

Growth, Leaf Gas Exchange, and Chlorophyll Fluorescence Responses of Two Cultivars of *Salix integra* Thunb. to Waterlogging Stress

H. F. Zhao¹, Y. Zhao¹, C. Zhang¹, X. Tao¹, and X. N. Xu^{1*}

ABSTRACT

Salix integra Thunb. is a shrub distributed in China along the Huai River riparian zone, and plays an important role in water and soil conservation. *S. integra* shrub land is easily submerged during July to October due to the flooding of Huai River. In order to characterize the physiological mechanisms of waterlogging tolerance of *S. integra* and to help rationally select waterlogging-tolerant species to alleviate the flood damage to agricultural production, we studied the effects of flooding on the growth, leaf gas exchange, and chlorophyll fluorescence of *S. integra* cuttings during a growth season (from August to October 2011). The biomass production and photosynthesis of *S. integra* cv. *qingpi* were promoted under moderate waterlogging stress, whereas its growth, chlorophyll content, net photosynthetic rate, and stomatal conductance were all significantly lower than those of the control group. A significant increase in non-photochemical quenching (NPQ) and a reduction in PSII maximal quantum photochemistry efficiency (F_v/F_m) were observed in flooded seedlings of *S. integra* cv. *hongpi*. In the case of *S. integra* cv. *qingpi*, however, there were no significant differences in NPQ and F_v/F_m between the treatment and the controls. Our results demonstrated that *S. integra* cv. *hongpi* exhibited slight damage to its photosynthetic apparatus under flooding. In contrast, a moderate flooding stress promoted the leaf and root production of *S. integra* cv. *qingpi*, indicating that *S. integra* cv. *qingpi* can tolerate prolonged soil flooding better than *S. integra* cv. *hongpi*. Thus, *S. integra* cv. *qingpi* is more suitable for afforestation in the riparian zone.

Keywords: Biomass, Flooding, Photosynthetic rate, Shrub, Tolerate.

INTRODUCTION

Waterlogging is one of the primary forms of abiotic stress confronting many plants. The problem develops when the water content in the soil surpasses the field moisture capacity, inducing waterlogging stress on plants growing in the soil. Waterlogging affects trees growing in coastal and riverside areas that are frequently flooded. Waterlogging stress generally inhibits plant root and shoot growth, leading to reduced biomass production and premature falling of fruit (Vartapetian and Jackson, 1997). However, several studies have

also shown that waterlogging stress does not significantly influence the growth of Japanese cedar and *Taxodium distichum* (L.) Rich. and promotes biomass accumulation in *Salix* trees and *Fraxinus velutina* (Hou, 2003; Tang *et al.*, 1998). The photosynthetic rate, stomatal conductance, and photochemical quantum efficiency of plants under flooding stress typically decrease over short term (in waterlogging-tolerant plants) or in the long term (in waterlogging-susceptible plants) (Carvalho and Amancio, 2002; Schwanz *et al.*, 1996). Waterlogging also induces degradation of chlorophylls, an increase in membrane

¹ Department of Forestry, College of Forestry and Landscape Architecture, Anhui Agricultural University, No. 130, West Changjiang Road, Hefei 230036, Anhui, People's Republic of China.

* corresponding author; e-mail: hongfeizhao@yahoo.cn



permeability, and an insufficient oxygen supply for the root system. Waterlogging-tolerant plants neutralize the stress by forming hypertrophied lenticels and aerenchymas and developing adventitious roots to adapt to the waterlogging (Kozłowski, 1997).

Salix integra Thunb. is a multipurpose shrub that is predominantly distributed in the riparian zone of the Huai River basin in China, where it plays key roles in soil and water conservation. The *S. integra* shrub frequently suffers widespread waterlogging stress during the season from July to October as a result of the often-overflowing Huai River. There have been studies on the growth, physiological, ecological, and photosynthetic properties under drought and waterlogging stress conditions for *Populus deltoides* and *S. matsudana* (Tang et al., 1998), *Populus alba* var. *pyramidalis* Bunge and *Populus nigra* L. var. *thevestina* (Li and Kakubari, 2001), and *S. gracilistyla* and *S. subfragilis* (Nakai et al., 2010, 2011). However, the effects of waterlogging stress on the growth and photosynthetic eco-physiology of *S. integra* have not been reported.

In the present study, we investigated the eco-physiological responses of *S. integra* to the simulated waterlogging stress for a 75-day period from August to October. The objectives of this study were to (1) characterize the impacts of waterlogging on the morphology, growth, leaf gas exchange, and chlorophyll fluorescence response of the two types of *Salix* trees, and (2) help rationally select waterlogging-tolerant species to alleviate the waterlogging damage to plants while investigating the physiological mechanisms of waterlogging tolerance of *S. integra*.

MATERIALS AND METHODS

Materials

Salix integra Thunb. belongs to the Salicaceae family and *Salix* genus and is a perennial deciduous shrub that is predominantly distributed in the riparian zone of the Huai River basin in China. Two

cultivars, *S. integra* cv. *hongpi* (SIh) and cv. *qingpi* (SIq), were selected for the experiment. Cuttings were collected from 1-year-old shoots of two cultivars of *S. integra* from Huanggang, Funan County Anhui Province (32°24'19"N and 115°16'30"E). The 1-year-old shoots were cut to lengths of 15 cm. The diameters at the centers of the cuttings ranged from 8.1 to 10.0 mm. Cuttings were planted in the experimental field of Anhui Agricultural University on 2nd of March 2011. In the middle of May 2011, uniformly growing seedlings were selected and transplanted to pots (23 cm in diameter and 28 cm in height), with three seedlings per pot. The pots were filled with a 3:1 nursery soil-to-merchandised peat substrate mixture. To guarantee a uniform growing environment, all of the seedlings were exposed to natural sunlight and rainfall, with well-watered conditions to promote growth.

To determine the response of *S. integra* cuttings to waterlogging stress, on 3rd of August, 2011, a total of 72 uniformly sized pots of each cultivar were selected and randomly distributed into three groups, each group receiving one treatment and each pot representing one replicate. The average base diameter of cuttings and average fresh weight were not significantly different among treatments at the outset.

The pots were placed into larger containers, the experimental design involved two waterlogging treatments (moderate and severe) and a control group with no flooding/no drought. Treatment duration was 75 days for waterlogging treatments from 3rd of August to 17th of October, 2011 to correspond to the typical duration of a flooding season in Funan. In the moderate waterlogging treatment, cuttings were watered to keep water-level 10 cm below the pot soil surface to simulate a shallow groundwater level. In the severe waterlogging treatment, water level was kept at 4-5 cm above the soil surface throughout the duration of the experiment. In the control, cuttings were watered with about 1500 ml daily and left to drain freely.

Measurements

The occurrence of leaf color changes, the leaf abscission, and the formation of adventitious roots and hypertrophied lenticels were regularly monitored and recorded. The cutting height was measured with a measuring tape with an accuracy of 0.1 cm. The base diameter at 5 cm above the ground was measured twice using a Vernier caliper with an accuracy of 0.01 cm, and the average value was recorded.

The biomass measurements were performed twice: once before the waterlogging and once after. The cuttings from 6 pots from each treatment group were analyzed prior to the waterlogging, and the remaining 18 pots in each treatment group were analyzed after the whole waterlogging experiment. At the end of the experiment, the cuttings were carefully removed from the pots and divided into above-ground and below-ground biomass. The weights of the roots, shoots, and leaves were measured separately, then, they were dried in an oven at 60°C for 48 hours. Finally, the biomass and its distribution were determined (Farquhar and Sharkey, 1982).

On the sunny day between 9:00 and 13:00, under a natural light level of 1000-1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, the net photosynthetic rate and stomatal conductance of the cuttings in the different treatment groups were measured in a 20×30 mm transparent leaf chamber with a Li-6400 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA). The leaves measured were fully expanded and were the fourth to seventh leaves from the top of the plant. The air temperature and humidity of the leaf chamber were maintained at 30°C and 40-60%, respectively, during the measurements. Chlorophyll fluorescence parameters (F_v/F_m) and non-photochemical quenching (NPQ) were measured in a standard fluorescence leaf chamber with a Li-6400 portable photosynthesis system. Prior to the measurement in the early morning, a clip was placed on each leaf for 30-40 min for dark adaptation (Yi et al., 2006).

Leaves that were in the same position and of the same maturity level as those measured using the Li-6400 system were sampled, and 0.1 g of the leaves was extracted with 80% acetone. The extractions were analyzed using UV- visible spectrophotometry to determine the concentrations of chlorophyll a (Chla), chlorophyll b (Chlb), and total chlorophyll (Chl (a+b)), which were calculated using the Aron revised method (Li, 2000). The chlorophyll content per gram of dry leaf (mg g^{-1}) was determined based on the chlorophyll concentration in the extract and the water content of the leaf.

Statistical Analysis

All statistical analyses were performed with Data Processing System for Windows. Repeated measurement analysis of variance (ANOVA) was used, and differences were considered significant at $P=0.05$.

RESULTS

Morphological Features

Throughout the 75-day waterlogging period, the survival rates of the two cultivars i.e. SIh and SIq, were 100%; however, the morphological characteristics of the cuttings exhibited remarkable differences. Under the moderate waterlogging treatment, the morphology of the leaves was almost identical to that of the control. Late in the severe waterlogging treatment period (55 days), about 11% (6-7 leaves) of the total number of leaves per cutting on the lower portion turned yellow and 9% (5 leaves) fell off for SIq. In the case of SIh, 13% (5-9 leaves) of leaves turned yellow on day 40, under the moderate waterlogging, and 10% (5-7 leaves) of the total number of leaves per cutting developed necrosis spots on day 33 under severe waterlogging condition, when 17% of the total leaves fell off and the tree growth slowed considerably.



Under the waterlogging treatment, both cultivars behaved similarly with regard to their root growth compared with the controls. After 15 days of severe waterlogging, white fattened lenticels and red adventitious roots were developed at the base of the submerged shoot for SIh, whereas the white attachment appeared at the base of the submerged shoot for SIq after 20 days, and enlarged lenticels and a few adventitious roots appeared after 24 days. The quantity of adventitious roots for SIq under severe waterlogging condition increased with experimental time and was significantly greater than that for SIh. At the end of the waterlogging treatment, the biomass

of adventitious roots of SIq ($2.39 \text{ g plant}^{-1}$) was as high as 227% of the SIh ($1.05 \text{ g plant}^{-1}$).

Growth and Biomass

In the first 20 days of the waterlogging treatment (under both the moderate and severe waterlogging), the base diameter and shoot height of the cuttings for SIh and SIq were similar and not significantly different ($P > 0.05$) from those in the controls. However, after 20 days of severe waterlogging, the growth of base diameter and the shoot height of SIh were remarkably

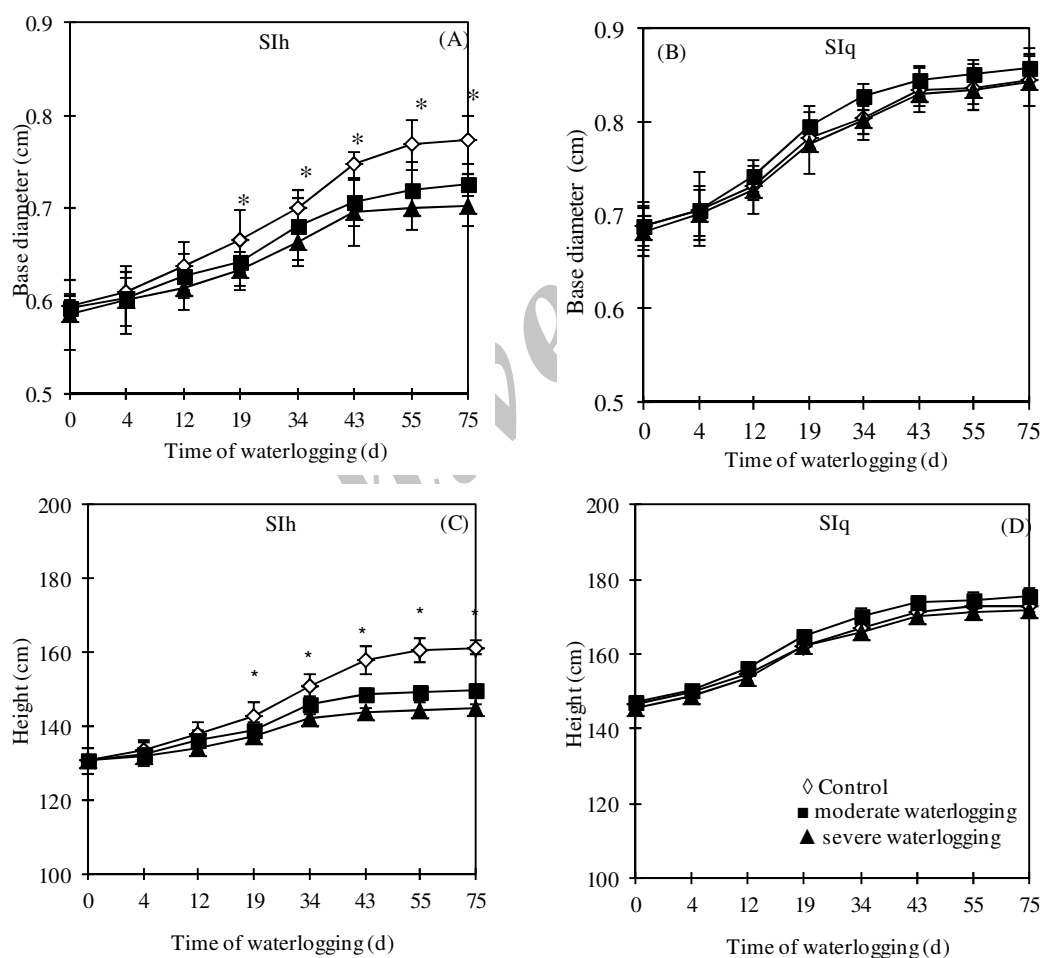


Figure 1. Variations in the base diameters and shoot heights of *Salix integra* cv. *hongpi* and cv. *qingpi* under the waterlogging stress (mean \pm sd). Asterisks(*) indicate significantly different ($P < 0.05$).

inhibited and became more severely inhibited as the waterlogging continued (Figures 1-a and -c). At the end of the waterlogging experiment, the base diameters of SIh under both waterlogging conditions decreased by, respectively, 6 and 9%, and its shoot heights decreased by 7 and 10%, respectively, compared with those in the control. During the entire experimental period, the base diameter and shoot height of SIq were not significantly different from those in the controls ($P > 0.05$).

Under the moderate waterlogging stress, the biomass production of SIh was not significantly different from that of the controls (Table 1). The severe waterlogging stress slowed the biomass accumulation of SIh, which was predominantly manifested by the decrease in leaf biomass. Under the moderate waterlogging, SIq significantly increased in biomass compared with the controls, while in severe waterlogging, no significant difference in biomass production was found compared to the control. The root biomass of SIq under both the moderate and severe waterlogging treatments significantly increased by, respectively, 35 and 23% greater than those of the controls. During the whole moderate waterlogging process, no adventitious root was developed at the base of the submerged shoot for SIq. The biomass of non-adventitious root for SIq under control and severe waterlogging treatment were 6.36 g plant⁻¹ and 5.45 g plant⁻¹, respectively, and there was no significant difference between them, indicating that the root biomass increase was due to adventitious root formation under severe waterlogging stress. Biomass productions increased in both cultivars despite the exposure to waterlogging stress in the end of the experiment.

Physiological Responses

After 19 days of waterlogging stress, the net photosynthetic rate decreased for SIh, compared with that for the control, by 25 and 48% under the moderate and severe

Table 1. The biomass and its components before and after the water logging stress.^a

	SIh			SIq		
	Control	Moderate waterlogging	Severe waterlogging	Control	Moderate waterlogging	Severe waterlogging
Before waterlogging						
Leaf (g plant ⁻¹)	2.95 ± 0.81	2.88 ± 0.78	3.01 ± 0.23	3.19 ± 0.45	3.18 ± 0.78	2.79 ± 0.35
Stem (g plant ⁻¹)	5.32 ± 1.12	5.31 ± 1.24	5.48 ± 2.11	5.34 ± 1.34	5.06 ± 1.88	5.26 ± 1.26
Root (g plant ⁻¹)	1.69 ± 0.56	1.75 ± 0.66	1.69 ± 0.21	2.10 ± 0.83	2.32 ± 0.55	2.65 ± 0.42
Cutting (g plant ⁻¹)	5.16 ± 1.09	5.12 ± 1.18	5.08 ± 1.33	6.24 ± 1.02	6.53 ± 1.96	5.89 ± 0.89
Total Biomass (g plant ⁻¹)	15.12 ± 2.55	15.06 ± 1.05	15.26 ± 2.45	16.87 ± 2.86	17.09 ± 3.05	16.59 ± 2.32
After waterlogging						
Leaf (g plant ⁻¹)	5.11 ± 1.12a	3.22 ± 0.97ab	2.72 ± 1.23b	5.3 x d0 ± 0.84a	7.84 ± 1.73b	5.98 ± 1.08a
Stem (g plant ⁻¹)	10.97 ± 2.24a	10.90 ± 1.56a	7.79 ± 1.04b	9.11 ± 1.72a	11.10 ± 2.10a	9.94 ± 1.95a
Root (g plant ⁻¹)	4.16 ± 0.78a	5.73 ± 0.44a	3.64 ± 0.66a	6.36 ± 0.54a	8.61 ± 1.46b	7.84 ± 1.14a
Cutting (g plant ⁻¹)	7.50 ± 1.91a	7.39 ± 1.35a	7.23 ± 2.11a	8.67 ± 1.86a	9.21 ± 1.56a	8.72 ± 1.72a
Total Biomass (g plant ⁻¹)	27.74 ± 2.73a	27.24 ± 3.21a	21.38 ± 3.07b	29.44 ± 3.51a	36.76 ± 3.72b	32.48 ± 2.97ab
Biomass Increment (%)	83.22	74.62	40.10	74.51	115.10	95.78

^a Note: Differences between treatments by species were considered significant at $P = 0.05$.



waterlogging stress, respectively (Figure 2a). At the end of the experiment, the net photosynthetic rate of SIh decreased by 34 and 46% under the moderate and severe waterlogging, respectively. There was no significant difference in the net photosynthetic rate of SIq among the three treatment groups ($P > 0.05$) (Figure 2-b).

Compared with the control, after 19 days of the waterlogging stress, the stomatal conductance of SIh under the moderate and severe waterlogging decreased by 35 and 56%, respectively (Figure 2-c). In contrast, the stomatal conductance of SIq was 24% greater than that of the control in the moderate waterlogging, but was not significantly different ($P > 0.05$) than the control under

severe waterlogging (Figure 2-d). At the end of the waterlogging treatment, the stomatal conductance of SIh under moderate and severe waterlogging decreased by 24 and 51%, respectively, compared with that of the control, whereas SIq showed no significant difference from the control.

The Chla and Chl(a+b) contents of SIh were significantly different among the treatments, whereas the Chla, Chlb, and Chl(a+b) contents of SIq exhibited no significant difference among the treatments ($P > 0.05$; Figures 3-a, -b, -d, -e, and -f). The chlorophyll contents of SIh began to decrease after 19 days and continued to decrease thereafter in

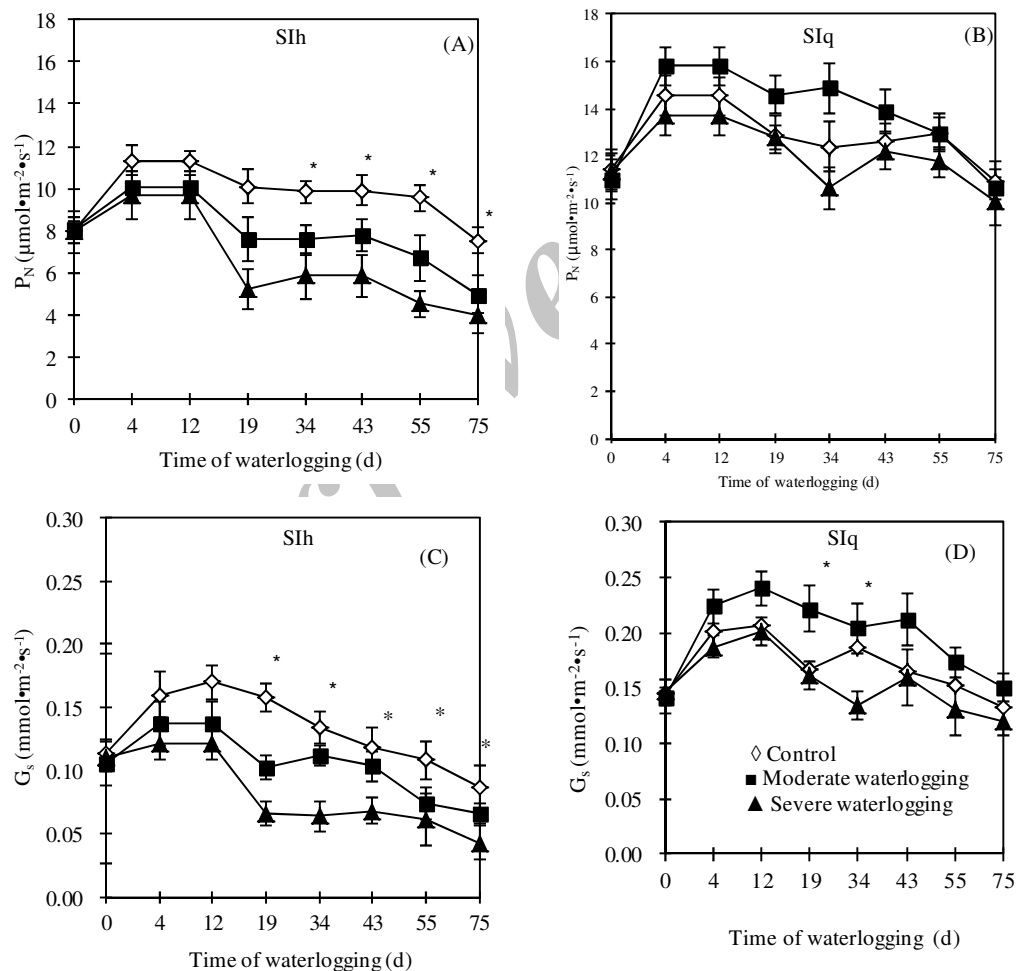


Figure 2. The effect of waterlogging stress on the photosynthetic rate and stomatal conductance of *Salix integra* cv. hongpi and cv. qingpi (mean \pm sd). Asterisks(*) indicate significantly different ($P < 0.05$).

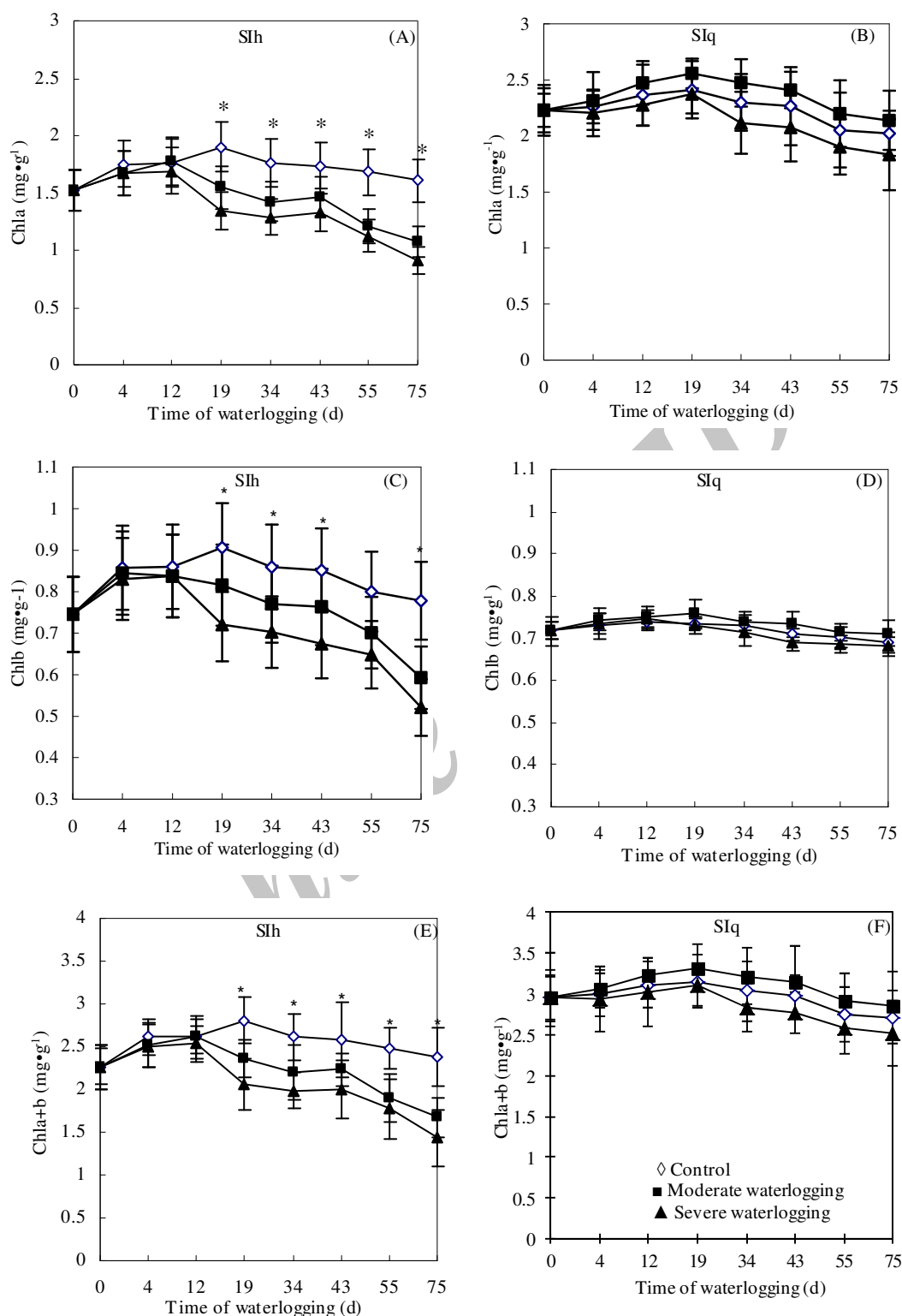


Figure 3. The effect of waterlogging stress on the chlorophyll contents of *Salix integra* cv. *hongpi* and cv. *qingpi* (mean ± sd). Asterisks (*) indicate significantly different ($P < 0.05$).



waterlogged trees compared to controls. The average contents of Chla, Chlb, and Chl(a+b) for SIh under the moderate waterlogging were 1.08 mg g^{-1} , 0.59 mg g^{-1} , and 1.67 mg g^{-1} , respectively, at the end of the experiment, and were 0.91 mg g^{-1} , 0.52 mg g^{-1} , and 1.43 mg g^{-1} , respectively, after the severe waterlogging. Compared with the control, the decrease in the Chla content of SIh was greater than that of the Chlb. The Chla and Chlb content of SIh under moderate waterlogging decreased by 32% and 24%, respectively, and the corresponding decrease was 43% and 33% under severe waterlogging. The fluctuation

in the chlorophyll content of SIq was relatively small throughout the study period; the chlorophyll content decreased only slightly after 43 days of the waterlogging stress (Figures 3-b, -d, and -f).

Under the two waterlogging stresses, the maximum photochemical efficiency (F_v/F_m) of SIh began to decrease after 34 days, with the average values of, respectively, 0.739 and 0.697 after 75 days, while the maximum efficiency of the control was 0.811. The F_v/F_m ratio of SIq fluctuated in the range of 0.8-0.85 during the waterlogging period, which was statistically similar to the values in the control (Figure 4a, b). The NPQ of

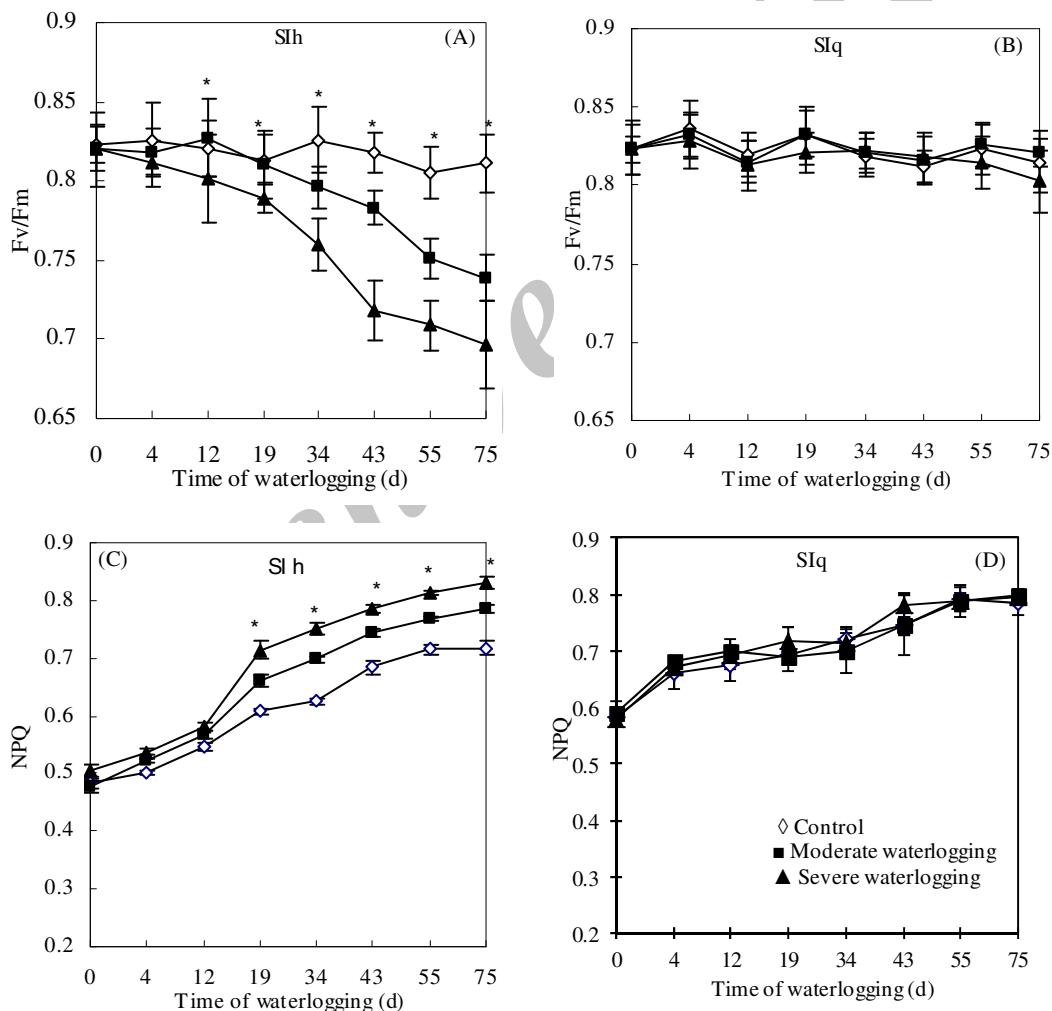


Figure 4. Variations in the maximum photochemical efficiency and non-photochemical quenching (NPQ) coefficient of *Salix integra* cv. *hongpi* and cv. *qingpi* under waterlogging stress (mean \pm sd). Asterisks(*) indicate significantly different ($P < 0.05$).

SIh began to increase after 34 days and continued to increase thereafter in waterlogged compared to the control. In the end of the experiment, the NPQ increased remarkably for SIh compared with that for the control, by 11 and 17%, respectively, under the moderate and severe waterlogging stress. There was no significant difference in NPQ of SIq among the three treatment groups ($P > 0.05$) (Figure 4-c and -d).

DISCUSSION

Effect of Waterlogging on the Morphology and Growth of *Salix integra*

Throughout the entire waterlogging experiment, the cuttings of both cultivars of *Salix integra* produced adventitious roots and hypertrophied lenticels, which are characteristic responses of tolerant wood species to waterlogging stress (Close and Davidson, 2003; Jing *et al.*, 2001; Yamamoto *et al.*, 1995). In a continuous waterlogging environment, the occurrence of adventitious roots is advantageous to the supply and regulation of water, nutrients, and growth regulators in plants (Emam and Bijanzadeh, 2012; Islam and Macdonald, 2004; Tsukahara and Kozlowski, 1995). The lenticel is the critical pathway of air exchange between the stem and root systems of a plant and the atmosphere (Kozlowski and Pallardy, 2002). Enlarged lenticels and aerenchymas increase gaseous diffusion, the total leaf area exposed to the oxygen in the air, and the amount of oxygen transported to the roots (Kozlowski, 1997; Yamamoto *et al.*, 1995). *Salix integra* cv. *qingpi* developed hypertrophied lenticels and adventitious roots earlier than *S. integra* cv. *hongpi* did and with more and longer adventitious roots.

The waterlogging stress had an obvious impact on the growth of base diameter, shoot height, and biomass accumulation of both cultivars. Severe waterlogging stress significantly reduced the shoot height and biomass accumulation for *S. integra* cv.

hongpi, whereas the base diameter decreased slightly. A previous study showed that waterlogging stress inhibited the growth of leaves and induced the abscission of leaves by hindering differentiation of leaf primordia and internode growth of plant (Cao *et al.*, 1993). The slight decrease in the base diameter may result from the formation of hypertrophied lenticels and aerenchymas at the base of the stem. The biomass of *S. integra* cv. *qingpi* was significantly different between moderate waterlogging and the control. However, the abovementioned growth parameters were not significantly different between the control and the severe waterlogging treatment. This finding is consistent with the results obtained in studies on *Taxodium distichum* (Hou, 2003), indicating that moderate waterlogging could promote the growth of *S. integra* cv. *qingpi*.

Effect of Waterlogging on Photosynthesis of *Salix integra*

The net photosynthetic rate and stomatal conductance of *S. integra* cv. *hongpi* under severe waterlogging decreased significantly in 19 days and, subsequently, decreased at a slower rate. The net photosynthetic rate and stomatal conductance of *S. integra* cv. *qingpi* under severe waterlogging recovered to the levels close to those of the control by the end of the experiment, which was similar to the observations in studies of *Liriodendron chinense*, *Cleistocalyx operculatus* (Jing *et al.*, 2000), and *Spartina patens* (Pezeshki *et al.*, 1993). The decrease in the net photosynthetic rate in the early stage of waterlogging was related to the closure of stomata and decreased stomatal conductance. The lowered CO₂ intake capability of the leaves leads to a smaller intercellular CO₂ concentration, and the substrate deficiency for photosynthetic enzymes directly leads to the lowering of the net photosynthetic rate (Farquhar and Sharkey, 1982; Malik *et al.*, 2001; Pezeshki *et al.*, 1996; Zoubeir *et al.*, 2012). The decrease in stomatal conductance may be



derived from the decrease in hydraulic conductivity between soil and plant or from the shortage in the oxygen supply to the root system (Mohd *et al.*, 2010; Vartapetian and Jackson, 1997). The emergence of adventitious roots and the formation of enlarged lenticels at the shoot closest to the water level in the middle and late stages of the waterlogging period alleviated the low oxygen status in the root system, thereby leading to the increase in the net photosynthetic rate and stomatal conductance. The increase in the net photosynthetic rate and stomatal conductance of *S. integra* cv. *qingpi* under the moderate waterlogging stress was similar to the reports on *Taxodium distichum* (Yang, 2007) and *Sapium sebiferum* (Cao *et al.*, 2010), which indicates that *S. integra* cv. *qingpi* is more tolerant to waterlogging.

Under waterlogging stress, leaves become less green and show signs of abscission, which is related to membrane damage, electrolyte leakage, and chlorophyll concentrations decrease (Christiane and Shabala, 2003; Islam and Macdonald, 2004; Kozlowski, 1997). However, several studies have shown that under waterlogging stress, sensitive plants exhibit no change in chlorophyll content (Pezeshki *et al.*, 1996). Early in the waterlogging period, the chlorophyll contents of the two cultivars of *S. integra* were similar to those of the control. After 12 days of waterlogging, the contents of Chla, Chlb, and Chl (a+b) in *S. integra* cv. *hongpi* began to decrease, and the leaves gradually became less green. In contrast, the chlorophyll contents in *S. integra* cv. *qingpi* changed little and were only slightly lower in the later stages of the waterlogging, with only a limited number of leaves on the lower part of the cuttings turning yellow and falling off. Moreover, the decrease in the Chla content in *S. integra* cv. *hongpi* was greater than that of the Chlb, which is similar to a report on *Hibiscus esculentus* (Ashraf and Arfan, 2005). This result indicated that the degradation of the photosynthetic reaction center in the leaves was higher than the degradation of the light-

harvesting chlorophyll a/b protein complex because in photosynthesis, Chla is predominantly bound to the photosynthetic reaction center, whereas Chlb is bound to the light-harvesting chlorophyll a/b protein complex (Larcher, 2003).

Changes in the chlorophyll fluorescence parameters reflected the degree of damage to photosystem II. The change in the F_v/F_m ratio revealed the influences on the net photosynthetic rate. A sustainable and stable F_v/F_m ratio indicates an undamaged photosystem II (Ball *et al.*, 1994; Jones *et al.*, 2006; Lavinsky *et al.*, 2007). NPQ reflects the degree of heat dissipation in the photochemical reactions, i.e. the quantity of the energy that was not used in the photochemical reactions (Christiane and Shabala, 2003; Maxwell and Johnson, 2007). The F_v/F_m ratio and NPQ of *S. integra* cv. *hongpi* under severe waterlogging continuously decreased and increased, respectively, in a pattern similar to that of the net photosynthetic rate, which is in accordance with previous findings (Close and Davidson, 2003; Yi *et al.*, 2006) and also revealed the change in the photochemical properties of *S. integra* cv. *hongpi* under waterlogging stress. The F_v/F_m ratio and NPQ of *S. integra* cv. *qingpi* under both moderate and severe waterlogging stresses were not significantly different from the control, which is in accordance with the previous studies (James *et al.*, 2002; Mielke *et al.*, 2003; Roohi *et al.*, 2013; Wu and Bao, 2011). This observation indicates that *S. integra* cv. *qingpi* was highly capable of adapting to waterlogging stress, with stable photosystem II activity and a fluctuating net photosynthetic rate that likely resulted from changes in stomatal conductance.

In general, the potential damage to a plant due to waterlogging stress increases with the duration of the stress. Few plants could only withstand continuous waterlogging for 1-3 months. The fact that both cultivars of *S. integra* survived 100% after 75 days of waterlogging indicates that *S. integra* has high waterlogging tolerance. The growth and photosynthesis mechanisms of *S.*

integra cv. *hongpi* were retarded by the waterlogging, whereas moderate waterlogging promoted biomass production for *S. integra* cv. *qingpi*, indicating that it was more tolerant to waterlogging than *S. integra* cv. *hongpi* and thus more suitable for the riparian and fluctuating groundwater level zones. Presently, laboratory experiments performed predominantly on seedlings provide important insights into the mechanisms of flooding tolerance. However, it is difficult to apply these results to adult trees or *in situ* conditions. To achieve a better understanding of the flooding tolerance of this tree species, the findings from the laboratory experiments must be compared and validated with field experiments. The photo-physiological recovery mechanism of *S. integra* after waterlogging also remains to be investigated, especially with *S. integra* cv. *hongpi*.

ACKNOWLEDGEMENTS

This study was supported by the National Key Support Program of Science and Technology for the Eleventh-Five-Year Plan of PR China (No. 2009BADA6B05). We are grateful to Zhang M.X. and Zhu L.L. for skillful technical assistance in the laboratory. Ying W.B., Wang L. and Yang J. assisted in data collection. We would like to thank three anonymous reviewers for their constructive comments.

REFERENCES

- Ashraf, M. and Arfan, M. 2005. Gas Exchange Characteristics and Water Relations in Two Cultivars of *Hibiscus esculentus* under Waterlogging. *Biol. Plantarum*, **49**: 459- 462.
- Ball, M.C., Butterworth, J.A., Roden, J.S., Christian, R. and Egerton, J. G. 1994. Applications of Chlorophyll Fluorescence to Forest Ecology. *Plant Physiol.*, **22**: 311-319.
- Cao, F. L., Cai, J. F., Wang, G. B. and Zhang, W. X. 2010. Effects of Waterlogging Stress on the Growth and Photosynthesis of *Sapium sebiferum*. *Scientia Silvae Sinicae*, **46(10)**: 57-61.
- Cao, F. L. and Robert, E. F. 1993. Effect of Artificial Flooding on Physiological Characteristics of *Populus Deltoides* Clones. *J. Nanjing Forestry Uni.*, **17(2)**: 29-44.
- Carvalho, L. C. and Amancio, S. 2002. Antioxidant Defence System in Plantlets Transferred From in Vitro to Ex Vitro Effects of Increasing Light Intensity and CO₂ Concentration. *Plant Sci.*, **162**: 33-40.
- Christiane, F. S. and Shabala, S. 2003. Screening Methods for Waterlogging Tolerance in Lunceme: Comparative Analysis of Waterlogging Effects on Chlorophyll Fluorescence, Photosynthesis, Biomass and Chlorophyll Content. *Funct.l Plant Biol.*, **30**: 335-343.
- Close, D. C. and Davidson, N. J. 2003. Long-term Waterlogging: Nutrient, Gas Exchange, Photochemical, and Pigment Characteristics of *Eucalyptus nitens* Saplings. *Russ. J. Plant Physiol.*, **50**: 843-847.
- Emam, Y. and Bijanzadeh, E. 2012. Water Uptake and Hydraulic Conductivity of Seminal and Adventitious Roots of Five Wheat Cultivars at Early Growth Stage. *J. Agr. Sci. Tech.*, **14**: 1605-1616.
- Farquhar, G. and Sharkey, T. D. 1982. Stomatal Conductance and Photosynthesis. *Annu. Rev. Plant Physiol. Mol. Biol.*, **33**: 317-345.
- Hou, E. Y. 2003. Effects of Water Stress on the One-Year Seedling Growth and Physiology Properties of *Pteroceltis Tatarinowii*, *Taxodium Distichum*, and *Fraxinus Velutina*. *J. Nanjing Forestry Uni.*, **15(1)**: 27-42.
- Islam, M.A. and Macdonald, S. E. 2004. Ecophysiological Adaptations of Black Spruce (*Picea mariana*) and Tamarack (*Larix laricina*) Seedlings to Flooding. *Trees*, **18**: 35-42.
- James, R. A., Rivelli, A. R., Munns, R. and Von Caemmerer, S. 2002. Factors Affecting CO₂ Assimilation, Leaf Injury, and Growth in Salt-stressed Durum Wheat. *Funct. Plant Biol.*, **29**: 1393-1403.
- Jing, Y. X., Cheng, Z. P., Cheng, H. Y. and Mo, X. M. 2000. The Relationship between Photosynthetic Character and Adventitious Roots in Flooded *Cleistocalyx Operculatus*



- seedlings. *J. Trop. Subtrop. Bot.*, **8(1)**: 361-364.
14. Jones, D. T., Sah, J. P., Ross, M. S., Oberbauer, S. F., Hwang, B. and Jayachandran, K. 2006. Responses of Twelve Tree Species Common in Everglades Tree Islands to Simulated Hydrologic Regimes. *Wetlands*, **26**: 830-844.
 15. Kozłowski, T. T. 1997. Responses of Woody Plants to Flooding and Salinity. *Tree Physiol.*, **1**: 1-29.
 16. Kozłowski, T. T. and Pallardy, S. G. 2002. Acclimation and Adaptive Responses of Woody Plants to Environmental Stresses. *Bot. Rev.*, **68**: 270-334.
 17. Larcher, W. 2003. *Physiological Plant Ecology*. Springer, Berlin, Heidelberg, New York, PP. 1-5.
 18. Lavinsky, A.O., De Souza Sant, C., Mielke, M.S., De Almeida, A.F., Gomes, F.P., Franca, S. and Da Costa Silva, D. 2007. Effects of Light Availability and Soil Flooding on Growth and Photosynthetic Characteristics of *Genipa Americana* L. seedlings. *New Forest.*, **34**: 41-50.
 19. Li, H. S. 2000. *Principles and Techniques of Plant Physiological Biochemical Experiment*. Higher Education Press, Beijing, PP.134-137. (in Chinese)
 20. Li, X. M. and Kakubari, Y. 2001. Photosynthesis and Chlorophyll a Fluorescence of Two Poplars under Water Stress. *J. Forest Res.*, **6**: 211-215.
 21. Malik, A.I., Colmer, T.D., Lambers, H., and Schortemeyer, M. 2001. Changes in Physiological and Morphological Traits of Roots and Shoots of Wheat in Response to Different Depths of Waterlogging. *Aust. J. Plant Physiol.*, **28**: 1121-1131.
 22. Maxwell, K. and Johnson, G. N. 2007. Chlorophyll Fluorescence: A Practical Guide. *J. Exp. Bot.*, **51**: 659-668.
 23. Mielke, M. S., De Almeida, A. F., Gomes, F. P., Aguilar, A.G. and Mangabeira, P. A. 2003. Leaf Gas Exchange, Chlorophyll Fluorescence, and Growth Responses of *Genipa Americana* Seedlings to Soil Flooding. *Environ. Expe. Bot.*, **50**: 221-231.
 24. Mohd, I., Shamsul, H., Qaiser, H., Shaheena, A. and Aqil, A. 2010. Physiological and Biochemical Changes in Plants under Waterlogging. *Protoplasma*, **241**: 3-17.
 25. Nakai, A., Hiromitsu, K. and Kisanuki, H. 2011. Stress Responses of *Salix gracilistyla* and *Salix subfragilis* Cuttings to Repeated Flooding and Drought. *J. Forest Res-Jpn*, **16**: 465-472.
 26. Nakai, A., Yurugi, Y. and Kisanuki, H. 2010. Stress Responses in *Salix gracilistyla* Cuttings Subjected to Repetitive Alternate Flooding and Drought. *Trees*, **24**: 1087-1095.
 27. Pezeshki, S.R., Pardue, J. H. and Delaune, R. D. 1993. The Influence of Soil Oxygen Deficiency on Alcohol Dehydrogenase Activity, Root porosity, Ethylene Production, and Photosynthesis in *Spartina patens*. *Expe. Bot.*, **33**: 565-573.
 28. Pezeshki, S. R., Pardue, J. H. and Delaune, R. D. 1996. Leaf Gas Exchange and Growth of Flood-tolerant and Flood-sensitive Tree Species under Low Soil Redox Conditions. *Tree Physiol.*, **16**: 453-458.
 29. Roohi, E., Tahmasebi-Sarvestani, Z., Modarres-Sanavy, S. A. M. and Siosemardeh, A. 2013. Comparative Study on the Effect of Soil Water Stress on Photosynthetic Function of Triticale, Bread Wheat, and Barley. *J. Agr. Sci. Tech.*, **15**: 215-228.
 30. Schwanz, P., Picon, C. and Vivin, P. 1996. Responses of Antioxidative Systems to Drought Stress in *Pendunculatae* oak and Maritime Pine as Modulated by Elevated CO₂. *Plant Physiol.*, **110**: 393-402.
 31. Tang, L. Z., Xu, X. Z. and Fang, S. Z. 1998. Influence of Soil Waterlogging on Growth and Physiological Properties of Poplar and Willow Seedlings. *Chinese J. Appl. Ecol.*, **9(5)**: 471-474.
 32. Tsukahara, H. and Kozłowski, T. T. 1995. Importance of Adventitious Roots to Growth of Flooded *Platanus occidentalis* Seedlings. *Plant Soil*, **88**: 123-132.
 33. Vartapetian, B. B. and Jackson, M. B. 1997. Plant Adaptation to an Aerobic Stress. *Ann. Bot-London.*, **79(Suppl. A)**: 3-20.
 34. Wu, X. and Bao, W. 2011. Leaf Growth, Gas Exchange, and Chlorophyll Fluorescence Parameters in Response to Different Water Deficits in Wheat Cultivars. *Plant Prod. Sci.*, **14(3)**: 254-259.
 35. Yamamoto, F., Sakata, T., and Terazawa, K. 1995. Physiological, Morphological and Anatomical Responses of *Fraxinus mandshurica* Seedlings to Flooding. *Tree Physiol.*, **15**: 713-719.

36. Yang J., He K.Y., Li X.C. and Hang L.B. 2008. Influences of Waterlogging Stress on the Growth and Leaf Anatomical Structures of Two Oak Species. *China Forestry Sci. Tech.* **22**:34-37
37. Yi, Y. H., Fan, D. Y., Xie, Z. Q. and Chen, F. Q. 2006. Effects of Waterlogging on the Gas Exchange, Chlorophyll Fluorescence and Water Potential of *Quercus Variabilis* and *Pterocarya Stenoptera*. *J. Plant Ecol-UK*, **30** (6): 960-968.
38. Zoubeir, B., Ali, A., Mohammed S., Lamhamedi, Mejda, A., and Mohamed, H. 2012. Adaptation and Morpho-physiology of Three *Populus deltoides* Marsh. × *P. nigra* L. Clones after Preconditioning to Prolonged Waterlogging. *Agroforest Syst.*, **86**: 433-442.

رشد، تبادل گازی برگ، و فلونورسنس کلروفیل دو رقم بید *Salix integra Thunb.* در پاسخ به تنش ماندابی

ح. ف. ژاوو، ی. ژاوو، س. ژانگ، ژ. تاوو، ژ. ن. ژوو

چکیده

بید *Salix integra* درختچه ای در چین است که در کناره های رودخانه هیوآی پخش است و نقش مهمی در حفاظت خاک و آب دارد. زمین های زیر این درختچه در طی ماه های ژوئیه تا اکتبر به آسانی زیر سیلاب رودخانه هیوآی می رود. مطالعه حاضر به منظور تعیین ساز و کار های فیزیولوژیکی تحمل شرایط ماندابی در *Salix integra* و نیز برای انتخاب منطقی گونه های مقاوم به ماندابی برای کاستن از صدمات سیل به تولیدات کشاورزی انجام شد و در آن ما اثر های سیل و ماندابی را روی رشد، تبادل گازی، و فلونورسنس کلروفیل قلمه های *Salix integra* در طی یک دوره رشد (اوت تا اکتبر ۲۰۱۱) بررسی کردیم. تولید زیست توده و فتوسنتز کالتیوار *S. integra* cv. Qingpi در شرایط تنش متوسط ماندابی بهبود یافت در حالی که رشد، محتوی کلروفیل و نرخ خالص فتوسنتز و هدایت روزنه ای این کالتیوار همگی به طور معنی داری کمتر از قلمه های گروه شاهد بود. همچنین افزایش معنی داری در خاصیت غیر-فتوشیمیایی ضد نوری (NPQ) و کاهش PSII در کار آبی بیشینه کوانتوم بیو شیمیایی (F_v/F_m) در قلمه های غرقاب شده کالتیوار *S. integra* cv. hongpi مشاهده شد. اما، در مورد کالتیوار *S. integra* cv. Qingpi تفاوت معنی داری در مقدار NPQ و F_v/F_m بین تیمار ماندابی و شاهد وجود نداشت. نتایج چنین نشان می دهد که در شرایط غرقابی، دستگاه فتوسنتز کالتیوار *S. integra* cv. hongpi اندکی صدمه دید. بر عکس، تنش متوسط ماندابی منجر به افزایش تولید برگ و ریشه *S. integra* cv. qingpi شد که چنین اشاره دارد که *S. integra* cv. qingpi می تواند سیلاب طولانی مدت را بهتر از *S. integra* cv. hongpi تحمل نماید. بنابراین، *S. integra* cv. qingpi برای درختکاری در کناره های رودخانه مناسب تر است.