South African Journal of Botany 98 (2015) 26–36

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Effects of silicon (Si) on growth, quality and ionic homeostasis of aloe under salt stress

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ARTICLE INFO

Article history:
Received 6 June 2014
Received in revised form 7 December 2014
Accepted 21 January 2015
Available online 18 February 2015

Edited by M Vaculik

Keywords:
Aloe
Silicon (Si)
Salt stress
X-ray energy spectrum analysis
Membrane vesicles
Proton pump

ABSTRACT

The physiological characteristics of aloe irrigated with brackish water and the relationship with ameliorative effects of silicon (Si) nutrition were studied. Results of cultivation and analysis showed that 2.0 mmol · L^{-1} added Si significantly alleviated growth inhibition and quality reduction of aloe under 100 mmol · L^{-1} NaCl stress; added Si significantly reduced Na^+ content while increased K^+ content and K^+/Na^+ ratio of salt stressed aloe plant, especially absorption and translocation selectivity of aloe root to K^+ and Na^+ were promoted, and ion homeostasis of aloe plant was kept well. Results of cross-sectional X-ray energy dispersive spectrum microanalysis of aloe root and leaf further confirmed the above findings. One of the mechanisms of silicon was its improvement to activity of plasma membrane H^+-ATPase, tonoplast membrane H^+-ATPase and H^+-pyrophosphates of aloe root under salt stress.

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1. Introduction

Silicon (Si) is the second most abundant element in soils. In spite of the prominence of Si as a mineral constituent of plants, it is not counted among the elements defined as 'essential', or nutrients, for any terrestrial higher plants except members of the Equisitaceae (Epstein, 1999). In recent years, the physiological function of Si and its relationship with plant resistance has been drawing attention (Currie and Perry, 2007; Epstein, 2009). Salt stress has been a major ecological factor affecting growth and yield of crop plants in coast area, but coast saline soil is also important land resources. Literatures as to exploit saline soil showed that added Silicon alleviates salt injuries of crop plants, such as rice (*Oryza sativa* L.), barley (*Hordeum vulgare* L.), cowpea (*Vigna unguiculata* L.) and several others (Liang et al., 2005; Manju and Aery, 2009; Shi et al., 2013). However, the effects of silicon on economic quality and ion homeostasis of plants under salt stress were rarely reported.

Aloe L. is a genus containing about four hundred species of flowering succulent plants (Viljoen and Wyk, 2001). *Aloe vera* L. (or *A. barbadensis* Miller) is the most famous species in *Aloe* L. The genus is native to Africa and is common in South Africa's Cape Province and the mountains of tropical Africa, and neighboring areas such as Madagascar, and the islands of Africa. Angiosperm Phylogeny Group II system (The APG II system) (2003) placed the genus in the family Asphodelaceae. In the past aloe was assigned to families Aloaceae and Liliaceae. Members of the closely allied genera *Gasteria*, *Haworthia* and *Kniphofia* which have a similar mode of growth, are also popularly known as aloes, while the plant sometimes called 'American aloe' (*Agave Americana* L.), belongs to Agavaceae, a different family. *Aloe* species are frequently cultivated as ornamental plants both in gardens and in pots (Feily and Namazi, 2009). Many *Aloe* species are highly decorative and are valued by collectors of succulents (Ma et al., 2010). Some species, especially *A. vera* L. are purported to have medicinal properties (Eriko et al., 2012; Woźnik and Paduch, 2012). Other uses of aloe include their role in alternative medicines and in home first aid (Yao et al., 2009; Dutta and Masakapalli, 2013). Both the translucent inner pulp as well as the resinous yellow exudates from the wounding aloe plant are used externally to relieve skin discomforts and internally as a laxative (Saini and Saini, 2011; He et al., 2013).

To date, some researches have shown that *A. vera* L. produces positive medicinal benefits for healing damaged skin. Some species of *Aloe* L. have also been used for human consumption. For example, drinks made from or containing chunks of aloe pulp are popular in Asia as commercial beverages and as a tea additive; this is notably true in Korea. Aloe is used externally to treat a number of skin irritations (Wang et al., 2013). It has antiseptic and antibiotic properties which make it highly valuable in treating cuts and abrasions. It has also been commonly used to treat first and second degree burns, as well as sunburns and

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http://dx.doi.org/10.1016/j.sajb.2015.01.008
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poison oak, poison ivy, and poison sumac infections, and eczema. It can also be used as a hair styling gel and works especially well for curly or fuzzy hair (Ma et al., 2010).

In the past several years, we studied the biological responses of aloe (*A.vera* L.) to irrigation water with different salinity and found that aloe not only has excellent drought resistance, also has certain degree of salt tolerance. Irrigating aloe with 50 mmol · L^{-1} NaCl for half a year didn't suppress its growth and development and the quality of leaf juice, but when the salinity of irrigation water reached 100 mmol · L^{-1} NaCl, the growth of aloe was significantly inhibited (Xu et al., 2006). In irrigation cultivation experiments, we also found that 2.0 mmol · L^{-1} added Si significantly alleviated growth inhibition of aloe under 100 mmol · L^{-1} NaCl stress, but the alleviative effects of 4.0 mmol · L^{-1} added silicon were not significant (Xu et al., 2007, 2008). In this article, we reported the effects of irrigating with 100 mmol · L^{-1} NaCl and 2.0 mmol · L^{-1} added Si on plant growth, important quality indexes and salt ion status of aloe and its enzyme kinetic mechanism so as to provide scientific bases for safe and efficient cultivation of aloe with brackish water irrigation.

2. Materials and methods

2.1. Experimental materials

Aloe (*Aloe vera* L.) maternal plant was introduced from China Kunming World Horticulture Exposition Garden and its young plantlets were acquired by substem propagation in our Plant Cell and Tissue Culture Laboratory.

2.2. Experimental methods

2.2.1. Material cultivation and treatments

The plantlets of aloe propagated in laboratory were transplanted into perlite medium for acclimation. After survival, the three-leaf-old aloe plants with the same size were selected and transplanted into plastic pots in greenhouse. The pots, with four holes at bottom, were 26 cm high and 22 cm diameter of the upper opening. The cultivation substrate was quartz sand, and the aloe plants were irrigated with Hoagland' solution. When the aloe grew to seven leaves (in the Mid-August), further selected the same-sized plantlets and treated with salt and/or silicon.

The source of salt was sodium chloride (NaCl). The source of silicon was potassium silicate ($K_2SiO_3 \cdot nH_2O$). Both NaCl and $K_2SiO_3 \cdot nH_2O$ were chemical pure. The molar mass of potassium silicate was in anhydride, i.e. chemically combined water in its molecular was not included in calculation. Based on our former experimental results, we designed four treatments in this study: (a) NaCl-Si- (Control), irrigated with Hoagland' solution; (b) NaCl-Si+, irrigated with Hoagland' solution containing 2.0 mmol · L^{-1} added Si; (c) NaCl + Si-, irrigated with Hoagland' solution containing 100 mmol · L^{-1} NaCl; (d) NaCl + Si+, irrigated with Hoagland' solution containing 100 mmol · L^{-1} NaCl and 2.0 mmol · L^{-1} added Si. Additional K^+ introduced by potassium silicate was subtracted from potassium nitrate (KNO_3) used for preparing Hoagland' solution and the resultant nitrate loss was supplemented with nitric acid (HNO_3) (65%). Each treatment had three replicates. Nutrient solution was prepared with distilled water. The pH value of the solution was adjusted to 6.4 with dilute (10.0 mmol · L^{-1}) sulfuric acid (H_2SO_4) or sodium hydroxide (NaOH). There is a table describing the formulations of four irrigation solutions in aloe cultivation experiment (Table 1). The basic irrigation solution was Hoagland' solution and its formulation was cited from Plant Physiology edited by Pandey and Sinha (1996), in which the microelement formulation was Arnon' nutrient solution. The concentration unit of each component in the Hoagland' solution was uniformly expressed as mg · L^{-1}; concentrations (mmol · L^{-1}) of the thirteen elements, i.e. N, P, K, Ca, Mg, S, Fe, Zn, Mn, Cu, B, Mo and Cl were 15.0, 1.0, 6.0, 5.0, 2.0, 2.0, 0.02, 7.6×10^{-4}, 0.01, 3.2×10^{-4}, 0.05, 1.1×10^{-4} and 0.02, respectively.

Table 1
Formulation of irrigation solution in aloe cultivation experiment.

Compound and molar mass (g · mol^{-1})	Treatment and recipe of irrigation solution (mg · L^{-1})*			
	NaCl-Si-	NaCl-Si+	NaCl + Si-	NaCl + Si+
KNO_3 (101.10)	510.00	112.86	510.00	112.86
$Ca(NO_3)_2 \cdot 4H_2O$(236.15)	1180.00	1180.00	1180.00	1180.00
KH_2PO_4(136.09)	136.00	136.00	136.00	136.00
$MgSO_4 \cdot 7H_2O$(246.47)	490.00	490.00	490.00	490.00
H_3BO_3(61.83)	2.86	2.86	2.86	2.86
$MnCl_2 \cdot 4H_2O$(197.91)	1.81	1.81	1.81	1.81
$ZnSO_4 \cdot 7H_2O$(287.54)	0.22	0.22	0.22	0.22
$CuSO_4 \cdot 5H_2O$(249.68)	0.08	0.08	0.08	0.08
$H_2MoO_4 \cdot H_2O$(179.97)	0.02	0.02	0.02	0.02
$FeC_4H_4O_6$(203.92)	5.00	5.00	5.00	5.00
NaCl (58.44)	-	-	5844.00	5844.00
$K_2SiO_3 \cdot nH_2O$(154.29 g · mol^{-1}) (in anhydride)**	-	400.86	-	400.86
HNO_3(63.01) (65%)***	-	381.26 (272 μl)	-	381.26 (272 μl)

*: The basic irrigation solution was Hoagland' solution. Its formulation was cited from Plant Physiology edited by Pandey and Sinha (1996), in which the microelement formulation was Arnon' nutrient solution. The concentration unit of each component was uniformly expressed as mg · L^{-1}. Additional K^+ introduced by potassium silicate was subtracted from potassium nitrate (KNO_3) used for preparing Hoagland' solution and the resultant nitrate loss was supplemented with nitric acid (HNO_3). The pH value of each irrigation solution was adjusted to 6.4 with dilute sulfuric acid (H_2SO_4) or sodium hydroxide (NaOH).
**: K_2O content of $K_2SiO_3 \cdot nH_2O$ was 47.0%; the ratio of K_2O/SiO_2 was 1.57; K and Si contents were 39.00% and 13.97%, respectively. Si concentration of added Si treatment was 2.0 mmol · L^{-1}.
***: The density of 65% HNO_3 was 1.4 mg · μl^{-1}.

Methods of Si addition and solution pH adjustment were based on the methods described by Liang et al. (2006) and Zhu et al. (2004). The addition of silicon to the culture solution did not result in any precipitation or floccules.

The cultivation was conducted under natural light condition in greenhouse. The irrigation quota of the solution was 400 ml per pot each time, and filter papers were covered on the surface of quartz sand in order to reduce moisture evaporation. Irrigation interval was 3 or 4 d according to the weather condition and rinsed quartz sand once every twice of irrigation, and kept night temperature of the greenhouse above 18°C. The first round of cultivation experiment finished in the Mid-September, i.e. the treatment time was 30 d; each treatment consisted of fifteen plantlets of aloe and three replicates, totally 180 aloe plantlets including control; they were for the determination of K^+, Na^+ and Cl^- contents, calculation of K^+/Na^+ selectivity (SK, Na) in root, stem and leaf, and X-ray microanalysis of mineral elements.

The second round of cultivation experiment finished in the Mid-November of the same year, the total treatment time was 120 d. Each treatment also consisted of fifteen plantlets of aloe, three replicates, totally 180 aloe plantlets including control. These aloes were for measurement and analysis of growth status, rate of leaf juice and its physicochemical property, preparation of cytoplasma membrane and tonoplast vesicles and assay of H^+-ATPase and H^+-PPase activities of aloe root tips as well.

2.2.2. Growth status measurement

When the sand culture finished, the leaf length, width, thickness and fresh weight per leaf, and the fresh weights of root, stem and leaf per plant were immediately measured. The fresh weight per leaf was the average weight of all leaves which were more than 50 mm long, and the leaf fresh weight per plant included all of the ground shoots. The fresh samples of root, stem and leaf per plant were fixed for 15 min at 115°C to deactivate enzyme activity and then dried to constant weight at 75°C, respectively. Fresh weight of the whole plant was sum of the

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