Environmental Pollution 240 (2018) 557–565

Contents lists available at ScienceDirect

Environmental Pollution

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

Arbuscular mycorrhizal fungi alleviate boron toxicity in *Puccinellia tenuiflora* under the combined stresses of salt and drought☆

Chunguang Liu*, Zheng Dai, Mengying Cui, Wenkai Lu, Hongwen Sun

Key Laboratory of Pollution Processes and Environmental Criteria (Ministry of Education), Tianjin Key Laboratory of Environmental Remediation and Pollution Control, Nankai University, Tianjin, 300350, China

ARTICLE INFO

Article history:
Received 29 December 2017
Received in revised form
27 April 2018
Accepted 29 April 2018

Keywords:
Boron contamination
Bioconcentration factor
Phytoremediation
Funneliformis mosseae
Claroideoglomus etunicatum

ABSTRACT

To investigate the effect of arbuscular mycorrhizal fungi (AMF) on boron (B) toxicity in plants under the combined stresses of salt and drought, *Puccinellia tenuiflora* was grown in the soil with the inoculation of *Funneliformis mosseae* and *Claroideoglomus etunicatum*. After three weeks of treatment, the plants were harvested to determine mycorrhizal colonization rates, plant biomass, as well as tissue B, phosphorus, sodium, and potassium concentrations. The results show that the combined stresses reduced mycorrhizal colonization. Mycorrhizal inoculation significantly increased plant biomass while reduced shoot B concentrations. Mycorrhizal inoculation also slightly increased shoot phosphorus and potassium concentrations, and reduced shoot sodium concentrations. *F. mosseae* and *C. etunicatum* were able to alleviate the combined stresses of B, salt, and drought. The two fungal species and their combination showed no significant difference in the alleviation of B toxicity. It is inferred that AMF is able to alleviate B toxicity in *P. tenuiflora* by increasing biomass and reducing tissue B concentrations. The increase in plant phosphorus and potassium, as well as the decrease in sodium accumulation that induced by AMF, can help plant tolerate the combined stresses of salt and drought. Our findings suggest that *F. mosseae* and *C. etunicatum* are potential candidates for facilitating the phytoremediation of B-contaminated soils with salt and drought stress.

1. Introduction

Boron (B) is an essential nutrient for plants, but in high concentrations, it may be toxic (Miwa et al., 2007). Excess B may exert detrimental effects on plant growth by changing the processes of metabolism, root cell division, leaf chlorophyll synthesis, and photosynthesis (Camacho-Cristóbal et al., 2008). Boron toxicity is a worldwide problem that restricts plant growth in North America, Southern Australia, the Middle East, Western Asia, North Africa, Malaysia, and China (Nable et al., 1997; Yau et al., 1995; Wang et al., 2014). High levels of B (10–100 mg/kg) often occurs naturally in the soils where excess B accumulates in topsoil due to the evaporation of B-laden groundwater (Power and woods, 1997; Tanaka and Fujiwara, 2008). Usually, about 10% of the total soil B is available to plants, and more than 5 mg/L available boron can be toxic to many agronomic crops (Ozturk et al., 2010). Boron may also be added to the soil as a result of mining, fertilization, irrigation, and even atmospheric deposition (Parks and Edwards, 2005; Wang et al., 2017). Soils in arid and semiarid regions may exhibit excess B, which is often present along with other stresses such as salt and drought (Yermiyahu et al., 2008; Ben-Gal and Shani, 2003). These additional stresses may aggravate the toxicity of B in the plant, posing a challenge to the alleviation of B toxicity.

To alleviate B toxicity in plants, an effective approach is to apply chemical amendments (e.g., gypsum and sulfuric acid) (Moraga et al., 2014). Unfortunately, most chemical amendments are not only phytotoxic (Evangelou et al., 2007), but also toxic to microorganisms that are beneficial to plant growth (Mühlbachová, 2009). A promising alternative to chemical amendments could be microorganisms associated closely with plant root. An advantage of using microorganisms is that the microbial metabolites are biodegradable and potentially less toxic (Rajkumar et al., 2012). *Bacillus pumilus*, a rhizobacterium, has been observed to be effective in the alleviation of B toxicity in tomato (*Lycopersicum esculentum* L.) and

☆ This paper has been recommended for acceptance by Joerg Rinklebe.

* Corresponding author. College of Environmental Science and Engineering, Nankai University, 38 Tongyan Rd., Haihe Education Park, Jinnan District, Tianjin, 300350, China.
E-mail address: liuchunguang@nankai.edu.cn (C. Liu).

https://doi.org/10.1016/j.envpol.2018.04.138
0269-7491/

rice (*Oryza sativa* L.) (Sirajuddin et al., 2016; Khan et al., 2016). *Glomus clarum* (Nicolson & Schenck), an arbuscular mycorrhizal fungus (AMF), has been recorded to reduce B uptake in wheat (*Triticum durum*) in high B soil but have no effect on biomass (Sonmez et al., 2009). This is the only study, to our knowledge, that investigated the effect of AMF of B toxicity in plant. As an important microorganism in the rhizosphere, AMF is able to form symbiotic associations with the root of most terrestrial plant species, enhancing plant tolerance to various soil and environmental stresses (Miransari, 2010). Increasing evidences have shown that AMF is able to assist phytoremediation of metal contamination by making metals more available for plant uptake or by reducing metal toxicity in their host plants (Coninx et al., 2017). It is possible for the application of AMF on the phytoremediation of B-contaminated soils. Since B toxicity often occurs along with salt and drought stress, it is crucial to investigate whether AMF alleviates B toxicity in plant under the combined stresses.

Puccinellia is a genus of plants in the grass family and is considered to be salt and drought tolerant. Recently, a few species of *Puccinellia* have also been observed to be tolerant to high levels of B (Stiles et al., 2010, 2011; Rámila et al., 2016). *Puccinellia tenuiflora* (Griseb.) Scribn. et Merr., for example, is a popular forage for livestock production, and is widely found in saline soils and frequently used in restoration projects (Yu et al., 2011; Zhang et al., 2013). According to our preliminary experiments, *P. tenuiflora* was able to survive over 300 mg B/L and −0.2 MPa osmotic potential (unpublished data). So far, no research has been conducted on the role of AMF in the alleviation of B toxicity in *P. tenuiflora*. The goal of the present study was to investigate the effect of two most common species of AMFs, *Funneliformis mosseae* (T.H.Nicol. & Gerd.) C. Walker & A. Schüßler and *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüßler, on the growth of *P. tenuiflora* and elements uptake under the combined stresses of B, salt, and drought.

2. Materials and methods

2.1. Seed germination and soil preparation

P. tenuiflora seeds were provided by Tianjin Landscape Institute. The seeds were surface sterilized with 0.1% (w/v) $KMnO_4$ for 2 h to remove fungi and then rinsed with distilled water. The seeds were then sown in a seedling tray filled with sterilized (121 °C, 30 min) vermiculite. The soil used for pot trial was collected from an agricultural area in Shouguang, Shandong. The soil was air-dried and sieved through a 2-mm screen mesh. The sieved soil was mixed with sand (<2 mm) at a rate of 3:1. The soil-sand mixture was then autoclaved high-pressure steam sterilizer (YXQ-LS-50A, Boxun Instrument, Shanghai, China) at 121 °C for 30 min followed by a three-day air drying.

2.2. Preparation of mycorrhizal inocula

Mycorrhizal inocula of *F. mosseae* and *C. etunicatum* were provided by the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences, Beijing, China. *F. mosseae* was originally isolated from the rhizosphere of *Astragalus adsurgens* Pall. in Ejin Horo Banner, Inner Mongolia, China. *C. etunicatum* was as originally isolated from the rhizosphere of *Solanum lycopersicum* L. in Langfang, Hebei. The inocula of the two species of mycorrhizal fungi were propagated respectively in a plastic pot containing a sterile mixture of sand and zeolite (1:1, v/v). The seedlings of white clover (*Trifolium repens* L.) were grown in the mixture as host plant at room temperature with natural light and humidity for three months. The culture substrate containing more spores, mycelium, and mycorrhizal root fragments was collected and air-dried for subsequent inoculation.

2.3. AMF inoculation and seedling transplantation

Eight hundred grams of the sterilized soil-sand mixture was filled in a plastic pot (12 cm in top diameter and 13 cm in depth) and then 45 g of AMF-inoculum was added to the mixture below the seedling root. Non-inoculated treatments received the same amount of autoclaved (121 °C, 30 min) inoculum together with a 20-mL aliquot of filtrate (<20 μm) of the AMF-inoculum, in order to provide a general microbial population free of AMF propagules (Aroca et al., 2013). Thirty *P. tenuiflora* seedlings (with a growth time of 3 weeks and a height of ~15 cm) were transferred to the plastic pot containing the autoclaved soil-sand mixture. The plants were cultivated for three weeks before exerting any stress, allowing adequate plant growth and symbiotic establishment.

The physicochemical properties of the soil were determined and presented in Table 1. Soil pH (soil:water, 1:2.5) was measured using a pH meter (PHE-3C, Instrument Electric, Shanghai, China). Soil organic matter was determined using the Walkley–Black wet oxidation method (Nelson and Sommers, 1982). Soluble salts were determined by measuring the electrical conductivity of a 1:5 soil/water extract (Rhoades, 1982). Cation exchange capacity (CEC) and the amounts of exchangeable bases (K, Ca, and Mg) were measured after successive extraction using 1 mol/L ammonium acetate (pH 7.0) and 100 g/L NaCl (Ultra et al., 2007). Field capacity was determined as follows: an excess of water was added to 400 g of dry soil in a pot, the pot was assumed to be at field capacity when formation of further droplets at the bottom of the pot after free percolation was fully ended, and the pot was reweighed and field capacity was estimated (Meers et al., 2005). Available nitrogen was determined by a micro-diffusion technique after alkaline hydrolysis (Fu et al., 2000). Available P (Olsen-P) was extracted with 0.5 mol/L of $NaHCO_3$ at 1:20 soil:solution ratio with a 30 min shaking period. Phosphorus concentrations in the extracts were determined by the ascorbic acid-molybdenum blue method (Kuo, 1996). Available potassium was determined by CH_3COONH_4 extraction method (Cheng et al., 2007). Available B was extracted from the soil by microwave heating and was determined using inductively coupled plasma-atomic emission spectrometry (ICP-AES) (IRIS Intrepid II XSP, Thermo Elemental, Waltham, MA, USA) (De Abreu et al., 1994). Particle size was analyzed using a hydrometer (TM-85, Jianxing Instrument, Hebei, China) (Ashworth et al., 2001).

2.4. Experimental setup

The experiment consists of two soil treatments: non-stress and

Table 1
Physicochemical properties of the soil used for the incubation experiment.

Soil parameters	Measurements
pH (soil:water, 1:2.5)	8.0 ± 0.2
Organic matter (%)	2.5 ± 0.2
Soluble salts (g/kg)	1.1 ± 0.1
CEC (cmol/L)	137.5 ± 3.3
Field capacity (%)	26.1 ± 1.5
Available N (mg/kg)	26.6 ± 2.2
Available P (mg/kg)	9.1 ± 0.7
Available K (mg/kg)	111.5 ± 5.6
Extractable B (mg/kg)	0.4 ± 0.0
Clay (%)	38.0 ± 2.8
Silt (%)	4.4 ± 0.4
Sand (%)	57.6 ± 3.3

Data are presented as mean values ± SD (n = 3).