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# Natural and Synthetic Hydrophilic Polymers Enhance Salt and Drought Tolerance of *Metasequoia* glyptostroboides Hu and W.C.Cheng Seedlings

Jing Li <sup>1,2</sup>, Xujun Ma <sup>1</sup>, Gang Sa <sup>1</sup>, Dazhai Zhou <sup>3</sup>, Xiaojiang Zheng <sup>3</sup>, Xiaoyang Zhou <sup>1</sup>, Cunfu Lu <sup>1</sup>, Shanzhi Lin <sup>1</sup>, Rui Zhao <sup>1</sup> and Shaoliang Chen <sup>1,\*</sup>

- Beijing Advanced Innovation Center for Tree Breeding by Molecular Design, College of Biological Sciences and Technology, Beijing Forestry University, Qinghua East Road 35, Beijing 100083, China; kaka19832008@163.com (J.L.); maxujun@lzb.ac.cn (X.M.); sg\_1214@126.com (G.S.); zhouxiaoyang@bjfu.edu.cn (X.Z.); lucunfu@bjfu.edu.cn (C.L.); szlin@bjfu.edu.cn (S.L.); ruizhao926@126.com (R.Z.)
- School of Architectural and Artistic Design, Henan Polytechnic University, Jiaozuo 454000, China
- Key Laboratory of Biological Resources Protection and Utilization in Hubei Province, Hubei University for Nationalities, Enshi 445000, China; sws0048@163.com (D.Z.); hbzxj123@126.com (X.Z.)
- \* Correspondence: Lschen@bjfu.edu.cn; Tel.: +86-(0)10-6233-8129

Received: 6 September 2018; Accepted: 11 October 2018; Published: 15 October 2018



**Abstract:** We compared the effects of hydrophilic polymer amendments on drought and salt tolerance of *Metasequoia glyptostroboides* Hu and W.C.Cheng seedlings using commercially available Stockosorb and Luquasorb synthetic hydrogels and a biopolymer, Konjac glucomannan (KGM). Drought, salinity, or the combined stress of both drought and salinity caused growth retardation and leaf injury in *M. glyptostroboides*. Under a range of simulated stress conditions, biopolymers and synthetic hydrogels alleviated growth inhibition and leaf injury, improved photosynthesis, and enhanced whole-plant and unit transpiration. For plants subjected to drought conditions, Stockosorb hydrogel amendment specifically caused a remarkable increase in water supply to roots due to the water retention capacity of the granular polymer. Under saline stress, hydrophilic polymers restricted Na<sup>+</sup> and Cl<sup>-</sup> concentrations in roots and leaves. Moreover, root K<sup>+</sup> uptake resulted from K<sup>+</sup> enrichment in Stockosorb and Luquasorb granules. Synthetic polymers and biopolymers increased the ability of *M. glyptostroboides* to tolerate combined impacts of drought and salt stress due to their water- and salt-bearing capacities. Similar to the synthetic polymers, the biopolymer also enhanced *M. glyptostroboides* drought and salt stress tolerance.

**Keywords:** hydrophilic polymers; Stockosorb; Luquasorb; Konjac glucomannan; photosynthesis; ion relation

## 1. Introduction

Soil salinity and drought pose major problems in agriculture and forestry [1–4]. Soil salinization often accompanies drought due to evaporative salt accumulation in upper soil layers. Together, these cause soil degradation and erosion [3]. Molecular physiology indicates that multiple stress signaling networks are involved in the plant response to dehydration and saline conditions. These networks specifically include the abscisic acid-activated signaling pathway, mitogen-activated protein kinase (MAPK) cascades, extracellular adenosine triphosphate (ATP) signaling, and hydrogen peroxide catabolic process [3,5–8]. Gene transformation, mycorrhization, and polymer amendments to soil can enhance drought and salt tolerance at the tissue and cellular level [1–3,9]. These interventions can

increase osmotic adjustment, antioxidative defense, water use efficiency, and ionic homeostasis in herbaceous and woody plants [2,3,6,9,10].

Hydrophilic polymers are commonly used as soil conditioners. They aid plant growth and development in drying soils [11,12] by increasing the plant's ability to absorb and retain large volumes of water and maintain osmotic balance. Amendments of hydrophilic polymers have been shown to increase available moisture levels around the root zone [13–16], thereby improving plant survival under drought stress [14,17–19]. Additional evidence shows that the presence of polymers increased the water holding capacity [14,20,21], decreased water percolation rates [22], and reduced the need for frequent irrigation [23].

In addition to enhancing plant survival in arid soils, hydrophilic polymers also aid plant growth in saline soils [24–29]. The water- and salt-retentive capacities of Stockosorb and Luquasorb polymers were shown to limit the accumulation of toxic ions in the plants [26–28]. Stockosorb hydrogel amendments to saline soil (potassium mine refuse) improved  $Ca^{2+}$  uptake in salt-resistant *Populus euphratica* Oliv. due to the polymer's cation exchange character [26]. We have previously shown that the exchangeable  $K^+$  contained in Stockosorb and Luquasorb enables *Populus simonii* × (*P. pyramidalis* + *Salix matsudana*) (*Populus popularis* cv. 35-44) (a salt-sensitive poplar species) to maintain  $K^+/Na^+$  homeostasis under saline conditions [27,28]. Hydrophilic polymers may also improve soil pore water quality available to plant roots, which enabled poplars under investigation to tolerate a combined stress of drought and salinity [28].

Most synthetic organic polymers (also known as plastic) may degrade under natural conditions, but typically persist in the environment for extended periods and are thus the subject of general environmental concern (Environmental issues and concern of synthetic polymers; https://prezi.com/5hthiclu4uwh/environmental-issues-and-concern-of-synthetic-polymers/). To consider the effectiveness of plastic alternatives, this study also investigated a biopolymer's effects on plant growth under adverse conditions. Konjac glucomannan (KGM) is a natural macromolecule made of  $\beta$ -1,4 linked D-mannose and D-glucose residues derived from *Amorphophallus konjac* K. Koch ex N.E. Br. The strong hydrogen bonds formed by hydroxyl groups in solution give this polysaccharide a high water absorbency [30,31]. We investigated KGM along with the synthetic Stockosorb 500 XL and Luquasorb hydrogels as a soil conditioner in soils subjected to drought and saline conditions. Amended soils were planted with seedlings of *Metasequoia glyptostroboides*, a valuable tree species widely used for coastal shelter, farmland protection, city greening, and as an ornamental. We compared the biopolymer and synthetic hydrogels in terms of their effects on plant growth, photosynthesis, water status, and ion relations under drought or saline conditions, or both drought and saline conditions.

## 2. Materials and Methods

# 2.1. Hydrophilic Polymers

This study used hydrophilic polymers Stockosorb 500 XL (granular type, cross-linked poly potassium-co-(acrylic resin polymer)-co-polyacrylamide hydrogel, Stockhausen GmbH, Krefeld, Germany), Luquasorb<sup>®</sup> product (powder type, potassium polyacrylate, BASF Corporation, Ludwigshafen, Germany), and Konjac flour-derived glucomannan (KGM purity >70%, Key Laboratory of Biological Resources Protection and Utilization in Hubei Province, Enshi, China).

# 2.2. Plant Material and Treatments

One-year-old seedlings of M. glyptostroboides (Hu and W.C.Cheng) were obtained from Xingdoushan Nature Reserve, Hubei province, China. In March, the seedlings were planted within individual 2.5 L pots containing sandy soil (sand:soil = 1:1, v/v). The potted plants were kept well-watered and received 500 mL of Hoagland's nutrient solution every two weeks. All plants were placed in a greenhouse at Beijing Forestry University prior to the initiation of salt and drought treatments. In early June, plants that exhibited healthy, uniform appearance were transferred to 5 L

pots filled with either control soil (no salt) or saline soil (soil was pretreated with 1 L 50 mM NaCl). These were left as controls or amended with one of the three polymer types (0.5% by dry weight). Plants were then divided into four groups, with each subjected to the following treatments:

- (1) Control (Non-polymer), Control + Stockosorb, Control + Luquasorb, Control + KGM;
- (2) NaCl (Non-polymer), NaCl + Stockosorb, NaCl + Luquasorb, NaCl + KGM;
- (3) Drought (Non-polymer), Drought + Stockosorb, Drought + Luquasorb, Drought + KGM;
- (4) Drought + NaCl (Non-polymer), Drought + NaCl + Stockosorb, Drought + NaCl + Luquasorb, Drought + NaCl + KGM.

Drought treatment consisted of withholding water after the initiation of water stress. Control plants were kept well-watered during the period of the experiment.

# 2.3. Shoot Height Measurement

The shoot height of three to five plant replicants (per treatment) was measured every seven days for a total 46-day exposure to saline and drought treatments (*M. glyptostroboides* leaves started to wilt after 46 days). Shoot height was measured from the growing tip to the base of the stem.

# 2.4. Whole-Plant Water Consumption

To simulate drought conditions (water stress), each pot containing one plant was covered with a plastic bag secured around the stem base. The whole-plant water consumption was measured under natural sunlight by the daily weight loss of the pot together with the plant over a 12 h period (07:00–19:00) [28,32]. At each sampling interval (day 7, 14, 21, 28, 35, and 42), three to five individual pots were examined for each treatment.

## 2.5. Leaf Gas-Exchange

Leaf transpiration rates (TRN), stomatal conductance (Gs), and net photosynthetic rates (Pn) of upper mature leaves were measured each week with a CIRAS-2 Portable Photosynthesis System (PP systems, Amesbury, MA, USA). TRN, Gs, and Pn were always measured between 8:30–11:00 a.m. under natural conditions, where photosynthetically active radiation (PAR) was ca. 1000  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>. During the measurements, leaf temperature ( $T_{leaf}$ ) ranged from 25 °C to 33 °C. Three to five individual seedlings per-treatment were measured at each interval.

## 2.6. Chlorophyll a Fluorescence

Chlorophyll *a* fluorescence was measured on a weekly basis using a PAM-2100 Fluorometer (Heinz Walz GmbH, Effeltrich, Germany) and methods described in Wang et al. (2007) [33]. The maximal efficiency of PSII photochemistry (Fv/Fm) was calculated based on measured fluorescence parameters [33].

## 2.7. Leaf Membrane Permeability

Leaf membrane permeability was examined after 20 days of saline and drought treatment. For each plant, 30 fresh leaf cubes (0.2 × 0.2 cm) were immersed in 10 mL of distilled water and then vacuumed for 30 min. Electrical conductivity ( $E_1$ ) was then measured with a DDS-307 conductivity meter (INESA Scientific Instrument Co., Ltd., Shanghai, China) at room temperature. Following this, leaf samples were incubated in boiling water (95 °C–100 °C) for 30 min and subjected to subsequent electrical conductivity ( $E_2$ ) measurements at room temperature. Leaf membrane permeability was calculated as  $E_1/E_2 \times 100\%$ .

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#### 2.8. Plant Harvest

Destructive harvests were conducted after 46 days of exposure to saline and drought treatments. Three to five replicated seedlings were harvested for each treatment. The roots were thoroughly rinsed free of soil with deionized water. All sampled materials (root, leaf, and stem) were then oven-dried at  $60\,^{\circ}\text{C}$  for five to eight days to determine the dry weight. Dried samples were ground into powder and stored for compositional analysis.

# 2.9. Ion Analysis of Leaves and Roots

Dried samples (0.5 g) of leaves and roots were extracted with 1 M HNO<sub>3</sub> as described by Storey (1995) [34]. Concentrations of Na<sup>+</sup> and K<sup>+</sup> were measured by an atomic absorption spectrometer (PerkinElmer 2280, Perkin-Elmer Corporation, Norwalk, CT, USA).  $Cl^-$  concentrations were determined by a modified silver titration method [35].

# 2.10. Ion Analysis of Soil

Soil pore water composition was analyzed immediately following plant harvest. Concentrations of Na<sup>+</sup>, Cl<sup>-</sup>, and K<sup>+</sup> were measured from aqueous soil extracts (dried soil rinsed in deionized water = 1.5, w/v). Na<sup>+</sup> and K<sup>+</sup> concentrations were measured by atomic absorption spectrophotometry (PerkinElmer 2280) at 589.0 and 766.5 nm, respectively, while Cl<sup>-</sup> concentrations were measured by silver titration [35].

# 2.11. Data Analysis

The software program SPSS (SPSS Statistics 17.0, 2008, SPSS Inc., Chicago, IL, USA) was used to calculate basic statistical parameters for measured data. Unless otherwise stated, differences are interpreted as statistically significant for the p < 0.05 level.

#### 3. Results

# 3.1. Occurrence of Leaf Injury under Water and Salt Stress

Observation during the experiment revealed that drought and/or salt caused leaf injury in plants without a polymer amendment after 16–26 day of the stress treatment (Table 1; Figure 1). Leaves exhibited chlorosis or necrosis prior to abscission (Figure 1). Hydrogels appear to have delayed stress-induced leaf injury until 30–46 day (Table 1; Figure 1). Plants amended with the biopolymer exhibited a pronounced delay of leaf injury relative to that of plants treated with synthetic polymer (Table 1).

**Table 1.** Effect of drought and saline stress on the timing (in days) of leaf injuries in *Metasequoia glyptostroboides* Hu and W.C.Cheng amended with synthetic (Stockosorb, Luquasorb) or natural (Konjac glucomannan, KGM) hydrophilic polymers and those not amended with polymers (Non-Polymer).

Treatment	Non-Polymer	Stockosorb	Luquasorb	KGM
Control	NI	NI	NI	NI
NaCl	$26 \pm 2$	NI	$36 \pm 2$	NI
Drought	$17\pm1$	$34 \pm 1$	$30 \pm 2$	$46 \pm 1$
Drought + NaCl	$16\pm1$	$34 \pm 2$	$30 \pm 2$	$46 \pm 1$

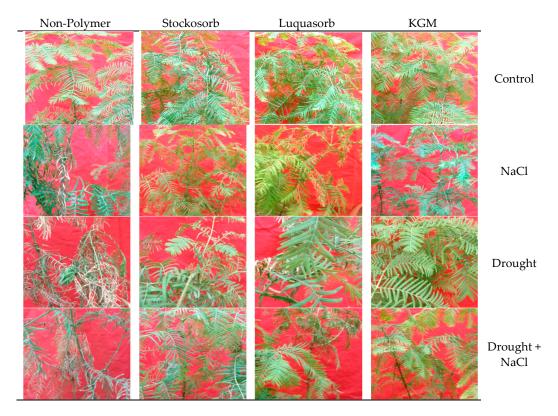
Legend: NI, non-injury. Each value ( $\pm SE)$  is the mean of three to five individual plants.

#### 3.2. Leaf Membrane Permeability

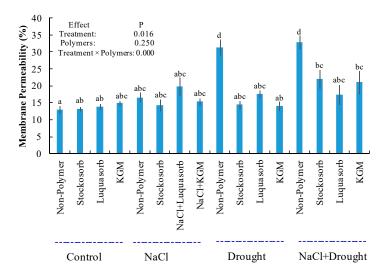
Membrane permeability (MP) was examined when the stress symptoms were visible in plants subjected to drought and saline conditions. Relative to control plants, leaf MP significantly increased in non-polymer-treated plants after 20 day of drought or combined drought and saline stress (Figure 2).

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However, the stress-induced increase in MP for these plants was markedly reduced by all polymers (Stockosorb, Luquasorb, or KGM) (Figure 2). Saline treatment did not cause a significant increase in MP, irrespective of polymer treatments at the observation time (Figure 2).



**Figure 1.** Images of *Metasequoia glyptostroboides* Hu and W.C.Cheng test plants subjected to drought and saline stress after 20 days. Columns indicate soils amended without (Non-Polymer) or with synthetic (Stockosorb, Luquasorb) and natural (Konjac glucomannan, KGM) hydrophilic polymers. Rows indicate *M. glyptostroboides* seedlings subjected to control, salt exposure, drought, and combined salt and drought exposure (see Materials and Methods section).



**Figure 2.** Effect of drought and salt stress on leaf membrane permeability in *Metasequoia glyptostroboides* seedlings supplemented with synthetic (Stockosorb, Luquasorb) and natural (Konjac glucomannan, KGM) hydrophilic polymers along with plants not amended with polymers (Non-Polymer). Each column represents the mean of three to five individual plants and bars represent the standard error of the mean. Columns labelled with different letters (a, b, c, and d) are significantly different at the p < 0.05 level.

#### 3.3. Shoot Height Increment

Amendment of 0.5% (by weight) Stockosorb, Luquasorb, or KGM caused no significant effect on height increment in control *M. glyptostroboides* plants (Figure 3A). Drought, salt, and combined drought and salt stress reduced shoot elongation in the absence of polymers (Figure 3A). The presence of hydrophilic polymers alleviated growth inhibition under stress conditions (Figure 3A). Plants subjected to salt stress and amended with Stockosorb exhibited a more pronounced effect in height enhancement than those amended with Luquasorb and KGM (Figure 3A). Plants subjected to combined drought and saline stress and amended with Luquasorb retained shoot growth better than those amended with Stockosorb and KGM (Figure 3A).

## 3.4. Plant Dry Weight

For plants not amended with polymers, dry weights of roots, stem, and leaves decreased significantly after 46 days of drought, salt, or combined drought and salt stress. These caused respective declines of 44%, 62%, and 65% in whole-plant biomass (Figure 3B). *M. glyptostroboides* roots and leaves were more sensitive to these stresses relative to stems (Figure 3B). Stockosorb, Luquasorb, and KGM alleviated whole-plant biomass inhibition by salt and drought stress, although specific tissue types did not show pronounced effects (Figure 3B). KGM also improved plant biomass for plants subjected to drought or salt stress (Figure 3B). For samples subjected to combined drought and salt stress, these effects were not as pronounced as those exhibited by plants treated with the two synthetic polymers (Figure 3B).

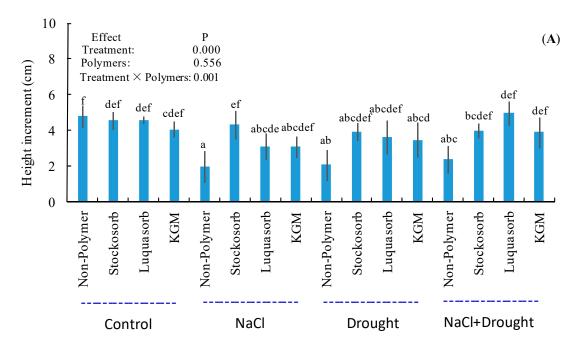
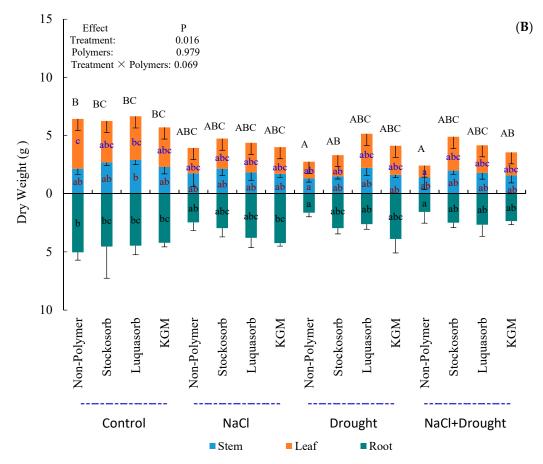


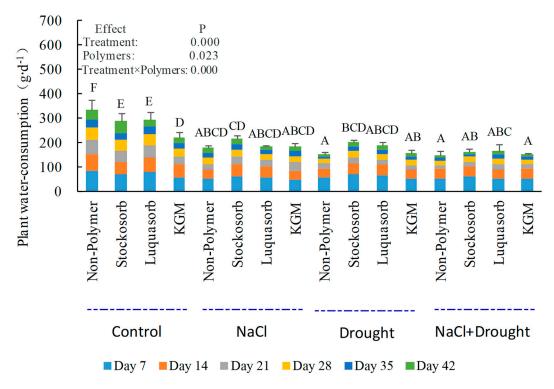
Figure 3. Cont.



**Figure 3.** Effect of drought and salt stress on height increment and biomass in *Metasequoia glyptostroboides* seedlings. (**A**): Shoot height increment. (**B**): Plant dry weight. Columns show samples amended with synthetic (Stockosorb, Luquasorb) and natural (Konjac glucomannan, KGM) hydrophilic polymers and those not amended (Non-Polymer). Each column represents mean analysis of three to five individual plants and bars represent the standard error of the mean. Columns labelled with different letters (a–f) are significantly different at the p < 0.05 level. (Note: Columns labelled with A, B, and C, indicate significant difference in whole-plant dry weight between treatments).

## 3.5. Whole-Plant Water-Consumption

Drought and/or salt stress decreased the daily water-consumption of plants not treated with polymers (Figure 4). Synthetic and natural polymer amendments increased the daily water-loss for plants subjected to stress (Figure 4). For plants subjected to drought and salt stress, water-consumption of plants treated with Stockosorb exceeded that of those treated with Luquasorb or KGM (Figure 4). For plants subjected to drought or combined drought and salt stress, plants treated with KGM exhibited less water loss than those treated with Stockosorb or Luquasorb (Figure 4).



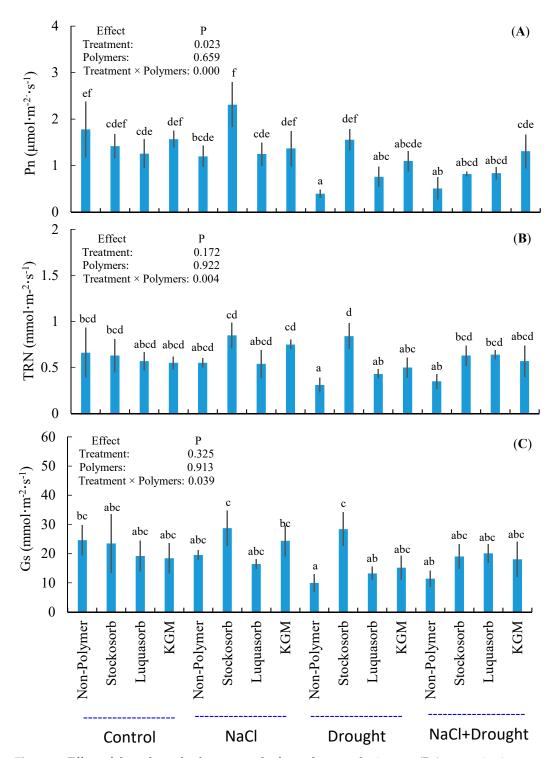
**Figure 4.** Effect of drought and salt stress on whole-plant water consumption in *Metasequoia glyptostroboides* seedlings. Columns show samples amended with synthetic (Stockosorb, Luquasorb) and natural (Konjac glucomannan, KGM) hydrophilic polymers and those not amended (Non-Polymer). Whole-plant water loss was measured after 7, 14, 21, 28, 35, and 42 days of salt and drought treatment. Each column represents the mean of three to five individual plants and bars represent the standard error of the mean. Columns labelled with different letters (A–F) are significantly different at the p < 0.05 level.

# 3.6. Leaf Gas-Exchange

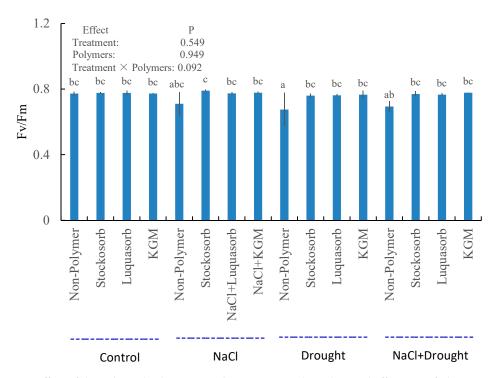
In the absence of hydrogels, drought and salt stress caused declines in net photosynthetic rates (Pn), transpiration rates (TRN), and stomatal conductance (Gs) (Figure 5). Hydrogel amendments increased Gs, Pn, and TRN under these stress conditions (relative to rates for plants not treated with polymers) (Figure 5). Relative to plants treated with Luquasorb or KGM, plants treated with Stockosorb showed the highest rates of gas exchange under drought or salt stress (Figure 5). The Pn of KGM-treated plants was 56%–60% higher than that of plants treated with synthetic polymers under combined drought and salt stress (Figure 5A). However, these combined-stressed plants treated with each polymer showed similar TRN and Gs values (Figure 5B,C).

# 3.7. Chlorophyll a Fluorescence

Seedlings subjected to drought and/or salt stress but not treated with polymers showed lower maximum values for PSII photochemistry (Fv/Fm) efficiency (Figure 6). This finding supports the photosynthetic response in water- and salt-stressed plants (Figure 5). Polymer amendments alleviated the drought and salt effects irrespective of powder and granular type of polymer (Figure 6).



**Figure 5.** Effect of drought and salt stress on leaf net photosynthetic rates (Pn), transpiration rates (TRN), and stomatal conductance (Gs) in *Metasequoia glyptostroboides* seedlings amended with synthetic (Stockosorb, Luquasorb) and natural (Konjac glucomannan, KGM) hydrophilic polymers along with those not amended with polymers (Non-Polymer). (**A**): Net photosynthetic rate. (**B**): Transpiration rate. (**C**): Stomatal conductance. Mean gas exchange values were measured on a weekly basis. Each column represents mean values for three to five individual plants and bars represent the standard error of the mean. Columns labelled with different letters (a–f) are significantly different at the p < 0.05 level.



**Figure 6.** Effect of drought and salt stress on the maximum photochemical efficiency of photosystem II in *Metasequoia glyptostroboides* seedlings amended with synthetic (Stockosorb, Luquasorb) and natural (Konjac glucomannan, KGM) hydrophilic polymers along with those not amended (Non-Polymer). Chlorophyll a fluorescence was measured on a weekly basis. Each column represents the mean value for three to five individual plants and bars represent the standard error of the mean. Columns labelled with different letters (a, b, and c) are significantly different at the p < 0.05 level.

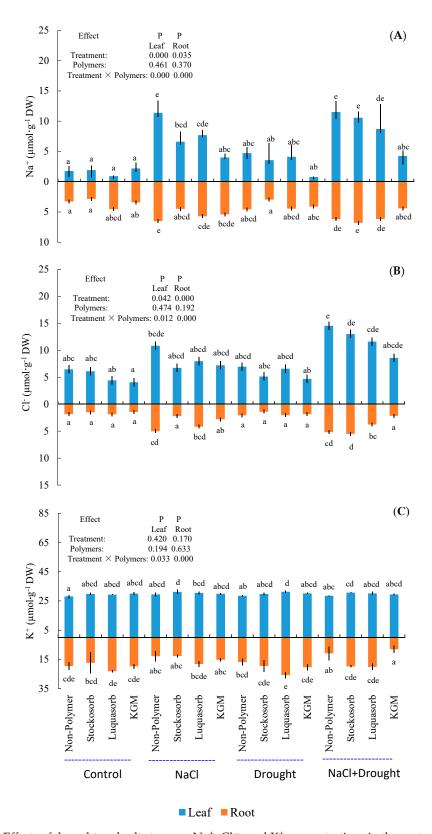
## 3.8. Ion Concentrations in Roots and Leaves

Salt (NaCl) treatment significantly increased Na<sup>+</sup> and Cl<sup>-</sup> levels in roots and leaves for plants not treated with polymers. However, leaves retained markedly higher salt concentrations than roots (Figure 7A,B). Samples treated with polymers and subjected to saline stress showed lower salt concentrations in roots and leaves (Figure 7A,B). Plants subjected to combined drought and salt stress showed more pronounced salt accumulation in leaves and roots relative to that measured from plants only subjected to salt stress (Figure 7A,B). For plants subjected to combined stresses, KGM was more effective than synthetic polymers at restricting Na<sup>+</sup> and Cl<sup>-</sup> buildup in roots and leaves (Figure 7A,B).

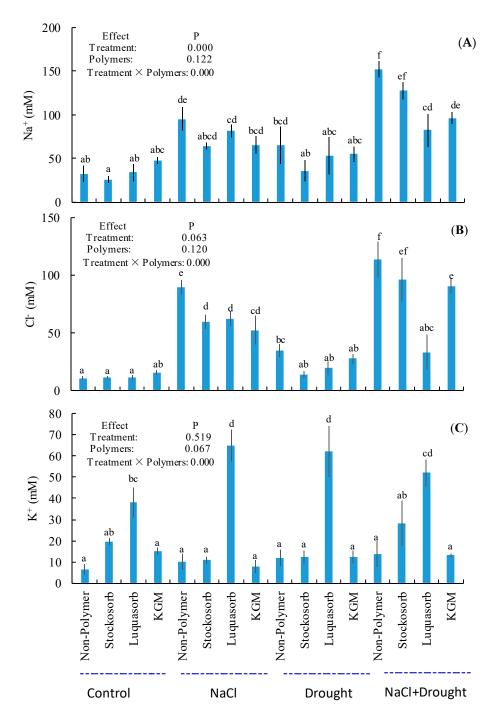
Plants subjected to salt stress but not treated with polymers exhibited lower  $K^+$  levels in roots, but not in leaves, regardless of drought stress (Figure 7C). In plants subjected to salt stress and amended with polymers, this decline in root  $K^+$  was reduced, and the effect was most pronounced in plants amended with Luquasorb (Figure 7C). In plants subjected to combined drought and salt stress, Stockosorb, and Luquasorb exhibited an increase in root  $K^+$  concentrations (relative to plants not amended with polymers), but KGM had no such effect (Figure 7C).

# 3.9. Ion Concentrations in Soils

Relative to control soils, soils subjected to saline and drought stress exhibited higher  $Na^+$  and  $Cl^-$  concentrations (Figure 8A,B). Hydrogel amendments lowered  $Na^+$  and  $Cl^-$  concentrations in salt-treated soils, regardless of drought stress (Figure 8A,B). Soils subjected to combined drought and salt stress and treated with Luquasorb showed the most significant decrease in  $Na^+$  and  $Cl^-$  levels relative to soils treated with Stockosorb and KGM (Figure 8A,B). Soils amended with synthetic polymers, in particular Luquasorb, exhibited increased  $K^+$  concentrations relative to soils treated with KGM, irrespective of control or stress treatment (Figure 8C).



**Figure 7.** Effects of drought and salt stress on Na<sup>+</sup>, Cl<sup>-</sup>, and K<sup>+</sup> concentrations in the roots and leaves of *Metasequoia glyptostroboides* seedlings amended with synthetic (Stockosorb, Luquasorb) and natural (Konjac glucomannan, KGM) hydrophilic polymers along with those not amended with polymers (Non-Polymer). (**A**): Na<sup>+</sup> concentrations. (**B**): Cl<sup>-</sup> concentrations. (**C**): K<sup>+</sup> concentrations. Each column represents the mean of three to five individual plants and bars represent the standard error of the mean. Columns labelled with different letters (a–e) are significantly different at the p < 0.05 level.



**Figure 8.** Effects of drought and salt stress on Na<sup>+</sup>, Cl<sup>-</sup>, and K<sup>+</sup> concentrations in soils supplemented without synthetic (Stockosorb, Luquasorb) and natural (Konjac glucomannan, KGM) hydrophilic polymers along with soils not supplemented with polymers (Non-Polymer). (**A**): Na<sup>+</sup> concentration. (**B**): Cl<sup>-</sup> concentration. (**C**): K<sup>+</sup> concentration. Each column represents the mean of three to five soil samples and bars represent the standard error of the mean. Columns labeled with different letters (a–f) are significantly different at the p < 0.05 level.

## 4. Discussion

# 4.1. Hydrophilic Polymers Increase Water Supply under Drought Stress

Synthetic hydrogels (Stockosorb and Luquasorb) and natural KGM polymer amendments caused relative increases in growth and photosynthesis (Figures 3, 5 and 6) in plants

subjected to drought conditions. These results support those reported in Syvertsen and Dunlop (2004), who found that polymer amendments improved the growth of drought-stressed citrus [36]. Similarly, Stockosorb polymers were shown to prolong the survival of *Pinus halepensis* [37], *Citrus* spp. [38], and *Populus Popularis* seedlings subjected to drought [28]. In this study, leaf injury caused by drought stress occurred due to increased leaf membrane permeability (Table 1; Figures 1 and 2). The application of Stockosorb, Luquasorb, and KGM decreased MP in drought-treated *M. glyptostroboides* leaves (Figure 2), thus delaying the occurrence of leaf injury (Table 1; Figure 1). Similarly, Shi et al. (2010) demonstrated that Stockosorb and Luquasorb polymers reduce the growth inhibition of *P. popularis* cuttings and delay leaf injury during drought stress [28]. Our results show that plants amended with the biopolymer exhibited a more pronounced delay of leaf injury relative to those treated with synthetic polymers (Table 1).

Growth and photosynthesis enhancement exhibited by plants treated with polymers occurred due to improved soil water retention under drought conditions [39]. Hydrogel amendments remarkably increased the available water capacity of the soil [28,39,40]. This water could then be absorbed by adjacent roots with up to 95% available to plants [22]. Increased root water supply in polymer-amended plants resulted in improved whole-plant water consumption and leaf transpiration per unit of surface area (Figures 4 and 5). Our data indicate that Stockosorb amendments provided plant roots with a larger amount of water than Luquasorb and KGM polymers (Figures 4 and 5). The larger Stockosorb granules were able to absorb and store larger amounts of water in the soil environment and can thus function as water sources during drought conditions [28]. M. glyptostroboides roots can grow interspersed with Stockosorb aggregates, which improves the water distribution [27,28]. The powder type polymers, KGM in particular, displayed an enhanced ability to moderate water supply to plant roots (Figures 4 and 5). This improved long-term water use efficiency under drought conditions [23,28]. The glucomannan structure of the KGM polymer confers excellent water retention capabilities [31,41]. This allows the KGM-amended soil to maintain the water supply to M. glyptostroboides roots over prolonged periods of water stress and thereby delays the occurrence of leaf injury (Table 1; Figure 1). These results support those of previous studies on hydrogel-treated P. popularis [28]. The powder polymers also more effectively slowed the rate of water supply to plants (and thus prolonged the duration of water supply) relative to the granular-type polymer [28].

## 4.2. Hydrophilic Polymers Alleviate Salt Stress

Under saline stress, plants amended with hydrophilic polymers exhibited improved shoot height increment, leaf gas exchange, and biomass relative to plants not amended with polymers (Figures 3 and 5). Plants subjected to saline conditions but amended with Stockosorb exhibited a more pronounced effect to lower the salt inhibition of transpiration (Figures 4 and 5) and photosynthesis (Figures 5 and 6) than those amended with Luquasorb and KGM. Enhanced tolerance of saline conditions mainly resulted from improved ion relations in polymer-amended plants. In this study, Stockosorb and KGM polymers markedly reduced Na<sup>+</sup> and Cl<sup>-</sup> concentrations in roots and leaves under salt stress (Figure 7). Excessive salt accumulation poses a significant threat to woody plants subjected to saline conditions [3,8,9,32,35,42–46]. Previous studies have shown that hydrophilic polymer amendments inhibited salt accumulation in poplar species' roots and shoots regardless of salt-resistant and salt-sensitive genotypes [26–28]. Reduced salt ion accumulation in roots and shoots resulted from the salt-retention and dilution capacity of the polymer amendments [28] (Figure 8). Water retention by Stockosorb, Luquasorb, and KGM polymers specifically causes the dilution of soil Na<sup>+</sup> and Cl<sup>-</sup> concentrations [28,47] and limits salt absorption by the surrounding roots.

In addition to reducing the uptake of salt ions, the two synthetic hydrogels, and especially Luquasorb, enhanced  $K^+$  uptake in salt-stressed roots (Figure 7). Maintaining  $K^+$  homeostasis allows plants to withstand toxicity from salt ions [45,48,49]. Plants amended with the two hydrophilic polymers exhibited improved  $K^+$  retention due to increased exchangeable  $K^+$  concentrations (Figure 8). These results were consistent with our previous studies in which *P. popularis* plants subjected to saline

conditions but treated with Stockosorb and Luquasorb exhibited increased K<sup>+</sup> concentrations in leaves and roots [27,28].

## 4.3. Hydrophilic Polymers Enhance Plant Tolerance of Combined Drought and Salt Stresses

When M. glyptostroboides plants were exposed to combined drought and salt stress, roots could not absorb enough water to compensate for the water loss in shoots (Figures 4 and 5). Excessive accumulation of Na<sup>+</sup> and Cl<sup>-</sup> also caused ion toxicity and membrane injury (Figures 1 and 7; Table 1). The hydrophilic polymers increased the soil available water for plants, which in turn increased water-consumption and leaf gas-exchange (Figures 4 and 5). In addition to improving root water supply, hydrophilic polymers lowered soil salt concentrations in the dryer, saline soil by increasing the water-holding capacity (Figure 8). As a consequence, the uptake and transport of salt ions were restricted in polymer-amended plants (Figure 7). Shi et al. (2010) similarly showed that hydrophilic polymers increased the ability of P. popularis to tolerate combined drought and salt stresses, primarily due to their water- and salt-retention capacities [28]. Plants amended with Stockosorb and Luquasorb exhibited improved K<sup>+</sup> nutritional status in roots. This enabled M. glyptostroboides plants to maintain K<sup>+</sup>/Na<sup>+</sup> homeostasis under water and salt stresses (Figures 7 and 8). Amendments with hydrophilic polymers improved water supply and K<sup>+</sup>/Na<sup>+</sup> homeostasis under drought and salt stresses, which enhanced the growth and survival of M. glyptostroboides.

Relative to drought- and salt-stressed plants amended with Stockosorb or Luquasorb polymers, drought- and salt-stressed plants amended with the KGM biopolymer exhibited less pronounced growth/biomass improvements (Figure 3). This arose due to (i) fewer K<sup>+</sup> ions and (ii) poor aeration in KGM-amended soil. KGM can absorb water and swell to form a sticky gel. This gel, however, can reduce the air supply to roots and thereby limit root cell respiration. Combined drought and salt stress therefore lowered root and shoot growth for KGM-amended plants relative to plants amended with Stockosorb and Luquasorb granules.

#### 5. Conclusions

The study showed that natural and synthetic hydrophilic polymers increased the growth and survival of *M. glyptostroboides* plants under drought, saline, and combined drought and saline stresses. We found that plants subjected to drought stress but amended with Stockosorb received more water delivery to roots relative to drought-stressed plants amended with Luquasorb and KGM polymers. KGM, a powder polymer, apparently limited the water supply to *M. glyptostroboides* roots and maintained a continuous water supply under long-term water stress. Under salt stress, the water retained in the hydrophilic polymers diluted salt concentrations in the soil, which in turn limited salt uptake by roots. Plants subjected to saline stress but amended with the two synthetic hydrogels, especially Luquasorb, exhibited enhanced K<sup>+</sup> absorption in roots and resisted Na<sup>+</sup> and Cl<sup>-</sup> toxicity. The high water- and salt-retention capacities of the hydrophilic polymers enhanced *M. glyptostroboides* tolerance of combined drought and saline stress.

**Author Contributions:** J.L., X.M., G.S., D.Z., and X.Z. (Xiaojiang Zheng) performed experiments, collected data, and carried out all analyses; X.Z. (Xiaoyang Zhou), C.L., and S.L. provided technical assistance to J.L., X.M., and G.S.; J.L. wrote this manuscript; R.Z. and S.C. supervised and revised the writing.

**Funding:** The research was supported jointly by the National Natural Science Foundation of China (Grant Nos. 31770643, 31570587), Beijing Natural Science Foundation (Grant Nos. 6182030, 6172024), the Research Project of the Chinese Ministry of Education (Grant No. 113013A), Fundamental Research Funds for the Central Universities (Grant No. 2017ZY07), the Program of Introducing Talents of Discipline to Universities (111 Project, Grant No. B13007), the Research Project from the Science and Technology Department of Henan Province (Grant No. 172102310677), and the Doctoral Fund of Henan Polytechnic University (Grant No. B2012-028).

**Acknowledgments:** We thank BASF Corporation (Germany) for providing the Luquasorb product, potassium polyacrylate (powder type).

**Conflicts of Interest:** The authors declare no conflict of interest.

#### References

- 1. Kumar, M. Crop plants and abiotic stresses. J. Biomol. Res. Ther. 2013, 3, e125. [CrossRef]
- 2. Harfouche, A.; Meilan, R.; Altman, A. Molecular and physiological responses to abiotic stress in forest trees and their relevance to tree improvement. *Tree Physiol.* **2014**, *34*, 1181–1198. [CrossRef] [PubMed]
- 3. Polle, A.; Chen, S. On the salty side of life: Molecular, physiological and anatomical adaptation and acclimation of trees to extreme habitats. *Plant Cell Environ.* **2015**, *38*, 1794–1816. [CrossRef] [PubMed]
- 4. Rennenberg, H.; Loreto, F.; Polle, A.; Brilli, F.; Fares, S.; Beniwal, R.S.; Gessler, A. Physiological responses of forest trees to heat and drought. *Plant Biol.* **2006**, *8*, 556–571. [CrossRef] [PubMed]
- 5. Xie, R.; Zhang, J.; Ma, Y.; Pan, X.; Dong, C.; Pang, S.; He, S.; Deng, L.; Yi, S.; Zheng, Y.; et al. Combined analysis of mRNA and miRNA identifies dehydration and salinity responsive key molecular players in citrus roots. *Sci. Rep.* **2017**, *7*, 42094. [CrossRef] [PubMed]
- 6. Kumar, M.; Choi, J.; An, G.; Kim, S.R. Ectopic expression of *OsSta2* enhances salt stress tolerance in rice. *Front. Plant Sci.* **2017**, *8*, 316. [CrossRef] [PubMed]
- 7. Fu, R.; Zhang, M.; Zhao, Y.; He, X.; Ding, C.; Wang, S.; Feng, Y.; Song, X.; Li, P.; Wang, B. Identification of salt tolerance-related microRNAs and their targets in Maize (*Zea mays* L.) using high-throughput sequencing and degradome analysis. *Front. Plant Sci.* **2017**, *8*, 864. [CrossRef] [PubMed]
- 8. Chen, S.; Polle, A. Salinity tolerance of *Populus. Plant Biol.* 2010, 12, 317–333. [CrossRef] [PubMed]
- 9. Chen, S.; Hawighorst, P.; Sun, J.; Polle, A. Salt tolerance in *Populus*: Significance of stress signaling networks, mycorrhization, and soil amendments for cellular and whole-plant nutrition. *Environ. Exp. Bot.* **2014**, 107, 113–124. [CrossRef]
- 10. Kumar, M.; Lee, S.C.; Kim, J.Y.; Kim, S.J.; Aye, S.S.; Kim, S.R. Over-expression of dehydrin gene, *OsDhn1*, improves drought and salt stress tolerance through scavenging of reactive oxygen species in rice (*Oryza sativa* L.). *J. Plant Biol.* **2014**, *57*, 383–393. [CrossRef]
- 11. Azzam, R.A. Polymeric conditioner gels for desert soils. *Commun. Soil Sci. Plant Anal.* **1983**, 14, 739–760. [CrossRef]
- 12. Woodhouse, J.; Johnson, M.S. Effect of superabsorbent polymers on survival and growth of crop seedlings. *Agric. Water Manag.* **1991**, 20, 63–70. [CrossRef]
- 13. Allahdadi, I.; Moazzen-Ghamsari, B.; Akbari, G.A.; Zohorianfar, M.J. Investigating the Effect of Different Rates of Superabsorbent Polymer (Superab A200) and Irrigation on the Growth and Yield of Zea Mays, 3rd Specialized Training Course and Seminar on the Application of Superabsorbent Hydrogels in Agriculture; IPPI: Tehran, Iran, 2005; pp. 52–56.
- 14. Al-Humaid, A.I.; Moftah, A.E. Effects of hydrophilic polymers on the survival of buttonwood seedlings grown under drought stress. *J. Plant Nutr.* **2007**, *30*, 53–66. [CrossRef]
- 15. Guiwei, Q.; Varennes, A.D.; Cunha-Queda, C. Remediation of a mine soil with insoluble polyacrylate polymers enhances soil quality and plant growth. *Soil Use Manag.* **2008**, *24*, 350–356. [CrossRef]
- 16. El-Hady, O.A.; El-Kader, A.A.; Shafi, A.M. Physico-bio-chemical properties of sandy soil conditioned with acrylamide hydrogels after cucumber plantation. *Aust. J. Basic Appl. Sci.* **2009**, *3*, 3145–3151.
- 17. Jobin, P.; Caron, J.; Bernier, P.Y.; Dansereau, B. Impact of two hydrophilic acrylic-based polymers on the physical properties of three substrates and the growth of *Petunia hybrida* 'Brilliant Pink'. *J. Am. Soc. Hort. Sci.* **2004**, *129*, 449–457.
- 18. Hüttermann, A.; Orikiriza, L.J.B.; Agaba, H. Application of superabsorbent polymers for improving the ecological chemistry of degraded or polluted lands. *Clean-Soil Air Water* **2009**, *37*, 517–526. [CrossRef]
- 19. Apostol, K.G.; Jacobs, D.F.; Dumroese, R.K. Root desiccation and drought stress responses of bare root *Quercus rubra* seedlings treated with a hydrophilic polymer root dip. *Plant Soil* **2009**, *315*, 229–240. [CrossRef]
- 20. Abedi-Koupai, J.; Sohrab, F. Evaluating the application of superabsorbent polymers on soil water capacity and potential on three soil textures. *Iran. J. Polym. Sci. Technol.* **2004**, *17*, 163–173.
- 21. Guterres, J.; Rossato, L.; Pudmenzky, A.; Doley, D.; Whittaker, M.; Schmidt, S. Micron-size metal-binding hydrogel particles improve germination and radicle elongation of Australian metallophyte grasses in mine waste rock and tailings. *J. Hazard. Mater.* 2013, 248–249, 442–450. [CrossRef] [PubMed]
- 22. Bhardwaj, A.K.; Shainberg, I.; Goldstein, D.; Warrington, D.N.; Levy, G.J. Water retention and hydraulic of cross-linked polyacrylamides in sandy soils. *Soil Sci. Soc. Am. J.* **2007**, *71*, 406–412. [CrossRef]

23. Agaba, H.; Orikiriza, L.J.B.; Obua, J.; Kabasa, J.D.; Worbes, M.; Hüttermann, A. Hydrogel amendment to sandy soil reduces irrigation frequency and improves the biomass of *Agrostis stolonifera*. *Agric. Sci.* **2011**, 2, 544–550. [CrossRef]

- 24. El-Sayed, H.; Kirkwood, R.C.; Graham, N.B. The effects of a hydrogel polymer on the growth of certain horticultural crops under saline conditions. *J. Exp. Bot.* **1991**, *42*, 891–899. [CrossRef]
- 25. Szmidt, R.A.K.; Graham, N.B. The effect of poly (ethylene oxide) hydrogel on crop growth under saline conditions. *Acta Hortic.* **1991**, 287, 211–218. [CrossRef]
- 26. Chen, S.; Zommorodi, M.; Fritz, E.; Wang, S.; Hüttermann, A. Hydrogel modified uptake of salt ions and calcium in *Populus euphratica* under saline conditions. *Trees* **2004**, *18*, 175–183.
- 27. Shao, J.; Chen, S.; Wang, R.; Zhang, X. Enhancement of hydrogel on salt resistance of *Populus popularis* '35–44' and its mechanism. *J. Beijing For. Univ.* **2007**, *29*, 79–84. (In Chinese)
- 28. Shi, Y.; Li, J.; Shao, J.; Deng, S.; Wang, R.; Li, N.; Sun, J.; Zhang, H.; Zhu, H.; Zhang, Y.; et al. Effects of Stockosorb and Luquasorb polymers on salt and drought tolerance of *Populus popularis*. *Sci. Hortic.* **2010**, 124, 268–273. [CrossRef]
- 29. Dorraji, S.S.; Golchin, A.; Ahmadi, S. The effects of hydrophilic polymer and soil salinity on corn growth in sandy and loamy soils. *Clean-Soil Air Water* **2010**, *38*, 584–591. [CrossRef]
- 30. Koroskenyi, B.; Mccarthy, S.P. Synthesis of acetylated konjac glucomannan and effect of degree of acetylation on water absorbency. *Biomacromolecules* **2001**, *2*, 824–826. [CrossRef] [PubMed]
- 31. Zhang, C.; Chen, J.; Yang, F. Konjac glucomannan, a promising polysaccharide for OCDDS. *Carbohyd. Polym.* **2014**, *104*, 175–181. [CrossRef] [PubMed]
- 32. Chen, S.; Li, J.; Wang, S.; Fritz, E.; Hüttermann, A.; Altman, A. Effects of NaCl on shoot growth, transpiration, ion compartmentation, and transport in regenerated plants of *Populus euphratica* and *Populus tomentosa*. *Can. J. For. Res.* **2003**, 33, 967–975. [CrossRef]
- 33. Wang, R.; Chen, S.; Deng, L.; Fritz, E.; Hüttermann, A.; Polle, A. Leaf photosynthesis, fluorescence response to salinity and the relevance to chloroplast salt compartmentation and anti-oxidative stress in two poplars. *Trees* **2007**, *21*, 581–591. [CrossRef]
- 34. Storey, R. Salt tolerance, ion relations and the effects of root medium on the response of citrus to salinity. *Aust. J. Plant Physiol.* **1995**, 22, 101–114. [CrossRef]
- 35. Chen, S.; Li, J.; Wang, S.; Hüttermann, A.; Altman, A. Salt, nutrient uptake and transport and ABA of *Populus euphratica*; a hybrid in response to increasing soil NaCl. *Trees* **2001**, *15*, 186–194. [CrossRef]
- 36. Syvertsen, J.P.; Dunlop, J.M. Hydrophilic gel amendments to sand soil can increase growth and nitrogen uptake efficiency of citrus seedlings. *Hortic. Sci.* **2004**, *39*, 267–271.
- 37. Hüttermann, A.; Zommorodi, M.; Reise, K. Addition of hydrogels to soil for prolonging the survival of *Pinus halepensis* seedlings subjected to drought. *Soil Till. Res.* **1999**, *50*, 295–304. [CrossRef]
- 38. Arbona, V.; Iglesias, D.J.; Jacas, J.; Primo-Millo, E.; Talon, M.; Gómez-Cadenas, A. Hydrogel substrate amendment alleviates drought effects on young citrus plants. *Plant Soil* **2005**, 270, 73–82. [CrossRef]
- 39. Chirino, E.; Vilagrosa, A.; Vallejo, V.R. Using hydrogel and clay to improve the water status of seedlings for dryland restoration. *Plant Soil* **2011**, *344*, 99–110. [CrossRef]
- 40. Narjary, B.; Aggarwal, P.; Singh, A.; Chakraborty, D.; Singh, R. Water availability in different soils in relation to hydrogel application. *Geoderma* **2012**, *187–188*, 94–101. [CrossRef]
- 41. Tatirat, O.; Charoenrein, S. Physicochemical properties of konjac glucomannan extracted from konjac flour by a simple centrifugation process. *LWT-Food Sci. Technol.* **2011**, *44*, 2059–2063. [CrossRef]
- 42. Chen, S.; Li, J.; Fritz, E.; Wang, S.; Hüttermann, A. Sodium and chloride distribution in roots and transport in three poplar genotypes under increasing NaCl stress. *For. Ecol. Manag.* **2002**, *168*, 217–230. [CrossRef]
- 43. Chen, S.; Li, J.; Wang, T.; Wang, S.; Polle, A.; Hüttermann, A. Osmotic stress and ion-specific effects on xylem abscisic acid and the relevance to salinity tolerance in poplar. *J. Plant Growth Regul.* **2002**, *21*, 224–233. [CrossRef]
- 44. Sun, J.; Chen, S.; Dai, S.; Wang, R.; Li, N.; Shen, X.; Zhou, X.; Lu, C.; Zheng, X.; Hu, Z.; et al. NaCl-induced alternations of cellular and tissue ion fluxes in roots of salt-resistant and salt-sensitive poplar species. *Plant Physiol.* **2009**, *149*, 1141–1153. [CrossRef] [PubMed]
- 45. Sun, J.; Dai, S.; Wang, R.; Chen, S.; Li, N.; Zhou, X.; Lu, C.; Shen, X.; Zheng, X.; Hu, Z.; et al. Calcium mediates root K<sup>+</sup>/Na<sup>+</sup> homeostasis in poplar species differing in salt tolerance. *Tree Physiol.* **2009**, 29, 1175–1186. [CrossRef] [PubMed]

46. Sun, J.; Wang, M.; Ding, M.; Deng, S.; Liu, M.; Lu, C.; Zhou, X.; Shen, X.; Zheng, X.; Zhang, Z.; et al. H<sub>2</sub>O<sub>2</sub> and cytosolic Ca<sup>2+</sup> signals triggered by the PM H<sup>+</sup>-coupled transport system mediate K<sup>+</sup>/Na<sup>+</sup> homeostasis in NaCl-stressed *Populus euphratica* cells. *Plant Cell Environ.* **2010**, *33*, 943–958. [CrossRef] [PubMed]

- 47. Liu, Z.; Han, G.; Jiang, Y.; Liu, W. Research on keep water properties of Konjac Powder. *Acad. Period. Farm Prod. Process* **2005**, 42, 16–18. (In Chinese)
- 48. Shabala, S.; Cuin, T.A. Potassium transport and plant salt tolerance. *Physiol. Plant.* **2008**, 133, 651–669. [CrossRef] [PubMed]
- 49. Munns, R.; Tester, M. Mechanisms of salinity tolerance. *Ann. Rev. Plant Biol.* **2008**, *59*, 651–681. [CrossRef] [PubMed]



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