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Research article

Tissue physiological metabolic adaptability in young and old leaves of reed (*Phragmites communis*) in Songnen grassland

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ABSTRACT

Common reed (*Phragmites communis*) is widely distributed as the dominant plant species in the Songnen Plain of China. The aim of this study was to investigate different physiological adaptive mechanisms to salinity tolerance between young and old leaves. The profiles of 68 metabolites were measured and studied in reed leaves by gas chromatography-mass spectrometer. The nitrogen, carbon, and pigment contents showed stronger growth inhibition for older leaves with salinity stress. In young leaves, high K^+ contents not only promoted cell growth, but also prevented influx of superfluous Na^+ ions in cells; the Ca^{2+} accumulation in old leaves implied that Ca^{2+} triggered the SOS-Na^+ exclusion system and reduced Na^+ toxicity. Thus, the mechanism of enhanced tolerance differed between young and old leaves. The metabolite results indicated that the young and old leaves had different mechanisms of osmotic regulation; sugars/polyols and amino acids played important roles in developing salinity tolerance in young leaves but high contents of fatty acids were important for old leaves. These results implied dramatically enhanced sugars and amino acid synthesis but inhibited energy metabolism in young leaves. In contrast, fatty acid synthesis was enhanced in old leaves. The results extended our understanding of the differences in physiological metabolism in adaptive to the salt-alkalization of soil in Songnen grassland between young and old leaves of reeds.

1. Introduction

As one of the three largest grasslands in northeast China and one of the ten key pasture areas in China, the Songnen grassland has high economic and ecological value (Shi and Guo, 2006; Zheng et al., 2015). However, because of poor parent material, hydrological conditions and overgrazing, soil salinization on the Songnen grassland has become increasingly serious, with resulting vegetation degradation and reduced productivity (Ye et al., 2009). As one of the important grasses of Songnen grassland vegetation, reed (*Phragmites communis*) is widely distributed in wetlands and neutral and salinized dry habitats, and has characteristics of strong ecological tolerance, habitat expansion capacity, and competitiveness (Li et al., 2009; Qiu, 2014). Reed is also a good forage grass, which can be used for medicine, paper making, and produces light industry raw material and architectural material. It can also adjust to the local climate and maintain biological diversity (Li et al., 2009, 2016). In addition, reeds growing in the Songnen sandy land and strongly alkaline meadow can stabilize sand and improve the soil environment (Qiu, 2014). The reed grassland on basification meadow can be used as pasture or for cut grass (Du et al., 2006; Hansen et al., 2007). Therefore, reed has an irreplaceable role in the maintenance of grassland ecological system stability and local eco-economical construction (Ruan and Silva, 2011; Barding et al., 2013).

Leaves are important in photosynthesis, and their functional characteristics can directly reflect plant hereditary characters and the effective utilization of resources (Gerloff-Elias et al., 2005; Diepenbrock, 2010). The functional characteristics of leaves varies with different positions, affecting the exchange of substances and energy between plants and the surrounding environment, as well as the plant survival strategies formed to adapt to environmental change (Ashraf and O'Leary, 1997; Gao et al., 2008). To deepen research on the photosynthetic production capacity of individual plants and groups, it is necessary to consider the influence of leaf position (Diepenbrock, 2010). As previous research has shown, the leaves at different positions of the same plant appearing at different time, so the leaf and the corresponding leaf activity are also different. (Hajlaoui et al., 2010; Wang et al., 2012a). From the top to the bottom, leaf vitality increased first and then descended, which is always related to plant type and leaf lifespan.

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Previous research showed that the chloroplasts of mesophyll cells inside reeds growing in saline-alkali soil were circular (Takahashi et al., 2009; Qiu, 2014). There was a large starch granule inside each chloroplast and there was a phenomenon of mitochondria embedded into chloroplast (Qiu, 2014; Liu et al., 2016). Previous research on the physical and ecological characteristics of reed under osmotic stress was focused on the influence of salinity stress on biomass, output, quality, water potential and proline, and not on analyzing the leaf metabolic response under natural salinity conditions (Takahashi et al., 2009; Gorai et al., 2010; Ding et al., 2015). It is of great theoretical and practical significance to investigate reed leaves in order to achieve a better understanding of adaptive growth regulation in natural saline habitats and the related biological mechanisms for plant individuals and groups (Li et al., 2016; Qiu et al., 2016).

P. communis is a cosmopolitan species distributed in heterogeneous habitats and shows large variation in genetic material, morphology and chromosome numbers, as well as plasticity in reproductive strategy (Hansen et al., 2007; Engloner, 2009). It widely distributed in Songnen grassland and formed a series of biological, ecological as well as genetic characters contributing to accommodate to the specific local climatic and edaphic conditions (Yang and Liang, 1998; Qiu, 2014). In this study, we used reed as experimental material to investigate whether soil salinization exerts different effects on ion balance and metabolism in old and young leaves of reed. The results should help in explaining the physiological adaptive mechanisms and reveal reed population development in a degenerated meadow.

2. Materials and methods

2.1. Study site and plot selection

The Songnen Plain is an area of about 17.0×10^6 ha located between the Greater Khingan Range and Changbai Mountain (121°30′–127°00′E, 43°30′–48°40′N), formed from alluviation by the Songhua and Nenjiang Rivers, and belonging to a temperate semi-arid and semi-humid monsoon climate zone (Shi and Guo, 2006). The mean annual rainfall and annual temperature is 400–500 mm and 4.6–6.4 °C, respectively, with annual evaporation capacity 2–3 times the rainfall. Salinized meadow soil is the main soil type of the Songnen Plain, with NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3 the main salt components of salinized soil (Shi and Guo, 2006; Qiu et al., 2016).

The sites were set up on salinized soil where reed coexisted with *Suaeda salsa* communities, which consisted of four 3 × 3 m plots, and the distance between plots was < 100 cm – consequently, they shared similar climatic conditions and soil types. Each plot represented one replicate in this research. Reeds were washed with distilled water, after which young (second leaf at up) and old (first leaf at bottom) leaves were separated and frozen immediately in liquid nitrogen in mid-July 2017. The samples of plant material were used for physiological index measurements.

2.2. Measurement of properties and nutrients elements of soil

Soil samples were collected in each plot at depths of 0–10, 10–20, 20–30, and 30–40 cm. Afterward, the obtained samples were mixed together to obtain a uniform sample and passed through a 0.25-mm soil sieve after being naturally dried. The EC and pH of salinity soil were measured using a conductivity meter (DDG-2080-S, Anhui, China) and a pH meter (PSH-3C, Jiangsu, China), respectively. The Na^+, K^+, Ca^{2+} and Mg^{2+} contents were determined using an ICP-OES spectrometer (iCAP 6000). The Cl^- and SO_4^{2-} contents were determined through ion exchange chromatography (DX300 ion chromatographic system). The CO_3^{2-} and HCO_3^- contents were determined using a dual indicator (phenolphthalein and bromophenol)-neutralization titrimetric method with H_2SO_4 as a standard acid. The contents of the eight ions were summed to give the total salinity content.

2.3. Measurement of physiological parameters of reed

About 1 mg dry power samples were utilized to analyze N and C contents (%) using a stable isotope mass analyzer according to the manufacturer's manual (IsoPrime Elemental Analyzer, Isoprime Ltd., Japan). Approximately 500 mg fresh leaf samples were measured spectrophotometrically at wavelengths of 440, 645, and 663 nm to determine chlorophyll a, chlorophyll b, and carotenoid contents in acetone extracts, with the detailed method according to Zhu (1993). All measures were replicated four times.

2.4. Measurement of cations and anions in leaves

The 10 mg dry samples were digested with HNO_3, and then 10 mL of deionized water was added. An ICP-OES spectrometer (iCAP 6000 series, Thermo Fisher Scientific Inc.) was used to determine the levels of cations: Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, and Mn^{2+}. The 100 mg dry samples were treated with 20 mL of deionized water at 100 °C for 20 min. Ion exchange chromatography (Dionex, Sunnyvale, CA, USA) with a mobile phase comprising (1.7/1.8 mM Na_2CO_3/$NaHCO_3$) was used to determine the levels of anions: NO_3^-, Cl^-, SO_4^{2-} and $H_2PO_4^-$. All measures were replicated four times.

2.5. Measurement of metabolic profiles

For metabolic profiling analyses, metabolites were extracted from reed leaves (100 mg FW) of different positions, and their contents were determined using an Agilent 7890 gas chromatograph system (CA, USA) with a DB-5MS coated capillary column (J&W Scientific, Folsom, CA, USA), as described by Guo et al. (2015). All measures were replicated four times.

2.6. Statistical analysis

The means of indices were calculated from four replicates obtained for each measurement. Statistical analyses of physiological parameters and ion balance data, which involved data processing and Duncan's analysis ($P < 0.05$), was performed using SPSS statistical software, version 16 (SPSS, Chicago, IL, USA). For metabolic profiles, principal component analysis (PCA) and orthogonal projections to latent structures discriminant analysis (OPLS-DA) were carried out to distinguish separation of samples and metabolites by SIMCA, version 14.

3. Results

3.1. Soil properties

The Na^+ and the total salt contents in soil samples were 41.56 and 116.93 μmol/g, respectively; the pH was 10.84 and the electrical conductivity was 698.41 μS/cm (Table 1). These important factors indicated a saline-alkali soil with nutrient contents not high in general nutrients, except for K^+ (Table 1).

3.2. Physiological parameters of young and old leaves

The C and N contents were higher in young than in old leaves under salinity soil environment; however, the C/N ratio was lower in young leaves (Fig. 1A, $P < 0.05$). The contents of chlorophyll a, chlorophyll b, and carotenoids were higher in young rather than in old leave (Fig. 1B, $P < 0.05$).

3.3. Ion balance of young and old leaves

In salinized soil, K^+, Na^+, Ca^{2+}, and Mg^{2+} were the dominant cations among total ions in reed leaves (Table 2). The K^+ contents were higher in young rather than in old leaves, whereas Na^+, Ca^{2+} and