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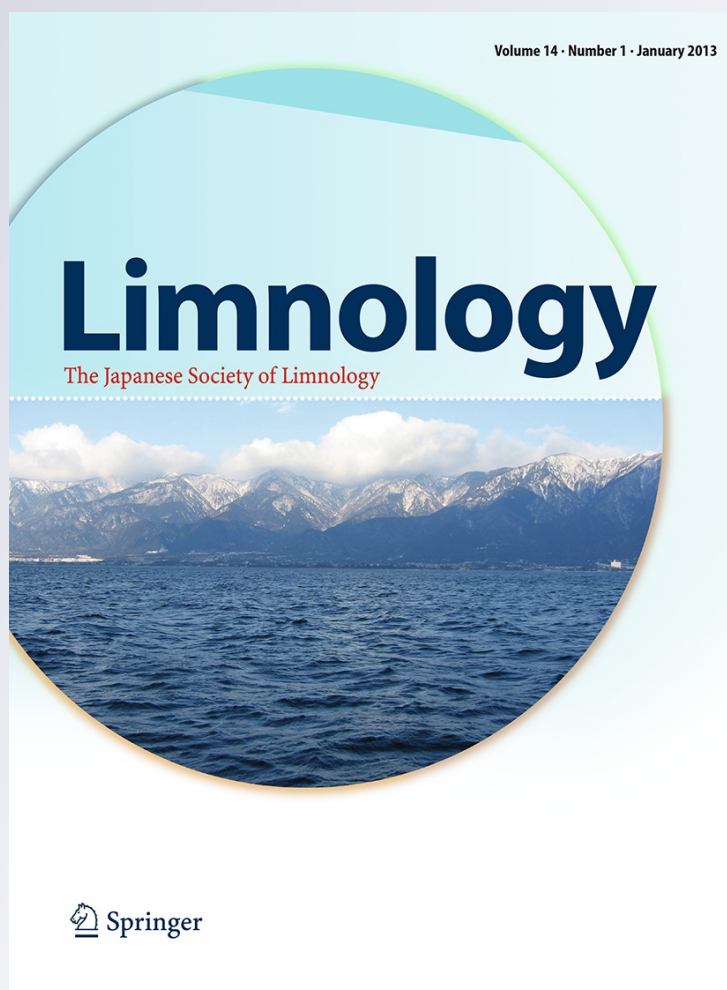
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Physiological mechanisms for plant distribution pattern: responses to flooding and drought in three wetland plants from Dongting Lake, China

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Abstract Both flooding and drought are important in determining plant distribution in wetlands. However, the roles of plant's physiological response to flooding and drought in accounting for plant distribution are far from clear. To this end, three typical wetland plants with different distribution patterns (high-elevation species *Miscanthus sacchariflorus*, low-elevation species *Carex brevicuspis* and *Polygonum hydropiper*) in Dongting Lake were treated with three water levels (flooding 25 cm, control 0 cm, drought –25 cm), and relative growth rate (RGR), malondialdehyde (MDA) content, electrolyte leakage and proline content were investigated. The RGR of the three species decreased significantly in both flooding and drought treatments. Compared to the control, the RGR of *M. sacchariflorus* decreased more in the flooding treatment but less in the drought treatment compared to the other two species. The contents of MDA in the three species increased in both flooding and drought treatments, except for *P. hydropiper* in the flooding treatment. MDA

contents increased more in *M. sacchariflorus* in the flooding treatment but less in the drought treatment compared to the other two species. Only *M. sacchariflorus* had a higher electrolyte leakage in the flooding treatment, and drought led to a higher electrolyte leakage in *P. hydropiper* and *C. brevicuspis*. Proline content increased 69.2, 66.7 and 39.6 % in *P. hydropiper*, *C. brevicuspis* and *M. sacchariflorus* in the flooding treatment, and increased 44.2, 13.0 and 45.3 % in the drought treatment, respectively. These results suggest that *M. sacchariflorus* has a higher tolerance to drought but a lower tolerance to flooding than do the other two species, which might be the intrinsic mechanisms accounting for their different distribution patterns.

Keywords Flooding · Drought · MDA content · Electrolyte leakage · Proline content

Introduction

Explaining species distribution pattern along environmental gradients has been a dominant subject in ecological research for decades (Engels et al. 2011). Many abiotic habitat conditions (e.g., salinity, water level, soil nutrients) and biotic factors (e.g., competition, facilitation and grazing) have been discussed as important drivers of the spatial variation in wetland plant distribution (Crain 2008; Luo et al. 2010; Engels et al. 2011). Among them, flooding is considered the most important factor in determining plant distribution (Blom 1999; Dwire et al. 2004), mainly because of the reduced oxygen availability in the soil (Van Eck et al. 2006; Li et al. 2011). However, wetland plants, especially in floodplains and river-connected lakes, also experience periodic drought stress because of large water-level fluctuation (Pagter et al. 2005; Parolin et al. 2010).

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Growing evidence has confirmed the role of drought events in determining plant composition and distribution (Lopez and Kursar 2007; Parolin et al. 2010), and drought may represent more limitations for survival than does flooding for the local vegetation in the Amazonian (Keel and Prance 1979) and Rio Balsas floodplains (Lopez and Kursar 2007).

The differences in tolerance to stresses among species are of decisive importance for the distribution of wetland plants (Bates et al. 1973; Fritz and Dodds 2004; Pagter et al. 2005). Tolerance, the ability of an organism to avoid displacement during a stress period, is usually related to the adjustments of plant morphology (e.g., biomass allocation, aerenchyma, root structure) and physiology [e.g., malondialdehyde (MDA), electrolyte leakage, proline and antioxidative enzymes]. To date, many studies have been performed on the morphological responses in order to account for plant distribution patterns (Qin et al. 2010; Li et al. 2011). However, the roles of physiological adaptation in determining plant distribution are far from clear.

Both flooding and drought can lead to oxidative stress through an increase in reactive oxygen species (ROS), then significantly disturbing plant's metabolism (Pagter et al. 2005; Hossain et al. 2009). The MDA content and electrolyte leakage, important indicators for lipid peroxidation and membrane damage, can accumulate significantly under waterlogging/drought conditions, and a higher increment can be found in stress-sensitive species (Arbona et al. 2008; Luo et al. 2008). Moreover, proline, an important compatible solute or osmoprotector for plants to acclimate to osmotic stress, can be accumulated more in stress-tolerant plants than in sensitive species (Sofa et al. 2004; Pagter et al. 2005). Therefore, MDA content, electrolyte leakage and proline content are important indicators reflecting plant's waterlogging/drought tolerance ability, and differences among species might account for their different distribution patterns.

Dongting Lake (E: 111°40'–113°10', N: 28°30'–30°20'), the second largest freshwater lake in China, lies to the south of the Yangtze River and is connected to the Yangtze River by three channels. The wetlands in this lake are characterized by its large seasonal fluctuations of water level, being completely flooded during May–October and experiencing drought during November–April. Plant zonation along an elevation gradient is a common phenomenon in this wetland, and dominant species, such as *Phragmites australis*, *Miscanthus sacchariflorus*, *Carex brevicuspis* and *Polygonum hydropiper*, usually occur in different elevations (Xie and Chen 2008).

The aim of this experiment was to elucidate the physiological mechanisms of plant distribution patterns by investigating the physiological responses of three typical wetland plants to flooding and drought in Dongting Lake. To this end, the high-elevation species *M. sacchariflorus* and low-elevation species *C. brevicuspis* and *P. hydropiper*

were chosen to grow in three different water-level treatments. Based on their different distribution patterns, the following hypotheses were tested: firstly, the relative growth rate (RGR) of *M. sacchariflorus* will decrease more in the flooding treatment but less in the drought treatment compared to the other two species; secondly, MDA content and electrolyte leakage of *M. sacchariflorus* will increase more under flooding conditions but less in the drought treatment compared to the other two species; thirdly, the proline content of *M. sacchariflorus* will increase more in the drought treatment but less in the flooding treatment compared to the other two species.

Materials and methods

Plant materials

Plants (*Miscanthus sacchariflorus*, *Carex brevicuspis* and *Polygonum hydropiper*) were collected in February 2009 from Junshan County (E: 112°59'40.0"; N: 29°22'17.7"), East Dongting Lake. The vegetation was cut into small blocks (20 cm × 20 cm) and transported to an outdoor net house in the Institute of Subtropical Agriculture, the Chinese Academy of Sciences (about 150 km away from the plant collection site). Plant fragments with roots were placed into plastic buckets, which contained 15 cm of soil to germinate new ramets. The plants were watered when necessary. The net house was covered with one layer of nylon net, and the light was about 50 % of full sun.

Experimental setup

On 13 May 2009, 24 ramets of each species with similar size (4–6 leaves and about 30 cm in height for *M. sacchariflorus*; 5–6 leaves and about 20 cm in height for *C. brevicuspis* and 6–7 leaves and about 25 cm in height for *P. hydropiper*) were selected and planted into PVC tubes (25 cm in height and 12 cm in diameter, one ramet per tube), which were filled with 25 cm soil (containing 1.45 % organic matter, 4.04 $\mu\text{g g}^{-1}$ exchangeable N, and 0.88 $\mu\text{g g}^{-1}$ exchangeable P), excavated from Junshan County. All plants were randomly placed into four large plastic bins (78 cm × 56 cm × 60 cm, 18 tubes per bin, two plants per treatment per species) to control the water level.

Therefore, there were four replicates per treatment for each species. Different water levels were obtained by elevating the tubes with bricks, and the plants of each species were randomly placed in each bin. Experimental treatments were initiated on 20 May, and three water levels were used in this experiment: –25 cm (drought), 0 cm (control) and 25 cm (flooding, relative to the soil surface). Prior to the experiment, the biomass of four plants per species was

measured. Tap water (containing $0.511 \mu\text{g l}^{-1} \text{NH}_4^+-\text{N}$, $1.760 \mu\text{g l}^{-1} \text{NO}_3^--\text{N}$ and $0.527 \mu\text{g l}^{-1} \text{PO}_4^{3+}-\text{P}$, pH 7.2) was supplied in a timely manner to keep a constant water level in the bins, and the water was replenished once a week to prevent algae.

Harvest

The plants were harvested after 70 days. First, a certain amount of fresh leaf samples was collected for the analysis of physiological traits. Then, plant roots were carefully dug out by hand and cleaned up using tap water. Plants were divided into leaves, stems and roots. Fresh weights of each plant tissue were measured first, then oven dried at 80°C for 48 h and weighed. Leaf mass for the analysis of physiological traits was also added to the corresponding plant biomass by the conversion of fresh weight to dry weight. RGR was determined as: $\text{RGR} = (\ln w_2 - \ln w_1) / (t_2 - t_1)$, where w_1 was the initial biomass, w_2 the biomass at harvest time t_2 and $(t_2 - t_1)$ the experimental time.

MDA content

The content of MDA was measured using the method described by Duan et al. (2005). Firstly, 0.5 g fresh leaves was homogenized in 10 ml of 10 % trichloroacetic acid (TCA) and centrifuged at $14,000 \times g$ for 10 min. After that, 2 ml 0.6 % thiobarbituric acid (TBA) in 10 % TCA was added to an aliquot of 2 ml of the supernatant. The mixture was then heated for 30 min in boiling water and cooled quickly in an ice bath. After centrifugation at $10,000 \times g$ for 10 min, the absorbance of the supernatant was determined with a 360 UV/visible light spectrophotometer (Shimadzu, Japan) at 450, 532 and 600 nm. The MDA concentration was calculated using the formula: $C (\mu\text{mol l}^{-1}) = 6.45(A_{532} - A_{600}) - 0.56A_{450}$, and was expressed as $\mu\text{mol g}^{-1} \text{FW}$ (Duan et al. 2005; Yang et al. 2009).

Electrolyte leakage

The electrolyte leakage was determined as described by Dionisio-Sese and Tobita (1998); 0.2 g of fresh leaves was cut into 5-mm-long pieces and placed in test tubes with 10 ml distilled deionized water. The tubes were incubated in a water bath at 32°C for 2 h, and the initial electrical conductivity of the medium (EC_1) was measured using a DDS-11C EC sensor (Shanghai Precision Instrument Co., Ltd., China). The samples were autoclaved for 20 min at 121°C to release all electrolytes, then cooled to 25°C , and the final electrical conductivity (EC_2) was measured. The electrolyte leakage (EL) was calculated by using the formula: $\text{EL} = (\text{EC}_1/\text{EC}_2) \times 100$ (Dionisio-Sese and Tobita 1998; Masood et al. 2006).

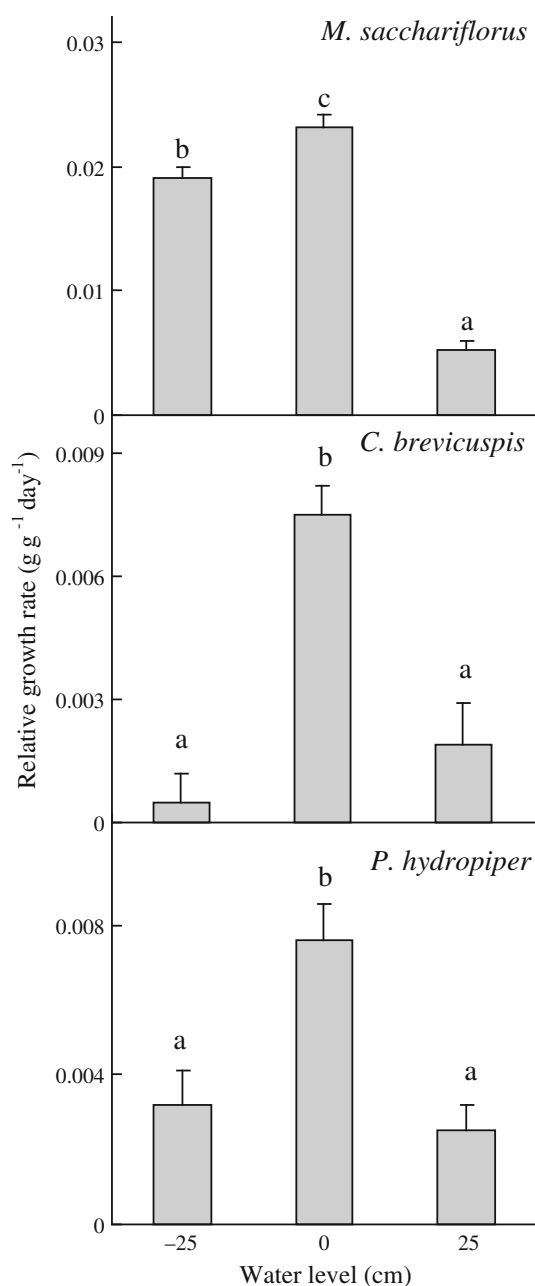


Fig. 1 Relative growth rate of three species (mean \pm SE, $n = 4$) growing in three water levels. Different letters indicate a significant difference among treatments at the 0.05 significance level

Proline content

The proline content was determined as described by Bates et al. (1973). About 0.5 g of leaf samples was homogenized in 5 ml 3 % sulfosalicylic acid and homogenate filtered through filter paper. After addition of 2 ml acid ninhydrin and 2 ml glacial acetic acid, the mixture was heated for 1 h in a 100°C water bath. The reaction was then stopped using an ice bath. The mixture was extracted with 4 ml toluene, and the absorbance of the fraction with the toluene aspirated from the

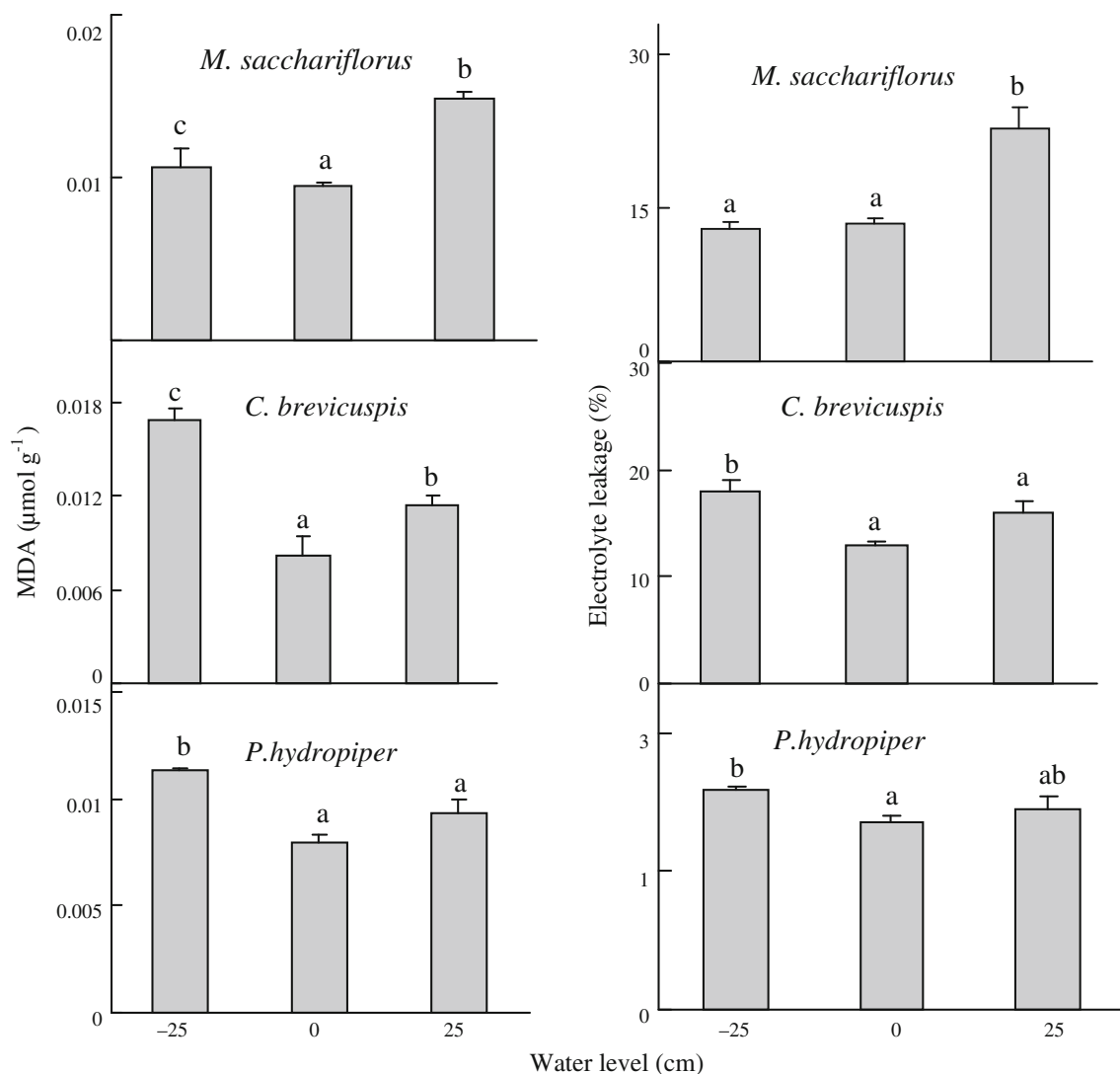


Fig. 2 MDA content and electrolyte leakage of three species (mean \pm SE, $n = 4$) growing in three water levels. Different letters indicate significant difference among treatments at the 0.05 significance level

liquid phase was measured at 520 nm using a 360 UV/visible light spectrophotometer (Shimadzu, Japan). The proline content was determined using the calibration curve as $\mu\text{mol proline g}^{-1}$ FW (Bates et al. 1973; Yazici et al. 2007).

Data analysis

In each bin, the mean values of two plants in each treatment were used for the data analysis. Two-way ANOVAs, with water level and species as the main factors, were performed to determine the main effects and interactions on RGR, MDA content, electrolyte leakage and proline content. Multiple comparisons of means were performed using Tukey's test at the 0.05 significance level, and a Bonferroni correction for multiple comparisons was made where necessary. Data were \log_{10} -transformed if necessary to reduce the heterogeneity of variances. Normality and

homogeneity were tested using Liljefors test and Levene's test, respectively. All analyses were performed using the software SPSS 16.0 for Windows.

Results

Relative growth rate

Relative growth rates of the three species decreased significantly in both flooding and drought treatments ($P < 0.001$; $F = 85.074$; Fig. 1). Compared to the control, the RGR of *M. sacchariflorus* decreased less (17.7 %) than that of *C. brevicuspis* (93.3 %) or *P. hydropiper* (57.9 %) in the drought treatment, while it decreased 77.2, 74.7 and 67.1 % in the flooding treatment in *M. sacchariflorus*, *C. brevicuspis* and *P. hydropiper*, respectively.

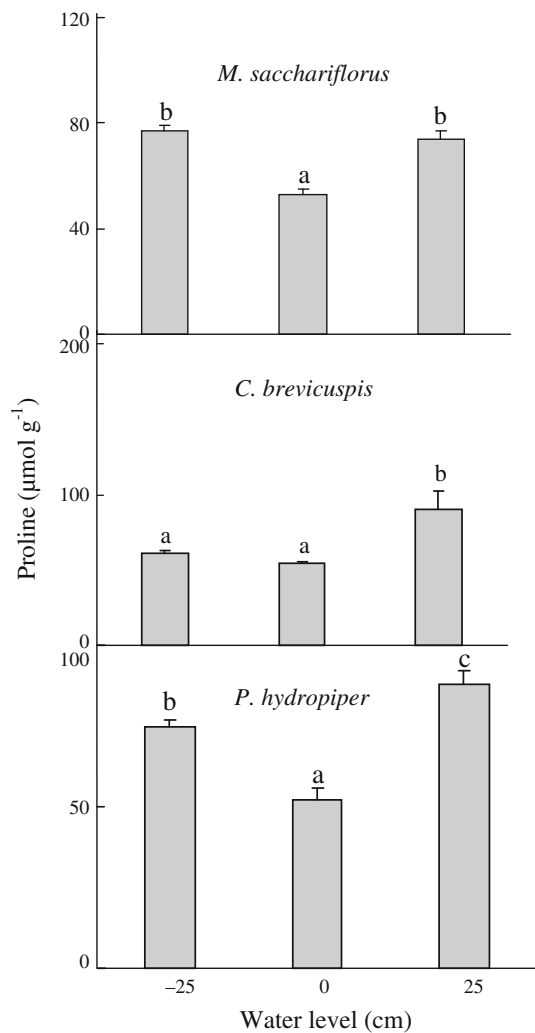


Fig. 3 Proline content of three species (mean \pm SE, $n = 4$) growing in three water levels. Different letters indicate significant difference among treatments at the 0.05 significance level

MDA content

Both flooding and drought led to a significant increment in MDA content in the three species ($P < 0.01$; $F = 7.38$; Fig. 2), except for *P. hydropiper* in the flooding treatment. Compared to the control, the MDA content of *M. sacchariflorus* (55.8 %) increased more in the flooding treatment than that in *P. hydropiper* (16.3 %) or *C. brevicuspis* (39.0 %). However, the MDA content increased more in *P. hydropiper* and *C. brevicuspis* than in *M. sacchariflorus* in the drought treatment.

Electrolyte leakage

Electrolyte leakages of the three species were significantly affected by the water level ($P < 0.001$; $F = 13.97$; Fig. 2). Compared to the control, only *M. sacchariflorus* had a higher electrolyte leakage in the flooding treatment, and

drought led to an increased electrolyte leakage in *P. hydropiper* and *C. brevicuspis*.

Proline content

The proline content was significantly affected by water level ($P < 0.001$; $F = 55.87$; Fig. 3). Compared to the control, the proline content increased 69.2, 66.7 and 39.6 % in *P. hydropiper*, *C. brevicuspis* and *M. sacchariflorus* in the flooding treatment, and increased 44.2, 13.0 and 45.3 % in the drought treatment, respectively. For *C. brevicuspis*, the proline content increased only in the flooding treatment. For the other two species, either flooding or drought treatment led to a higher proline content.

Discussion

The changing pattern of RGR of the three species in different water level treatments suggested that *M. sacchariflorus* has a higher drought tolerance but a lower flooding tolerance than does *C. brevicuspis* or *P. hydropiper*, which supports our hypothesis 1. Moreover, these results are also consistent with Luo et al. (2008), who found that a trade-off exists between tolerance to drought and tolerance to flooding in three wetland plants with different distribution elevations from the Sanjiang Plain.

In this experiment, a higher enhancement of MDA content and electrolyte leakage under the flooding condition in *M. sacchariflorus* suggested that flooding damage to *M. sacchariflorus* was stronger compared to *C. brevicuspis* and *P. hydropiper*, which is consistent with our hypothesis 2.

More enhancement of electrolyte leakage in *M. sacchariflorus* may be due to an increase in membrane permeability or loss of membrane integrity, which in turn leads to an increase in solute leakage (Masood et al. 2006). Electrolyte leakage and MDA content increased less in *C. brevicuspis*, and *P. hydropiper* might account for lower lipid peroxidation levels and less affected membrane permeability, which might be caused by their developed aerenchyma systems (Qin et al. 2010).

Less enhancement of MDA content and electrolyte leakage in *M. sacchariflorus* indicated that the damage caused by drought was less in *M. sacchariflorus* than in *C. brevicuspis* and *P. hydropiper*. Higher drought tolerance ability of *M. sacchariflorus* might be explained by its relatively deeper root systems. Four years of field investigation by the researchers in the Dongting Lake Station for Wetland Ecosystem Research, the Chinese Academy of Sciences, found that the maximum root depth of *M. sacchariflorus* was more than 100 cm, while it was <20 cm for *C. brevicuspis* and *P. hydropiper*. Therefore, *M.*

sacchariflorus has a greater water absorption ability than do the other two species.

Compared to *P. hydropiper* and *C. brevicuspis*, more increment of proline content in *M. sacchariflorus* in the drought treatment but less in flooding stress suggested that proline was more effective in *M. sacchariflorus* to acclimate to the drought stress compared to the other two species, which also can be reflected by their different changing patterns of RGR, MDA and membrane permeability. These results support our hypothesis 3. Moreover, the positive correlation between proline content and the tolerance ability of plants to withstand stressed conditions has been confirmed in other studies (Sofa et al. 2004; Pagter et al. 2005).

Our study indicated that the different distribution pattern of the three species in the Dongting Lake can be reflected by their different physiological responses to flooding and drought stresses. Moreover, the results of our study also can be used to explain the expansion of the *M. sacchariflorus* community in accord with the more frequent drought events in recent years due to human activities and climate changes (Xie and Chen 2008). However, besides flooding and drought, plant distribution in wetlands can also be determined by other environments, such as competition and soil characteristics (Rogel et al. 2001; Luo et al. 2010). Therefore, more studies are also needed to understand the physiological mechanisms controlling plant distribution in wetlands.

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