Influence of silicon priming on seedling growth, root xylem anatomy and ion accumulation of barley (Hordeum vulgare L.) under drought stress

Ehsan Bijanzadeh*, Rohullah Naderi, and Vahid Barati

*College of Agriculture and Natural Resources of Darab, Shiraz University, Iran
(Received: 24/10/2017-Accepted: 12/12/2017)

Abstract

The detrimental drought effects can cause dry weight loss and silicon is known to enhance crop tolerance to drought by increasing seedling growth and hydraulic conductivity. To investigate the effects of silicon priming (0, 1 and 2 mM as sodium silicate), on seedling growth and root anatomy of three barley cultivars (Khatam, Rihane, and Nimrooz) a laboratory experiment was conducted under drought stress induced by 0.8 MPa polyethylene glycol (PEG). Under 0.8 MPa PEG, the highest root length was observed in Nimrooz barley cultivar (7.86 mm) at 2 mM silicon. In Nimrooz, seedling dry weight increased from 0.19 mg plant⁻¹ in control to 0.27 mg plant⁻¹ at 2 mM silicon (29.6% increase). At 2 mM silicon, Nimrooz with the highest root surface area had the highest hydraulic conductivity (6.29×10⁻⁹ m s⁻¹ MPa⁻¹) while in Rihane and Khatam was low as 5.38 and 5.19×10⁻⁹ m s⁻¹ MPa⁻¹ under 0.8 MPa PEG, respectively. In Nimrooz, positive relationship was observed between application of 2 mM silicon and increase of the mean diameter of peripheral metaxylem vessels under drought. In all barely cultivars, drought stress affected the diameter of the vessels more than the number of the vessels. Also, under 0.8 MPa PEG, K⁺ and Ca²⁺ content increased from 0 mM silicon to 2 mM. Overall, silicon priming at 2 mM, especially in Nimrooz appeared to be a promising and cost-effective procedure to alleviate the drought stress.

Keywords: Peripheral metaxylem, Polyethylene glycol, Ion accumulation, Root surface area, Hydraulic conductivity

Introduction

Silicon is present in plants in amounts equivalent to calcium, magnesium and phosphorus and is the second most abundant element after oxygen in soil. In Poaceae family, silicon often is present in higher quantity than any other inorganic constituent (Shakoor, 2014). Drought, is one of the serious environmental stresses, and is the most significant factor restricting plant growth and productivity in a majority of agricultural fields of the world (Ma, 2004; Malhotra, 2016). Silicon nutrition alleviates many abiotic stresses such as drought, UV radiation, high temperature, flooding, lodging, freezing, salt, metal toxicity and nutrient imbalance. Silicon plays a very important role in drought tolerance because silicon fed plants maintains higher leaf water potential. In addition, endodermal tissue, which plays an important role in water transport across the root, accumulates large amounts of silicon in mature drought-tolerant plants (Ma and Yamaji, 2006; Al-Mayahi, 2008; Epstein, 2009).

Silicon has been proved to be useful in seed plant species mainly Poaceae and Cyperaceae especially during environmental stress (Hattori et al., 2005). Different plant species accumulate different amount of silicon e.g. rice accumulates maximum amount of silicon (Ma et al., 2006). Polyethylene glycol (PEG), a non-penetrated and nontoxic osmotic lowers the water potential of the medium and has been used to stimulate water stress without harmful effects or toxic on the plant, but inhibits growth by lowering water potential of the medium stated that the adverse effects of PEG on plant growth were alleviated by adding Si to drought stressed treatments in terms of shoot length, shoot mass, and root mass (Ober and Sharp, 2003; Al-Mayahi, 2008). It has been widely reported that silicon increases drought tolerance in plants such as wheat (Gong and Chen 2012), maize (Gao et al., 2006), sorghum (Ahmed et al., 2011a) and sunflower (Gunes et al., 2008). Gong and Chen (2012) reported that the water potential of silicon-applied drought-stressed wheat leaves is maintained to a greater extent compared with stressed plants without silicon supplementation, suggesting that silicon can improve the water status of wheat plants during drought. A similar phenomenon was observed by Pei et al. (2010) in wheat exposed to polyethylene glycol induced water stress.

Priming hastens the germination, seedling emergence and early growth in such a way that soil moisture and nitrogen are engaged and utilized (Shakoor, 2014). Priming could be done with a number of chemicals including silicon, which is an effective barrier against water losses by cuticular transpiration and/or fungal infections. Silicon enables the plant to tolerate drought and increase uptake of water, which in
return will help the plant to produce higher dry mass and yield (Ahmed et al., 2013).

Root anatomy plays a major role in root hydraulic, influencing axial conductance and the distribution of water uptake along the root with a more localized role for aquaporins (Bramely et al., 2009). These membrane integral proteins form water-conducting channels, responsible for the variable hydraulic conductivity of root systems (Javot and Maurel, 2002). Root hydraulic properties could be changed with the magnitude of water flow induced across roots (Passiourea and Munns, 1984; Mureen et al., 2010). The hydraulic conductivity of a root is a complex parameter because of the complicated structure of the osmotic barrier made up of exodermis, cortex and endodermis (Kramer and Boyer, 1995). Bijanzadeh (2017) declared that the data regarding root hydraulic conductivity are required to understand the mechanisms of water uptake by wheat roots especially under stress conditions. The main aim of this study was investigating the effect of silicon priming on the seedling growth, root xylem anatomy, and ion accumulation of three barley cultivars under drought stress stimulated by polyethylene glycol (PEG).

Materials and Methods

Seed priming by silicon: To investigate the effect of silicon priming on root anatomy and hydraulic conductivity of three barley cultivars including ‘Khatam’, ‘Rihane’, and ‘Nimrooz’ under drought stress induced by PEG (6000) a laboratory experiment was conducted at College of Agriculture and Natural Resources of Darab, Shiraz University in 2014. All seeds were prepared from Research Center of Hassan Abad, Fars Province, Iran. All seeds of barley cultivars were surface sterilized by dipping them in 2% hypochlorite sodium solution for 60 second and rinsed thoroughly with sterilized distilled water. Then, seeds were primed with 0, 1, and 2 mM silicon solutions (Sodium silicate) for 3hrs. in the dark at 25 °C. and distilled water was also used as control. After priming, seeds were washed with tap water for two minutes, rinsed with distilled water and then dried between two filter papers Whatman grade 2 (22 °C and 60% relative humidity).

Plant growth in Hoagland nutrient solution: The seeds were first bubbled in distilled water for one day and then put in CaSO₄ solution in a 10-liter beaker and were aerated for 3 days. When seedlings had a root length of 5–10 mm, they were transferred to a hydroponic system, containing a modified half-strength Hoagland nutrient solution [KH₂PO₄ (1.5 mM), KNO₃ (2.0 mM), CaCl₂ (1.0 mM), MgSO₄ (1.0 mM), FeNa (18.0 μM), H₃BO₃ (8.1 μM), MnCl₂ (1.5 μM)] (Fricke et al., 1997). Then, seedlings of each barley cultivar were grown at two concentrations of PEG (0 and 0.8 MPa) and were compared in a completely randomized design with five replications.

Four seedlings were kept in each 1-liter glass beaker and the nutrient solution was ventilated by a gas exchange pump at a flow rate of 400 mL min⁻¹ in growth chamber. Plants were kept at a day/night photoperiod of 16/8 hours and temperature of 20/15°C. Relative humidity was 70% and photosynthetic active radiation was 350–450 μmol m⁻² s⁻¹. Then, seedling growth, hydraulic conductivity, root anatomy, root surface area, and ion analysis at 14 days old plants were measured according to the following procedures.

Determination of root hydraulic conductivity using root exudation method: Surface area of the roots was determined by measuring the length and the radius of the main axis of seminal and adventitious roots and the number, length, and diameter of the lateral roots of the 14 days old plants. Surface area was calculated by treating roots as cylinders (Knipfer and Fricke, 2010). Total root area (A₅) was calculated as below:

\[ A₅ = 2πr₁L₁ + 2πr₂L₂ + 2πr₃L₃ + 2πr₄L₄ \]

Where, \( r₁ \approx \) Main root radius ≈ 250 μm; \( L₁ \approx \) Main root length; \( r₂ \approx \) Lateral root (I) radius ≈ 125 μm; \( L₂ \approx \) Lateral root (I) length; \( r₃ \approx \) Lateral root (II) radius ≈ 62.5 μm; \( L₃ \approx \) Lateral Root (II) length; \( r₄ \approx \) Lateral root (III) radius ≈ 31.25 μm, \( L₄ \approx \) Lateral root (III) length. Then, an individual root was attached to the excised root base to a glass capillary (diameter 0.5 mm). The rise of the xylem sap in the capillary was measured in 5 minutes intervals for one hour. Exudate volume (Ve) was used to determine the hydraulic properties of the roots. Ve and the hydraulic conductivity of the root (Lpr) were determined as below (Knipfer and Fricke, 2010):

\[ Lpr = \frac{Δp}{Vₜ}(\frac{1}{Δt})(\frac{1}{Δr})(\frac{1}{A₅}) \]

\[ Vₜ = \pi r₄ h \]

\[ Δp = Pe-Pm \]

Where, \( r₄ \approx \) radial of glass capillary (250 μm); \( h \approx \) height of the root exudates in glass capillary, \( t \approx \) time of going up the root exudates in the glass capillary, \( Pe \approx \) osmotic potential of the root exudates, and \( Pm \approx \) osmotic potential of the medium. The osmolality of root exudates (Pe) and medium (Pm) was determined by PicolitreOsmometry (Model P302, UK). Samples were either analyzed or stored under a of liquid paraffin layer(to minimize evaporation) in 2ml centrifuge tubes at 4°C for up to 3 days (Fricke and Peters, 2002).

Root anatomy structure and surface area: Root anatomical structures were investigated on free-hand cross-sections that were made from 5-10 mm root tips, at 14 days old seedlings (Steudle, 2000). For detection of number and diameter of vessels in central and peripheral metaxylem of ceminal roots, (see Fig. 1), sections were stained for 3 minutes with 0.5% toluidine blue and counterstained with distilled water (Hachez et al., 2006). Then, sections were observed with a Ceti microscope (Magnum-T, Ceti, UK) and images were captured with a digital camera (SX60 HS, Canon, Japan). Surface area of the roots was determined by measuring the length and the radius of the main axis of seminal and adventitious roots and the number, length, and diameter of the lateral roots of the 7 days old plants. Surface area was calculated by treating roots as
cylinders (Knipfer and Fricke, 2010).

Total root area ($A_r$) was calculated as below:

$$A_r = 2\pi r_1 L_1 + 2\pi r_2 L_2 + 2\pi r_3 L_3 + 2\pi r_4 L_4$$

Where, $r_1$ = Main root radius \(\approx 250 \mu m\); $L_1$ = Main root length; $r_2$ = Lateral root (I) radius \(\approx 125 \mu m\); $L_2$ = Lateral root (I) length; $r_3$ = Lateral root (II) radius \(\approx 62.5 \mu m\); $L_3$ = Lateral root (II) length; $r_4$ = Lateral root (III) radius \(\approx 31.25 \mu m\); $L_4$ = Lateral root (III) length. Final length of shoot and root was measured 14 days after germination and seedlings were weighted using an analytical balance (EQ-120, AND, Japan) and then were dried at 60 °C for 24 hours and finally weighted.

**Ion analysis:** Ion determination of $K^+$ and $Ca^{2+}$ was done by the methods described by (Yu et al., 2001). The plants were harvested after 14 days of treatment. The dried root, stem and leaf samples were ground to pass a 30-mesh screen for chemical analysis. Then 50 mg of the particles were taken by weighing, 15 ml of distilled water was added, test tubes were put into boiling water for 90 min. After cooling of the abstraction solutions, 50 ml pure water was added to each tube. $K^+$ and $Ca^{2+}$ contents were analyzed with a 6400-A flame photometer (FPF 7, Jenway, UK).

**Statistical analysis:** The experimental data were statistically analyzed for variance using the SAS system (SAS Institute, Cary, NC, USA). When analysis of variance showed significant treatments effects, a Fisher’s Least Significant Difference (LSD) test was applied to compare the means at the 0.05 level of probability.

**Results and Discussion**

**Shoot and root length and seedling dry weight:** In barley cultivars, drought stress (induced by 0.8 MPa PEG) decreased root length compared to the control, while silicon priming at 1 and 2 mM improved root length (Table 1). Under 0.8 MPa PEG, the highest root length was observed in Nimrooz barley cultivar (7.86 mm) at 2 mM silicon. Contrary to Nimrooz, the root length of Rihane and Khatam cultivars showed no significant difference between 1 and 2 mM silicon priming, under 0.8 MPa PEG. Likewise, shoot length was affected by PEG especially in Khatam and Rihane cultivars (Table 1). Interestingly, in Nimrooz, under 0.8 MPa PEG, shoot length from 5.39 mm in control increased to 5.91 and 6.01 mm at 1 and 2 mM silicon, respectively. In Nimrooz, seedling dry weight increased from 0.19 mg plant$^{-1}$ in control to 0.27 mg plant$^{-1}$ in 2 mM silicon (29.6% increase), while in Khatam and Rihane, seedling dry weight was not affected by silicon under 0.8 MPa PEG (Table 1). In drought-stressed sorghum (Sorghum bicolor), Hattori et al. (2005) observed a significant higher root and dry mass accumulation in silicon-applied plants compared to the control, indicating that silicon facilitates root growth during drought. Ahmed et al. (2011b) suggested that silicon application is mainly beneficial to the growth of sorghum root, allocating more matter to the plant root system grown hydroponically. Also, Kaya et al. (2006) reported that use of silicon improves growth and biological yield of maize (Zea mays L.) under water stress conditions. Habibi et al., (2013) reported that silicon supplementation (2.73 mM kg$^{-1}$ soil) in pistachio (Pistacia vera L. ‘Ahmadaghai’) plants under field conditions significantly increased plant dry weight and relative water content under drought stress. Al-Mayahi (2016) showed that drought stress caused significant decreases in response percentage of root induction and the lengths of the roots of date palm (Phoenix dactyliflora) plantlets cv. Barhee while inclusion of 0.8 MPa silicon to the PEG containing medium significantly increased the response percentage of root induction and root lengths compared with media containing PEG only. Similar to our results especially in Nimrooz barley cultivars, Epstein and Bloom (2005) reported that silicon does not appear to be beneficial to plants until some stress is imposed.

**Root surface area and hydraulic conductivity:** In all barley cultivars, 0.8 MPa PEG decreased root surface area of seminal root significantly, while decreasing rate in Khatam and Rihane was pronounced more than Nimrooz (Table 2). On the other hand, silicon priming could improve root surface area especially in Nimrooz, so that from $1.61 \times 10^{-3}$ m$^2$ in control increased to 1.92 and $1.97 \times 10^{-3}$ m$^2$ in 1 and 2 mM silicon, respectively. When barley seedling were exposed to 0.8 MPa PEG, silicon priming increased hydraulic conductivity of seminal root especially in Nimrooz, so that in 2 mM silicon increased 4.1% compared to the control (Table 2). Overall, under 0.8 MPa PEG and 2 mM silicon, Nimrooz with highest root surface area had the highest hydraulic conductivity ($6.29 \times 10^{-9}$ m s$^{-1}$ Pa$^{-1}$) while in Rihane and Khatam was low as 5.38 and $5.19 \times 10^{-9}$ m s$^{-1}$ Pa$^{-1}$. On the other hand, Zhu and Gong (2014) declared that nutrient uptake is related to root surface area and length. An increase in surface area provides more exposed sites for uptake of diffusible ions. Silicon-mediated enhancement of root growth may therefore stimulate nutrient absorption and enhance drought tolerance. Sonobe et al., (2011) declared that although silicon did not stimulate root growth under drought, silicon application in fact increased water uptake, thereby contributing to stimulation of nutrient uptake. The increased water uptake upon silicon addition under drought is due to improved hydraulic conductance of roots (Hattori et al., 2008) and root growth (Chen et al., 2011). Likewise, in sorghum, for example, silicon increased root and whole-plant hydraulic conductance, under osmotic stress (Liu et al., 2014 and 2015). Our findings are in agreement with Hattori et al. (2005) who reported that the stimulative effect of silicon on root growth may be due to enhanced root elongation and surface area and hydraulic conductivity as a consequence of enhanced cell wall extensibility in the growth zone, as observed in sorghum.

**Anatomical structure of xylem in seminal root:**

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When barley seedlings were 14 days old (the developmental stage at which they were analyzed), they had two to three seminal roots. In all of the cultivars, seminal roots had one large central metaxylem vessels typically, and a number of smaller and circularly arranged peripheral metaxylem vessels ranged from 7±2 to 9±1 under control and drought stress conditions (Table 3 and Fig.1). Overall, in both central and peripheral metaxylem number of vessels was not affected by silicon priming and drought stress induced by 0.8 MPa PEG (Table 3 and Fig.1). At 0.8 MPa PEG, the diameter of central metaxylem was more responsive to 1 and 2 mM silicon than control especially in Nimrooz (Table 3 and Fig.1). At 14 days old seedling in seminal roots the mean diameter of the central metaxylem ranged from 25.4 to 37.1 μm by silicon and PEG interaction (Table 3). Mean diameter of peripheralmetaxylem vessels was affected by 0.8 MPa PEG in Khatam and Rihane negatively, more than Nimrooz cultivar (Table 3 and Fig. 1). Generally, in Nimrooz, positive relationship was observed between application of 2 mM silicon and increasing the mean diameter of peripheral metaxylem vessels under drought stress. In agreement with our results, Bijanzadeh and
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Table 3. Effect of silicon (Si) and drought stress (PEG) on number and diameter of mature xylem vessels of seminal root at 14 days old of barley cultivars. Number of vessels are means ±SD.

<table>
<thead>
<tr>
<th>Barley cultivar</th>
<th>PEG (MPa)</th>
<th>Si (Mm)</th>
<th>Central metaxylem</th>
<th>Peripheral metaxylem</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Number of vessels</td>
<td>Mean diameter of vessels (µm)</td>
</tr>
<tr>
<td>Khatam</td>
<td>0</td>
<td>0</td>
<td>1±0</td>
<td>35.6</td>
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<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>1±0</td>
<td>35.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>1±0</td>
<td>36.7</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>0</td>
<td>1±0</td>
<td>25.4</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>1±0</td>
<td>26.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>1±0</td>
<td>26.3</td>
</tr>
<tr>
<td>Rihane</td>
<td>0</td>
<td>0</td>
<td>1±0</td>
<td>31.6</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>1±0</td>
<td>31.7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>1±0</td>
<td>32.1</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>0</td>
<td>1±0</td>
<td>26.5</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>1±0</td>
<td>26.8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>1±0</td>
<td>27.3</td>
</tr>
<tr>
<td>Nimrooz</td>
<td>0</td>
<td>0</td>
<td>1±0</td>
<td>36.1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>1±0</td>
<td>36.8</td>
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<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>1±0</td>
<td>37.1</td>
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<tr>
<td></td>
<td>0.8</td>
<td>0</td>
<td>1±0</td>
<td>30.7</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>1±0</td>
<td>33.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>1±0</td>
<td>34.3</td>
</tr>
</tbody>
</table>

LSD (0.05) ns: 1.8 ns: 0.63

Figure 1. Effect of silicon priming on anatomical structure of the xylem in the seminal root of three barley cultivars of the 14 days old seedlings under drought stress. Hand cross-sections of seminal roots taken at 5–10 mm from seminal root tip and sections were stained for 1–3 minutes with 0.5% toluidine blue and counterstained with distilled water and viewed under fluorescence light (390–420 nm) to visualize xylem structure. CMX: central metaxylem; PMX: peripheral metaxylem. Scale bar is 50 μm.

Emam (2015) reported that in all barely cultivars, salt stress affected the diameter of central and peripheral metaxylem vessels more negatively in comparison to the number of the vessels and the diameter of central metaxylem was more than peripheral metaxylem in seminal roots. Also, Bijanzadeh (2017) declared that central metaxylem of barley cultivars could be classified as immature xylem vessels and had more diameter, compared to the peripheral metaxylem.

Ion accumulation: In all of the silicon and PEG
levels, K⁺ accumulation in Nimrooz was more than the other cultivars (Fig. 2). On the other hand, in all barley cultivars, under 0.8 MPa PEG, K⁺ increased from 0 mM silicon to 2 mM especially in Nimrooz, so that from 193 mg g⁻¹ dry weight reached to 215 mg g⁻¹ dry weight. With increasing the silicon level, the highest Ca²⁺ was observed in 2 mM silicon in both 0 and 3.6mM PEG (Fig. 3). Likewise, Ca²⁺ accumulation in Nimrooz was significantly more than Khatam and Rihane, (Fig. 3). Overall, 0.8 MPa PEG, in all cultivars decreased K⁺ and Ca²⁺ accumulation compared to the control (Fig. 2 and 3). Pei et al. (2010) reported that silicon decreased the Ca²⁺, K⁺, and Mg²⁺ concentrations in wheat shoots under water-deficit stress induced by PEG; when the improvement of shoot dry matter by silicon was taken into consideration, however, the total content of each of these minerals in shoots actually increased. Similar to our results, especially in Nimrooz barley cultivar, Kaya et al. (2006) has observed that water stress reduced leaf calcium (Ca²⁺) and potassium (K⁺) of maize plants, while silicon addition increased Ca²⁺ and K⁺ levels in water-stressed maize leaves.

**Conclusion:**
This study suggested that silicon priming of barley seeds may improve seedling growth under drought stress by increasing root hydraulic conductivity and ion accumulation including K⁺ and Ca²⁺. Overall, silicon priming at 2 mM as silicate sodium in some suitable barley cultivars such as Nimrooz appears to be a promising and cost-effective procedure to confer resistance to major stresses such as drought. Further investigations will be needed to determine how silicon regulates water uptake by roots and affects root anatomical characteristics to better understand the mechanisms of silicon promoted plant growth.
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