Journal of Integrative Agriculture 2017, 16(8): 1800–1807

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RESEARCH ARTICLE

Effects of neutral salt and alkali on ion distributions in the roots, shoots, and leaves of two alfalfa cultivars with differing degrees of salt tolerance

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Abstract

The effects of neutral salt and alkali on the ion distribution were investigated in two alfalfa (*Medicago sativa* L.) cultivars, including Zhongmu 1, a high salt-tolerant cultivar, and Algonquin, a low salt-tolerant cultivar. The alkali stress expressed more serious growth inhibition than the neutral salt stress at the same Na^+ concentration. Compared with Algonquin, Zhongmu 1 did not exhibit a higher alkali tolerance under the Na_2CO_3-$NaHCO_3$ treatment with the low Na^+ concentration (50 mmol L^{-1}). The alkali increased the accumulation of Na^+, Ca^{2+}, and Mg^{2+} in the root and changed the Ca^{2+} and Mg^{2+} balance in the entire alfalfa plant. The salt and alkali stresses decreased the K^+ and Fe^{3+} contents of the roots and leaves, the root Mn^{2+} content, and the shoot Zn^{2+} content, but they increased the Fe^{3+} accumulation of the shoots, the shoot and leaf Cu^{2+} contents, and the leaf Zn^{2+} content in both alfalfa cultivars. Based on the results obtained under the conditions of this experiment, we found that the salt and alkali stresses reduced the plant growth in both alfalfa cultivars, while the alkali caused a stronger stress than the neutral salt in alfalfa. Thus, we conclude that under hydroponic conditions, the deleterious effects of the alkali on plants are due to the distribution change of some trophic ion balance in the roots, shoots, and leaves of the plants by causing of Na^+, CO_3^{2-}, and/or HCO_3^- stresses.

Keywords: alfalfa, ion distribution, neutral salts, alkali, stress

1. Introduction

Soil salinization is a worldwide problem that significantly limits food production. Approximately 20% of the irrigated agricultural land in the world is adversely affected by salinity (Viswanathan *et al.* 2005). Salinization and alkalinization, which frequently co-occur in the soil, are two major environmental factors that limit plant growth and crop productivity, and the conditions in natural salt-alkaline soil are very complex (Shi and Wang 2005). In China,

Received 6 June, 2016 Accepted 7 November, 2016
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doi: 10.1016/S2095-3119(16)61522-8

the land area of salt-alkaline soils is 99 million hectares (Tang *et al.* 2009). Saline soil (mainly containing NaCl and Na_2SO_4) and alkaline soil (mainly containing $NaHCO_3$ and Na_2CO_3) are two typical types of salt-alkaline soils in China. Therefore, the problem of the salt-alkali mixed stress should be recognized and investigated as thoroughly as that of neutral salt stress. However, the mixed stress caused by salt-alkaline soil is not well studied and reported.

Salt stress is caused by neutral salts (NaCl and Na_2SO_4), whereas alkali stress is caused by alkaline salts ($NaHCO_3$ and Na_2CO_3) (Shi and Yin 1993; Yang *et al.* 2007). In a saline soil, CO_3^{2-} and/or HCO_3^- adversely affect plants not only *via* salt stress but also *via* alkali stress (Shi and Sheng 2005; Li *et al.* 2010a, b). The reduction in the growth response to salinity is usually attributed to either ion cytotoxicity and/or a low external osmotic potential (Munns and Termaat 1986). Ion cytotoxicity occurs when K^+ is replaced by Na^+ in biochemical reactions and conformational changes, and the functions of proteins are lost when Na^+ and Cl^- penetrate into the hydration shells and interfere with the noncovalent interaction between amino acids (Zhu 2002). Salinity also affects the nutrient balance in plant tissues (Pessarakli *et al.* 1991). An increased salt treatment causes Na^+ and Cl^- contents to increase, and K^+, Ca^{2+}, and Mg^{2+} contents to decrease in some plants (Khan *et al.* 1999, 2000; Khan 2001; John *et al.* 2003). In contrast, recent investigations in alkali-resistant halophytes, *Suaeda glauca* and *Kochia sieversiana*, have shown that Na^+, K^+, Ca^{2+}, and Mg^{2+} contents in the shoots increased with an increasing salinity under both salt and alkali stresses (Yang *et al.* 2007, 2008b, 2009). However, the knowledge is still limited on how neutral and alkaline salts affect the micronutrient content and composition of plants. Hu and Schmidhalter (2001) declared that micronutrients are generally less affected by salt stress than macronutrients. However, salinity increased the Zn^{2+} contents in the roots and leaves of pepper (Cornillon and Palloix 1997) and the Zn^{2+}, Cu^{2+}, and Mn^{2+} contents in wheat and rice (Alpaslan *et al.* 1998). The concentration of Fe^{3+}, Mn^{2+}, and Zn^{2+} in the leaves of zucchini increased with increasing salinity; however, Cu^{2+} decreased (Villora *et al.* 2000). In addition, Sanchez-Raya and Delgado (1996) reported that the salinity reduced the Fe^{3+} and Mn^{2+} contents in sunflower seedlings.

Alfalfa is one of the most important forage crops with high protein and highly digestible fiber contents. Although alfalfa can be grown under moderate saline-alkaline conditions, alfalfa plants are sensitive to high salt conditions. Salt stress imposed by 50–200 mmol L^{-1} NaCl significantly limits productivity and growth level of alfalfa (Li and Brummer 2012). To explore the molecular mechanism of salt tolerance in alfalfa, transgenic alfalfa plants have been developed by over-expressing foreign genes encoding compatible solutes (Li *et al.* 2014), ion transporters (Zhang *et al.* 2014), protein kinase (Wang *et al.* 2014) and transcription factors (Jin *et al.* 2010; Tang *et al.* 2013, 2014) in recent years. An *et al.* (2016) conducted transcriptional profiling of the whole seedling alfalfa treated with saline-alkaline solution to assess changes in gene expressions. Differentially expressed gene (DEG) profiles as responses to stress were presented, and DEG response to saline-alkaline stress was annotated. DEG-specific responses to saline-alkaline conditions were identified through comparison with the saline stress response pathway, and specific mechanisms of saline-alkaline stress tolerance were elucidated. Salt effects on the physiology of alfalfa have been well documented (Wang and Han 2007, 2009; Steppuhn *et al.* 2012; Palma *et al.* 2013; Quan *et al.* 2016). The biomass, root activity, and proline accumulation of alfalfa under mixed salt-alkali stress have been reported (Peng *et al.* 2008). To understand the entire plant response, further knowledge of ion accumulations under complex salt and alkali stresses is still required.

In this study, exogenous neutral salt (NaCl and Na_2SO_4) and alkali ($NaHCO_3$ and Na_2CO_3) were applied to investigate the ion distribution, plant growth, and Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Mn^{2+}, Cu^{2+}, and Zn^{2+} contents in the roots, shoots, and leaves of two alfalfa (*Medicago sativa* L.) cultivars, including a high salt-tolerant cultivar, Zhongmu 1, and a low salt-tolerant cultivar, Algonquin. The main objectives of this study were (1) to evaluate the effects of salt and alkali stresses on the growth of both alfalfa cultivars, (2) to investigate the effects of salt and alkali stresses on the distribution of the main macronutrient and micronutrient ions in the various parts of the alfalfa plant, and (3) to test the tolerance of the two alfalfa cultivars to salt and alkali stresses.

2. Materials and methods

2.1. Plant culture

Two alfalfa cultivars, Zhongmu 1 (high salt tolerance) and Algonquin (low salt tolerance), were used in this study. The seeds were sterilized with 6% sodium hypochlorite solution for 5 min. Following the germination in a sand medium at 25/20°C for 8 h/16 h (L:D) in a dark room, four seedlings were placed into the holes of a quadrate foam and transplanted into plastic vessels (13 cm high, 28 cm wide, 35 cm long). The vessels were wrapped with aluminum foil to minimize irradiation-induced heating and to suppress algae growth. Each vessel contained a 4.4-L standard nutrient solution composed of 2.5 mmol L^{-1} $Ca(NO_3)_2$, 2.5 mmol L^{-1} KNO_3, 1 mmol L^{-1} $MgSO_4$, 0.5 mmol L^{-1} $(NH_4)H_2PO_4$, 2×10^{-4} mmol L^{-1} $CuSO_4$, 1×10^{-3} mmol L^{-1} $ZnSO_4$, 0.1 mmol L^{-1} EDTA·FeNa, 2×10^{-2} mmol L^{-1} H_3BO_3, 5×10^{-6} mmol L^{-1} $(NH)_4Mo_{27}O_4$, and 1×10^{-3} mmol L^{-1} $MnSO_4$. The plants grew in a growth chamber at 45% relative humidity, alternating day and

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