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Saline soil desalination by honeysuckle (*Lonicera japonica* Thunb.) depends on salt resistance mechanism

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ABSTRACT

Honeysuckle (*Lonicera japonica* Thunb.) is a traditional medicinal plant in China. This study aimed to investigate the relation between salt tolerance mechanism in honeysuckle and phytodesalination of saline soil through field trial. In April, 2014, honeysuckle plants were transplanted to non-saline and moderate saline plots. Six months later, Na^+ concentration, Na^+ adsorption ratio and electrical conductance in tilth soil were significantly lowered by honeysuckle in moderate saline plots, suggesting that saline soil was desalinized. Due to the inhibition on plant growth, the estimated phytodesalination capacity by shoot Na^+ accumulation was only 8.71 kg Na^+ ha^{-1}, which seemed very limited compared with succulent halophytes. Therefore, soil desalination by honeysuckle should depend on Na^+ leaching rather than shoot Na^+ accumulation. Respiration rate and Na^+ extrusion in roots were elevated by salinity, and they were significantly and positively correlated, indicating the importance of root respiration for resisting Na^+ uptake. The elevated root respiration might aid in dissolving calcite by releasing more CO_2 into soil, and consistently, Ca^{2+} concentration in tilth soil was remarkably increased by honeysuckle in moderate saline plots in contrast to no significant change in non-saline plots. As a result, Na^+ leaching could be facilitated, as Na^+ at the exchange site would be efficiently replaced by Ca^{2+}. In conclusion, salt-induced elevation of root respiration enhanced salt resistance of honeysuckle by increasing Na^+ extrusion and could assist in desalinizing saline soil by improving Na^+ leaching.

1. Introduction

There is large area of abandoned saline land in coastal zone in the world, and a promising choice is to develop saline agriculture by using salt tolerant economic crops in these regions (Rozema and Flowers, 2008; Panta et al., 2014; Qin et al., 2015). Salinity inhibits plant growth and reduces crop yield. However, saline soil can be rehabilitated by plants with the decrease of soil salinity and Na^+ concentration, and this plant-based method is called phytoremediation (Qadir et al., 2007). In contrast to applying chemical reagents, phytoremediation is low-cost and environmentally friendly for desalinizing saline soil (Qadir and Oster, 2004).

Na^+ can interfere with metabolism in plant cell and is the primary toxic component for plant growth (Zhu, 2003; Munns and Tester, 2008). High soil Na^+ concentration is considered as the principle limiting factor for plant growth in coastal land, and accordingly, phytoremediation mainly aims to reduce Na^+ concentration in saline soil (Qadir et al., 2007). There are two major phytoremediation mechanisms including shoot Na^+ accumulation and improving Na^+ leaching to deeper soil. In regions with poor drainage and low precipitation, shoot Na^+ accumulation seems more important. Compared with glycophytes, succulent halophytes can efficiently sequester Na^+ into vacuole for protecting cytoplasm against ionic toxicity (Flowers and Colmer, 2008; Lv et al., 2012), and the accumulated Na^+ serves as a cheaper osmolyte than organic compounds to resist salt-induced osmotic stress (Munns and Tester, 2008). Consequently, succulent halophytes can accumulate a large amount of Na^+ in the shoot and are optimal materials for soil remediation in these regions (Zhao, 1991; Ravindran et al., 2007; Rabhi et al., 2009). Noticeably, Jlassi et al. (2013) estimated that *Sulla carnosa*, an indifferent halophyte, could desalinize moderate saline soil by accumulating considerable Na^+ in the shoot, because Na^+ concentration was diluted to prevent toxicity on shoot tissues by virtue of high leaf expansion rate. Thereby,

Abbreviations: EC, electrical conductivity; NMT, non-invasive micro-test technique; SAR, sodium adsorption ratio; TF, translocation factor.

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the phytoremediation approach through shoot Na^+ accumulation depends on special salt tolerant mechanisms in plants.

In contrast to non-leaching condition, Qadir et al. (2003) reported that Na^+ removal by shoot harvest of alfalfa contributed to only 1–2% of the total removed Na^+ in saline soil under irrigated condition, and root activity favored Na^+ leaching to deeper soil. Root respiration increases partial pressure of CO_2, assists in dissolving calcite, provides a source of Ca^{2+} to replace Na^+ at the exchange site of soil, and then facilitates Na^+ leaching (Qadir et al., 2005). Similarly, Na^+ removal through leaching was mainly responsible for total soil sodium removal in spite of the great shoot Na^+ accumulation in a halophyte, *Atriplex halimus* (Gharaibeh et al., 2011). Even for the succulent halophyte, *Suaeda fruticosa*, Na^+ leaching enhancement resulting from root effect was inferred to help remediate saline natural biotope (Rabhi et al., 2010). Thus, root respiration may play an important role in phytoremediation of saline soil under leaching condition. However, the role of root respiration in relation between salt tolerance and Na^+ removal from the soil is still unclear.

Honeysuckle (*Lonicera japonica* Thunb.) is a traditional medicinal plant and native to East Asia. Its flower is rich in two bioactive phenolic constituents, chlorogenic acid and galuteolin, and has been used as Chinese medicine for a long time (Chen et al., 2005). Honeysuckle is an ideal material to exploit coastal saline land due to its strong environmental adaptability and high economic value. Salinity can stimulate the synthesis of phenolic biosynthesis (Ksouri et al., 2007; Lim et al., 2012; Petridis et al., 2012; Colla et al., 2013; Borgognone et al., 2014), and the bioactive constituents of honeysuckle belong to phenolic compounds. Thus, there is a potential advantage that salinity may enhance medicinal quality of honeysuckle. In a recent study, we identified a salt tolerant honeysuckle cultivar by using hydroponic experiment and revealed that its tolerance originated from the control of Na^+ uptake and internal Na^+ transport (Yan et al., 2015a). In this study, we aimed to investigate phytoremediation effect of honeysuckle on moderate saline soil and explore the role of root respiration in the relation between salt tolerance and phytoremediation through field trial. Accordingly, honeysuckle plants were grown in non-saline and moderate saline plots for investigating the effects of salinity on root respiration, root Na^+ efflux, plant growth and shoot Na^+ accumulation. In addition, salinity and Na^+ concentration were contrasted between soil samples from plant inside (distributed with roots) and outside (distributed without roots) in non-saline and moderate saline plots. Our study can deepen the knowledge about phytoremediation of saline soil and may provide a reference for developing saline agriculture in coastal zone.

2. Materials and methods

2.1. Experimental site

The experiment site was established in Dongying Halophyte Arboretum, Dongying Academy of Agricultural Sciences, Shandong province, China (37°24′N, 118°39′E and 8.8 m above sea level). This area belongs to warm temperate continental monsoon climate. The annual average temperature and precipitation are 12.8 °C and 555.9 mm in this site.

2.2. Field trial design

Bare-rooted honeysuckle plants (two years old, Jiufengyihao cultivar) were bought from Jiujianpeng Agricultural Technology Limited Company (Pingyi, Shangdong, China) and planted in non-saline area in the arboretum in November, 2013. Four replicate plots were, respectively, constructed in non-saline and moderate saline areas, and the plot size was 3 m × 4 m. The initial soil nutrients, salinity and pH are listed in Table 1. To avoid border effects, plots were separated with ridges of 0.6 m wide and 0.3 m high, and 0.5 m around the plots was left as isolation belt. The plots were ploughed up and applied with compound fertilization at 750 kg ha^{-1}. In April, 2014, forty five plants were transplanted to each plot, and plant and row spacing were 0.5 m and 0.75 m. Plant inside and outside, respectively, indicate the sites which are less than 0.15 m and at least 0.35 m distance from the plants. Roots were mainly distributed in plant inside and hardly reached the outside. Thus, root metabolism could affect soil properties in plant inside rather than the outside, and soil from plant outside was sampled as control to reflect the effects of planting honeysuckle on soil salinity.

Table 1
Soil nutrients, salinity and pH in non-saline and moderate saline areas. Data in the table indicate the mean of five replicate samples (±SD). Within each row, different letters indicate significant difference at $P < 0.05$.

Parameters	Non-saline area	Moderate saline area
Organic matter content (mg g^{-1})	27.72 ± 0.51a	27.40 ± 1.46a
Total nitrogen content (mg g^{-1})	1.12 ± 0.11a	1.14 ± 0.11a
Available phosphorus content (mg g^{-1})	9.41 ± 1.21a	11.56 ± 2.45a
Available potassium content (mg g^{-1})	0.35 ± 0.11a	0.31 ± 0.09a
Na^+ content (mg g^{-1})	0.31 ± 0.05a	0.61 ± 0.20b
Electrical conductance (μs cm^{-1})	486 ± 29a	910 ± 119b
Sodium adsorption ratio	9.51 ± 0.92a	16.43 ± 2.12b
Soil pH	7.65 ± 0.31a	7.81 ± 0.27a

2.3. Soil sampling and analysis

In October, 2014, composite soil samples from 0–20 and 20–40 cm depth were collected from three randomly selected sites from plant inside and outside in each plot. Honeysuckle roots were mainly distributed in tilth soil with depth of 0–20 cm. Soil samples were ground to pass through 1 mm sieve, extracted with deionized water at the ratio of 1:5 (w/v) and then filtrated. The filtrate was collected for measuring Na^+, K^+, Ca^{2+} and Mg^{2+} content by using an atomic absorption spectrophotometer (TAS-990, China). Electrical conductivity (EC) was detected by using a conductivity meter (DDSJ-308A, China). Sodium adsorption ratio (SAR) is a classic parameter to indicate soil sodicity and calculated as: $SAR = Na^+/[(Ca^{2+} + Mg^{2+})/2]^{1/2}$, and the unit of ions concentration in this equation is mM (Murtaza et al., 2009). Before honeysuckle plants were transplanted to the plots, soil was sampled for analyzing the initial nutrients. Soil organic matter, total nitrogen, available phosphorous and available potassium contents were measured, respectively, by using $K_2Cr_2O_7$ external heating, Kjeldahl digestion, Mo-Sb colorimetric, and flame photometric methods (Jiao et al., 2013).

2.4. Plant sampling and analysis

In June and October, 2014, plants were harvested, rinsed with deionized water and wiped with tissues. Roots, leaves and veins were separated, dried at 105 °C for 10 min, and then dried at 70 °C to constant weight. The extraction of Na^+ was performed according to Song et al. (2011). Deionized H_2O (25 ml) was added to 0.1 g dried plant powder and boiled for 2 h. The supernatant was diluted 50 times with deionized H_2O for measuring Na^+ content by using an atomic absorption spectrophotometer (TAS-990, China). Na^+ translocation factors (TF) indicate Na^+ transport from root to the aerial part. TF1 and TF2 were calculated as: TF1 = Vine Na^+ concentration/Root Na^+ concentration and TF2 = Leaf Na^+ concentration/Root Na^+ concentration. Shoot Na^+ concentration was calculated as vine dry weight × Na^+ concentration of vine + leaf dry weight × Na^+ concentration of leaf. On the basis of plot area, shoot