential for NaCl tolerance of *Saccharomyces cerevisiae*. *J Biol Chem.*
 734 269: 8792–8796.
- 735 Mohamed, A.I., Ebead, B.M. 2013. Effect of magnetic treated irrigation water on salt removal
 736 from sandy soil and on the availability of certain nutrients. *International Journal*
 737 *Engineering and Applied Sciences*. 2: 36–44.

- Munns, R. 2002. Comparative physiology of salt and water stress. *Plant Cell Environ.* 25(2): 239–250. doi:10.1046/j.0016-8025.2001.00808.x.
- Nemchinov, L.G., Shabala, L., Shabala, S. 2008. Calcium efflux as a component of the hypersensitive response of *nicotiana benthamiana* to *Pseudomonas syringae*. *Plant Cell Physiol.* 49(1): 40–46. doi:10.1093/pcp/pcm163.
- Oleshko, K.P., Vadyunina, A.F., Zhilyayeva, V.A., Trukhin, V.I. 1981. Effect of a magnetic field on the properties of soil and plants. *Pochvovedeniye*, 7: 91-98
- Poinapen, D., Beeharry, G.K., Bahorun, T., Bunwaree, M., Préfumo, S. 2005. Effect of static magnetic fields on the growth and yield of butterhead lettuce seeds (*Lactuca sativa* var. Salina). MAS 2005 Food and Agricultural Research Council, Proceedings 7th Meeting of Agricultural Scientists. Réduit, Mauritius. Lalouette, J.A., Bheenick, K.J., Nundalallee, C (Eds), pp. 207–216.
- Pardo, J.M., Quintero, F.J. 2002. Plants and sodium ions: keeping company with the enemy. *Genome Biol.* 3(6): 1017–1021. doi:10.1186/gb-2002-3-6-reviews1017.
- Phirke, P.S., Patil, M.N., Umbarkar, S.P., Dudhe, Y.H. 1996. The application of magnetic treatment to seeds: methods and responses. *Seed Sci Technol.* 24: 365–373.
- Răcuciu, M., Creangă, D., Horga, I. 2008. Plant growth under static magnetic field influence. *Rom J Phys.* 53: 353–359. doi: <https://www.researchgate.net/publication/228824131>.
- Rauf, M., Shahzad, K., Ali, R., Ahmad, M., Habib, I., Mansoor, S., Berkowitz, G.A., Saeed, N.A. 2014. Cloning and characterization of Na⁺/H⁺ antiporter (*LfNHX1*) gene from a halophyte grass *Leptochloa fusca* for drought and salt tolerance. *Mol Biol Rep.* 41(3): 1669–1682. doi:10.1007/s11033-013-3015-3.
- Selim, M.M. 2008. Application of magnetized technologies in correcting underground brackish water for irrigation in the arid and semi-arid ecosystem. The 3rd International Conference on Water Resources and Arid Environments and the 1st Arab Water Forum.
- Shabala, S. 2000. Ionic and osmotic components of salt stress specifically modulate net ion fluxes from bean leaf mesophyll. *Plant Cell Environ.* 23(8): 825–837. doi:10.1046/j.1365-3040.2000.00606.x.
- Shabrangi, A., Majd, A. 2009. Effect of magnetic fields on growth and antioxidant systems in agricultural plants. PIERS Proceedings, Beijing, China, March 23–27, pp. 1142–1147.
- Shi, H., Quintero, F.J., Pardo, J.M., Zhu, J.K. 2002. The putative plasma membrane Na⁺/H⁺ antiporter *SOS1* controls long-distance Na⁺ transport in plants. *Plant Cell.* 14(2): 465–477. doi:10.1105/tpc.010371.
- Shi, R., Zhang, Y., Chen, X., Sun, Q., Zhang, F., Römhelt, V., Zou, C. 2010. Influence of long-term nitrogen fertilization on micronutrient density in grain of winter wheat (*Triticum aestivum* L.). *J Cereal Sci.* 51(1): 165–170. doi:10.1016/j.jcs.2009.11.008.

- 775 Silva, P., Gerós, H. 2009. Regulation by salt of vacuolar H^+ -ATPase and H^+ -pyrophosphatase
776 activities and Na^+/H^+ exchange. *Plant Signal Behav.* 4(8): 718–726.
777 doi:10.4161/psb.4.8.9236.
- 778 Song, J., Wang, B. 2015. Using euhalophytes to understand salt tolerance and to develop
779 saline agriculture: *Suaeda salsa* as a promising model. *Ann Bot.* 115(3): 541–553.
780 doi:10.1093/aob/mcu194.
- 781 Sun, J., Chen, S., Dai, S., Wang, R., Li, N., Shen, X., Zhou, X., Lu, C., Zheng, X., Hu, Z.,
782 Zhang, Z., Song, J., Xu, Y. 2009a. NaCl-induced alternations of cellular and tissue ion
783 fluxes in roots of salt-resistant and salt-sensitive poplar species. *Plant Physiol.* 149(2):
784 1141–1153. doi:10.1104/pp.108.129494.
- 785 Sun, J., Dai, S., Wang, R., Chen, S., Li, N., Zhou, X., Lu, C., Shen, X., Zheng, X., Hu, Z.,
786 Zhang, Z., Song, J., Xu, Y. 2009b. Calcium mediates root K^+/Na^+ homeostasis in
787 poplar species differing in salt tolerance. *Tree Physiol.* 29(9): 1175–1186.
788 doi:10.1093/treephys/tpp048.
- 789 Sze, H., Liang, F., Hwang, I., Curran, A.C., Harper, J.F. 2000. Diversity and regulation of plant
790 Ca^{2+} pumps: insights from expression in yeast. *Annu Rev Plant Physiol Plant Mol Biol.*
791 51(1): 433–462. doi:10.1146/annurev.arplant.51.1.433.
- 792 Takatshinko, Y. 1997. Hydromagnetic systems and their role in creating micro-climate.
793 International symposium on Sustainable Management of Salt Affected Soils, Cario,
794 Egypt, September, pp. 22-28.
- 795 Teakle, N.L., Teyrman, S.D. 2010. Mechanisms of Cl^- transport contributing to salt tolerance.
796 *Plant Cell Environment.* 33(4): 566–589. doi:10.1111/j.1365-3040.2009.02060.x.
- 797 Toledo, E.J.L., Ramalho, T.C., Magriotis, Z.M. 2008. Influence of magnetic field on
798 physical-chemical properties of the liquid water: Insights from experimental and
799 theoretical models. *J Mol Struct.* 873(1-3): 409-415. doi:10.1016/j.
800 molstruc.2008.01.010.
- 801 Turner, N.C., Colmer, T.D., Quealy, J., Pushpavalli, R., Krishnamurthy, L., Kaur, J., Singh, G.,
802 Siddique, K.H.M., Vadez, V. 2013. Salinity tolerance and ion accumulation in
803 chickpea (*Cicer arietinum* L.) subjected to salt stress. *Plant Soil.* 365(1): 347–361.
804 doi:10.1007/s11104-012-1387-0.
- 805 Waditee, R., Hibino, T., Nakamura, T., Incharoensakdi, A., Takabe, T. 2002. Overexpression
806 of Na^+/H^+ antiporter confers salt tolerance on a freshwater cyanobacterium, making it
807 capable of growth in sea water. *Proceedings of the National Academy of Science of the*
808 *United States of America.* 99(6): 4109–4114. doi:10.1073/pnas.052576899.
- 809 Wang, W.M., Jiang, Y.J., Zheng, D.M., Wang, J.Q., Liu, W.Y., Liu, G.D. 2010. Influence of
810 magnetization water irrigation on photosynthesis and transpiration of jujube tree.
811 *Xinjiang. Agric Sci.* 47(12): 2421–2425.
- 812 Yang, X.Y., Zhang, H.X., Zhang, L., Liu, Z.X., Yang, S., Wu, X. 2013. Effects of NaCl stress
813 on growth and absorption, transportation and distribution of ions in *Nitraria*

- 814 *tangutorum*. Scientia Silvae Sinicae. 49(9): 165-171.
815 doi:10.11707/j.1001-7488.20130924.
- 816 Yaycili, O., Alikamanoglu, S. 2005. The effect of magnetic field on Paulownia tissue cultures.
817 Plant Cell Tissue Organ Cul. 83: 109–114. doi:10.1007/s11240-005-4852-0.
- 818 Zai, X.M., Qin, P., Wu, G.R., Yan, D.L. 2005. Effects of Ca^{2+} on the activities of Ca^{2+} -ATPase,
819 Mg^{2+} -ATPase in chloroplast and Ca^{2+} distribution in peanut seedlings. Journal Oil Crop
820 Sciences. 27(4): 41–44. doi:10.3321/j.issn:1007-9084.2005.04.010.
- 821 Zhu, J.K. 2001. Plant salt tolerance. Trends Plant Sci. 6: 66–71.
822 doi:10.1016/S1360-1385(00)01838-0.
- 823 Zhu, Y., Li, Y.P., Huang, G.H., Guo, L. 2013. Risk assessment of agricultural irrigation water
824 under interval functions. Stoch Environ Res Risk Assess. 27(3): 693–704.
825 doi:10.1007/s00477-012-0632-7.

Table 1 Contents of Na⁺ and K⁺ in the leaves and roots of Neva by magnetic and non-magnetic treatments under NaCl stress (*p* < 0.05).

Treatment	Leaf			Root		
	K ⁺ %	Na ⁺ %	K ⁺ /Na ⁺	K ⁺ %	Na ⁺ %	K ⁺ /Na ⁺
T4	0.0419±0.0007b	0.0187±0.0003a	2.244±0.0360b	0.0247±0.0006a	0.0178±0.0002b	1.396±0.1571b
M4	0.0448±0.0002ab	0.0167±0.0001a	2.324±0.0604b	0.0268±0.0007a	0.0174±0.0002ab	1.539±0.1156ab
T0	0.0477±0.0003a	0.0060±0.0003b	8.280±0.7062a	0.0288±0.0009a	0.0167±0.0002a	1.743±0.1232ab
M0	0.0488±0.0004a	0.0053±0.0004b	9.374±0.3708a	0.0295±0.0006a	0.0154±0.0002b	1.924±0.0949a

Note: Data in the table are the Means ± SE of three replicates. Values followed by different lowercase letters are extremely remarkable differences at 0.05 probability level, the same below.

Table 2 Contents of Ca²⁺ and Mg²⁺ in the leaves and roots of Neva by magnetic and non-magnetic treatments under NaCl stress (*p* < 0.05).

Treatment	Leaf		Root	
	Ca ²⁺ %	Mg ²⁺ %	Ca ²⁺ %	Mg ²⁺ %
T4	0.2825±0.0048a	0.0126±0.00006a	0.2835±0.0001a	0.0120±0.0003c
M4	0.2793±0.0076a	0.0127±0.00002a	0.2795±0.0005ab	0.0135±0.0003b
T0	0.0667±0.0018b	0.0132±0.00005a	0.2775±0.0003ab	0.0138±0.0002b
M0	0.0639±0.0012b	0.0133±0.00004a	0.2751±0.0005b	0.0144±0.0001a

Table 3 Changes of plant growth of Neva by magnetic and non-magnetic treatments under NaCl stress (*p* < 0.05).

Treatmen t	Height /cm	Ground diameter /mm	Leaf area / cm ²	Root cap ratio	Total root length	Root surface- area	Root tips
T4	62.50±2.47d	10.50±0.22c	62.70±0.77 c	0.175±0.0002 b	783.7±11.34d	68.8±0.52d	2270.75±23.54 d
M4	99.80±4.20c	11.80±0.52c	69.63±0.28 b	0.182±0.0003 b	1041.8±14.41 c	97.5±0.98c	2541.00±14.30 c
T0	141.50±8.40 b	17.90±0.55b	70.24±1.52 b	0.251±0.0009 a	1242.1±34.65 b	107.2±1.93 b	2829.00±12.68 b
M0	195.50±3.28 a	21.50±0.49a	78.61±0.50 a	0.262±0.0003 a	1743.9±60.76 a	147.3±0.50 a	3579.50±53.01 a

Table 4 Changes of leaf gas exchange parameters of Neva by magnetic and non-magnetic treatments under NaCl stress (*p* < 0.05).

Treatment	Net photosynthetic rate/(μmol CO ₂ m ⁻² s ⁻¹)	Stomatal conductance/(mmol m ⁻² s ⁻¹)	Transpiratio n rate/(mmol m ⁻² s ⁻¹)	Intercellular carbon dioxide concentration /(mmol mol ⁻¹)	Stomatal limitation value	Water use efficiency /(μmol CO ₂ mmol ⁻¹ H ₂ O)
T0	10.09±0.04b	294.4±1.54b	3.44±0.02a	314.6±0.55c	0.172±0.001 b	3.10±0.02b
T4	8.72±0.15d	143.9±1.91d	2.81±0.04b	297.0±1.26d	0.218±0.003	2.72±0.03c

					a	
					0.106±0.002	
M0	12.20±0.15a	334.8±1.30a	3.40±0.10a	373.8±0.60a	d	3.58±0.15a
					0.119±0.005	
M4	9.18±0.123c	186.0±1.86c	2.79±0.04b	334.8±1.94b	c	2.93±0.03bc

Draft

Supplementary material: Effects of magnetized water treatment on growth characteristics and ion absorption, transportation, and distribution in *Populus × euramericana* ‘Neva’ under NaCl stress

Xiumei Liu, Fengyun Ma, Hong Zhu, Xuesong Ma, Jianyao Guo, Xiao Wan, Lu Wang, Huatian Wang, and Yanping Wang

Fig. S1 The ion selective transportation capacity of K^+ , Mg^{2+} and Ca^{2+} in leaves and roots of Neva by magnetic and non-magnetic treatments. Different letters (a, b, c) denote ion selective transportation capacity $S_{K,Na}$ (a), $S_{Mg,Na}$ (b) and $S_{Ca,Na}$ (c), respectively.