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Growth, photosynthesis and stress-inducible genes of *Phragmites australis* (Cav.) Trin. ex Steudel from different habitats

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ABSTRACT

The present study investigates growth of *Phragmites australis* in relation to photosynthesis and stress-inducible genes in its natural habitats. *P. australis* was collected from fresh water (FW), brackish water (BW), mesophytic (M), salt marsh (SM) and sand dune (SD) habitats. These habitats were categorized according to their soil properties. FW and BW habitats are mainly characterized by flooded soil (hypoxia). SM and SD habitats are typical habitats for salt and drought conditions. Shoot growth parameters estimated in the present study would indicate that the optimum growth was in M habitat. The growth in other habitats was lower than that in M one and this reduction coincided with a reduction in photosynthetic rate (*A*). Internal CO_2 concentration (C_i) did not show any significant reduction in all studied habitats except in M one. Starch, total soluble sugars and Rubisco contents could suggest that the growth reduction in FW habitat was a consequence of the reduction in photosynthetic rate while the reduction in growth was a cause of photosynthetic inhibition in BW habitat. Alcohol dehydrogenase (*ADH*) transcript level was constitutively expressed, and it was significantly increased in leaves collected from BW and SD habitats, suggesting its involvement in the fermentative metabolism. The expression of leaf plasma membrane Na^+/H^+ antiporter (*SOS1*) and high affinity potassium uptake (*HKT*) were stimulated in SM leaves assuming their roles in Na^+ re-circulation and K^+ uptake to maintain K^+/Na^+ ratio higher than one inside the leaf. Moreover, the reduced leaf water content could play a role in ion homeostasis inside this species. *P. australis* had the ability to maintain a stable water use efficiency (WUE) in all studied habitats assuming its ability in economizing water and maintaining the assimilation rate.

1. Introduction

Common reed (*Phragmites australis* (Cav.) Trin. ex Steudel) is a perennial, emergent aquatic grass that is considered to be one of the most widespread and productive plant in the world (Brix and Cizkova, 2001). It grows in a wide range of habitats, including fresh and brackish aquatic areas of swamps, lakesides and riversides, salt marshes and sand dunes (Haslam, 1970).

Plants that grow in aquatic areas, especially in stagnant water, have to adapt to unique soil condition (flooded soil) that is mainly characterized by oxygen depletion (hypoxia) where dissolved oxygen becomes reduced in concentrations that will be detrimental to aquatic species living in this system (Keddy, 2010).

Under hypoxia, oxygen supply is depleted therefore plants tend to maintain a steady supply of O_2 for various physiological processes (Vartapetian and Jackson, 1997). One of the major pathways depending on a steady supply of O_2 is mitochondrial respiration that is replaced by fermentative metabolism in order to maintain ATP generating under flooding conditions (Drew, 1997). Alcohol dehydrogenase (ADH) plays a key role in this fermentation pathway where it converts acetaldehyde to ethanol and regenerates NAD^+, a process which is thought to be the most important function in ethanol fermentation pathway and is critical for sustaining glycolysis under hypoxia (Kumutha et al., 2008).

Salt marsh and sand dune habitats are mainly characterized by salt and drought stresses that are united by the fact that at least a part of their detrimental effects is caused by disruption of plant water status, where both of them decrease soil water potential and hence limit water availability to plants causing osmotic stress (Verslues et al., 2006). Salt stress imposes two phases; the first is similar to drought (osmotic stress) and the second is ion-specific phase (ionic stress) that starts when salt

Abbreviations: *NHX1*, vacuolar sodium hydrogen antiporter; *SOS1*, plasma membrane sodium hydrogen antiporter; *HKT*, high affinity potassium uptake; *ADH*, alcohol dehydrogenase.

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accumulates to toxic concentration inside leaves (Munns and Tester, 2008).

Plants possess many mechanisms to prevent the accumulation of toxic ions inside the cytoplasm including minimizing Na^+ influx, intracellular compartmentalization of Na^+ via vacuolar Na^+/H^+ antiporter (NHX1), maximizing Na^+ efflux via plasma membrane Na^+/H^+ antiporter (SOS1) and recirculation of Na^+ out of the shoot through the phloem (e.g., Shi et al., 2002; Ward et al., 2003). Monocotyledonous species tend to maintain their K^+ concentration higher than that of Na^+ (K^+/Na^+ ratio higher than one) (Chen et al., 2007) throughout Na^+ extrusion mechanism as well as an efficient uptake of K^+. High K^+ affinity uptake transporter (HKT) is considered to mediate K^+ affinity uptake in many plants but it has been shown to function as K^+ and Na^+ co-transporter in some species (Takahashi et al., 2007)

The reduction in net photosynthesis in response to stress conditions (like salt, drought or hypoxia) has been attributed to many reasons among them the reduction in photosynthetic enzymes such as Rubisco (Bradford, 1983), the decrease in leaf chlorophyll content (Jackson and Drew, 1984) and the disruption in photosynthetic transport caused by alteration in source-sink relationship and reduced sink need (Wample and Thornton, 1984). Control of stomatal closure, reducing transpiration rate and regulating photosynthesis and growth rates are considered as adaptive strategies to various environmental stresses (Zhu, 2001).

Many studies have investigated the mechanisms involved in *P. australis* adaptation to different stressful conditions but these studies have ended with many contradictory results; moreover, many of them examined these adaptive strategies under controlled conditions (laboratory conditions) (reviewed in Engloner, 2009), which might not greatly reflect the effect of the natural habitats. Additionally, information available on examining the expression of stress-inducible genes like *NHX1, SOS1, HKT* or *ADH* and evaluating their roles in *P. australis* adaptation mechanism is limited and also the effect of different habitats conditions on growth-photosynthesis relationship is not clearly discussed. Therefore, the present study aims to investigate the acclimation of this cosmopolitan taxon to different, natural habitats in terms of growth, photosynthesis and stress-inducible genes. We hypothesized that *P. australis* will exhibit the highest growth and photosynthetic rate in mesophytic habitats. Since whole belowground parts of *P. australis* are difficult to access, the present study was concentrated on the acclimation of the aboveground parts to different habitats.

2. Materials and methods

2.1. Study area and plant materials

A field survey was carried out during July–August, 2012 and July–August, 2013 (maximum growth period) at 35/24 °C day/night temperature, 81% average humidity and 14 km/h wind speed along the North-eastern section of the Nile Delta, Egypt to identify the habitat types of *P. australis* community. These habitats were classified according to the measured electric conductivity and soil moisture as follows: salt marsh (SM), sand dune (SD), mesophytic (M), fresh water (FW) and brackish water (BW) habitats (Mashaly et al., 2009). Ten stands (5×5 m) in each natural habitat were selected and used as replicates for every habitat.

2.2. Soil sampling

From each stand, five soil samples were collected from the rhizosphere around the plant's roots in each habitat. Soil moisture was determined by weighing a known weight of each soil sample and drying it in oven at 105 °C for 72 h or until a constant weight is reached. The difference between fresh and oven-dry weight of the soil is considered to represent the soil moisture content and was expressed as percentage of the fresh weight. Electric conductivity (EC) was measured using Sporule model/10 meter. Soil Na^+ and K^+ were measured in the diluted soil extract using Flame Photometry (PFP7, Jenway). Total soil N content was estimated following semi-micro Kjeldahl method by acid digestion, distillation and titration (Anderson and Ingram, 1993). Total soil P content was measured as described by Jackson (1985). Soil samples from each stand were digested by using a triacid mixture. The mixture was reacted with ammonium molybdate and stannous chloride (colorimetric reaction).

2.3. Shoot sampling

For growth parameters from each stand, whole 5–10 shoots were kept in wet, plastic bags to determine stem and leaf fresh and dry weights and water content. Also leaf number, area, shoot length and diameter were measured in each stand.

For biochemical analyses form each stand, the third top leaf from 5–10 other shoots was collected at 11:00–13:00 in a clear, bright day and immediately kept in liquid nitrogen and stored at −80 °C until use in further analyses.

2.4. Gas exchange measurements

The third top leaf of 5–10 shoots from each stand was used in gas exchange measurements. Photosynthetic rate (A), transpiration rate (E), internal CO_2 (C_i) and stomatal conductance (g_s) were measured at 11:00–12:30 in a bright and clear day in late July(s) by using LCi-SD gas exchange system (Analytical Development Company Ltd., England) using external air as a source of CO_2 and sunlight as a source of light. The range of light intensity and leaf temperature were 1630–1690 $\mu mol\ m^{-2}\ s^{-2}$ and 35.5–36 °C, respectively. Leaves were left for 3 min to acclimate leaf chamber before recording the data.

2.5. Na^+ and K^+ measurements

Na^+ and K^+ were extracted as described by Hansen and Munns (1988). About 100 mg of frozen leaf was homogenized in liquid nitrogen, and then 2 ml of ultrapure H_2O was added. The samples were heated in a water bath at 100 °C for 1 h. After cooling the samples, the residues were removed by centrifugation at 14,000 rpm for 20 min. Then the diluted supernatants were used in measuring Na^+ and K^+ by flame photometer (PFP7, Jenway, Essex, UK). Five replicates from five independent leaves were used for each stand.

2.6. Determination of soluble sugars, starch and total chlorophyll contents

Soluble sugars were extracted from 100 mg frozen leaf homogenized with 1000 μl 80% ethanol overnight. The samples were then centrifuged and the extract (supernatant) was dried in a water bath. After complete drying, the residue was re-dissolved in 1000 μl distilled water and used for determination of soluble sugars. About 100 μl aliquots of the reconstituted samples were carefully mixed with 3 ml anthrone (8.6 mM anthrone in 80% v/v H_2SO_4), heated in a water bath at 100 °C for 10 min and then cooled on an ice bath for 30 min. The absorbance was recorded at 623 nm. Soluble sugar concentration was calculated from glucose standard curve in range of 20–100 μg (Schluter and Crawford, 2001). Five replicates from five independent leaves were used for each stand.