

The effects of flooding on several hybrid poplar clones in Northern China

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Abstract The ecophysiological, morphological, and growth characteristics of 14 poplar clones were studied during 37 days of flooding and a 13-day recovery period. Cuttings were subjected to three soil water regimes, viz. drained (control), shallow flooding to 10 cm above the soil, and deep flooding to a depth of 120 cm. All hybrids modified their ecophysiological and morphological patterns to decrease carbon loss and maintain water balance. In response to flooding, all 14 hybrids reduced their expansion and initiation of new leaves, reduced height and root collar growth, and reduced the number of leaves. For shallowly flooded plants, adventitious roots developed by day 14, and their number increased with flooding duration; net photosynthesis, stomatal conductance, and growth decreased significantly compared with the control; dry weights of roots, leaves, and total biomass decreased and the allocation of growth to shoots and roots changed. After flooding ended, net photosynthesis recovered, but stomatal conductance recovered before net CO₂ assimilation since photosynthesis was

limited by stomatal factor at the initial stage of stress and it was limited by non-stomatal factors over relatively long periods of stress. Transpiration and the amount of water obtained from the roots both decreased. In the deeply flooded plants, similar but often more severe changes were observed. Based on our results, we classified the hybrids into three types using hierarchical cluster analysis. Clones 15-29, 196-522, 184-411, 306-45, 59-289, DN-2, DN-182, DN-17, DN-14274, NE-222, DTAC-7, and R-270 were flood-tolerant, clone NM-6 was flood-susceptible, and clone 328-162 was moderately flood-tolerant.

Keywords Biomass allocation · Flooding · Flood tolerance · Leaf gas exchange · Photosynthesis · *Populus*

Introduction

Natural forest in China occupies about 10% of the country's area, and is far from sufficient to meet the ever-increasing demand for wood products. As a multi-functional species, hybrid poplar (*Populus* spp.) has been identified as a promising candidate for plantation establishment to fill the gap between the world's supply and demand for wood (Jain and Singh 2000; Schreiner 1959; White et al. 1988). The cultivation of poplars in China can be traced back to the 7th century A.D.

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However, due to degeneration of varieties, slower growth rates, increased susceptibility to disease, simplified approaches to breeding and inefficient management, the clones that were developed can no longer meet modern demands. As a result, it is necessary to select and introduce superior genotypes identified based on local experiments in China. There are large areas of flood plain in China, especially along the middle and lower reaches of the Yangtze River and the Yellow River, and these areas have a high potential for supporting plantations of fast-growing poplars in the future. However, these areas are frequently flooded. To ensure that the correct clones are chosen, it is important to study the eco-physiological and morphological characteristics of hybrid poplar under flooding to serve as a reference for selection and introduction of flood-tolerant clones for use in these areas.

A large body of literature exists on the characteristics of plants that are responsible for tolerance of flooding (Brandle 1991; Fernandez et al. 1997; Pezeshki and Chambers 1985; Pezeshki 2001; Rood et al. 2003). Under flooded conditions, root tolerance of flooding is a key factor that governs plant survival. The ability to form adventitious roots is an important indicator of the adaptation mechanisms (Gill 1970; Kawase 1981; Kozłowski 1997). Adventitious roots play a positive role in supporting shoot growth by enhancing water and nutrient uptake during prolonged flooding (Armstrong et al. 1994; Hook 1984; Jackson 1985). One of the initial responses to flooding is stomatal closure (Marcelo et al. 2003; Pezeshki 1994, 2001) and in accordance with the reduced stomatal conductance, photosynthesis decreases rapidly under continued flooding (Pezeshki 2001). As a result, biomass accumulation and shoot and root growth decrease, and the allocation patterns of biomass are affected (Kozłowski 1997; Lopez and Kuesar 1999; Rubio et al. 1995). However, studies on the effects of flooding on popular hybrid clones are limited (Cao and Conner 1999; Levine and Stromberg 2001; Peng 2003). Our goal in the present study was not to demonstrate new mechanisms of adaptation for wetland plants, but rather to show what adaptations occurred in a range of hybrid poplar clones under flooding. Furthermore,

because poplars have a highly variable genotype, there have been no comprehensive studies on how interactions between physiological processes and the morphology of different hybrid poplar genotypes determine a clone's production potential, especially under field conditions. Thus, evaluations of the relative performance of different clones under flooding are required to identify superior clones that would be suitable for use in areas of China subject to flooding.

We chose 14 hybrid clones first introduced into China in 2004 for our studies of the responses of poplar to flooding. The objectives of our study were: (i) to investigate how different degrees of flooding change the eco-physiological responses and morphological adaptations of hybrid poplar; and (ii) to select flood-tolerant clones suitable for use on flood plains, especially those along the middle and lower reaches of the Yangtze River and the Yellow River.

Materials and methods

Plant material and experiments

Our study were performed at a centre for the introduction of hybrid poplar in the southwestern suburbs of Beijing, China (39°45' N, 116°51' E). This region has a warm, temperate climate, with an annual average of 200 frost-free days, 655 mm of precipitation, and a mean yearly temperature of 11.6 °C. About 1-year-old shoots of 14 American clones were selected: 15-29 (*P. trichocarpa* × *P. deltoids*, female), 196-522 (*P. trichocarpa* × *P. deltoids*), 184-411 (*P. trichocarpa* × *P. deltoids*, male), 59-289 (*P. trichocarpa* × *P. deltoids*, male), 328-162, 306-45 (*P. trichocarpa* × *P. nigra* male), DN-2 (*P. deltoides* × *P. nigra*), DN-182 (*P. deltoides* × *P. nigra*, male), DN-17 (*P. deltoides* × *P. nigra*, male), DN-14274 (*P. deltoides* × *P. nigra*), NE-222 (*P. deltoides* × *P. nigra* var. *caudina*), DTAC-7 (*P. deltoides* × *P. trichocarpa*), R-270 (*P. deltoides* × *P. nigra*), and NM-6 (*P. nigra* × *P. maximowiczii*, female). Before planting, the shoots were cut into pieces 10–15 cm long with similar diameter of stem and each of the

cuttings had three to four buds after soaking in water for 12 h. In May 2004, we planted these cuttings in 40 × 45 cm pots containing natural field soil (a light loam), then grew them in the open air. We initiated the flooding treatment when the cuttings had reached a mean height of 80–90 cm. Cuttings of each clone were randomly assigned to three treatments with a 49-day study duration. Each treatment group consisted of nine cuttings of each clone. In the control, we held the soil water content at field capacity. In the shallow flooding treatment, the cuttings remained in a pond where they were continuously flooded to a depth of 10 cm above the soil surface; water was periodically added whenever the water level decreased due to evapo-transpiration. In contrast, cuttings in the deep flooding treatment were submerged completely, to a depth of 120 cm, in ponds. We observed and measured the cuttings at 0, 2, 3, 4, 7, 17, and 37 days. During the measurement at each time, water in the pond was pumped out and the measurement was accomplished within 40 min. After 36 days of flooding, we removed the cuttings from the flood treatment and allowed them to recover for 13 days. We recorded the survival, level of defoliation (number of leaves that were lost), and visible morphological changes in the stems, leaves, and roots throughout the experimental period.

Measurement of leaf gas exchange

We measured gas exchange in the third fully expanded and mature leaf from the top of the stem in all cuttings from 09:00 to 11:00 h using the Li-Cor 6,400 portable photosynthesis system (Li-Cor, Inc., Nebraska, USA); these measurements included net CO₂ assimilation rate (P_n), transpiration rate (E), and stomatal conductance (g_s), as well as the corresponding ambient environmental conditions (temperature, relative humidity, and photosynthetically active radiation, PAR). We measured the leaf water potential (Ψ) of the same leaf using a ZLZ-4 pressure chamber (Lanzhou Univ., China) after the gas exchange was measured. Water was pumped from pond for a 40 min interval each time measurements were made.

Growth parameters

The height, root-collar diameter, leaf number, and leaf area of each cutting of the 14 clones were measured at 0, 7, 17, and 37 days of the flooding treatment, and the treatment was stopped at the 13 days after flooding. The root-collar diameter and the length of the stems were measured with calipers and a ruler, respectively. Leaf areas were estimated using an LI-3000 automatic Leaf Area Meter (Li-Cor, Inc., USA). Biomass production and partitioning were determined only at the end of the experiment by dividing each plant into stem, leaves, and roots; in addition, the stems and leaves were combined to produce a total shoot value. Biomass samples were dried at 75°C to constant weight, and then weighed using a milligram balance. Then the root/shoot ratio was calculated. To compare the effects of each flooding treatment on plant growth, the rate of change in the canopy areas and biomass were calculated as follows:

$$\text{Change} = (C - F)/C$$

where C represents the control value and F represents the value in the flooding treatment.

Statistical analysis

The data for all eco-physiological and morphological parameters were subjected to analysis of variance (ANOVA) using the SPSS software (version 10.0; SPSS Inc., Chicago, Illinois, USA) supplemented with a multiple-comparison test of the means using the least significant difference (LSD) method with a significance level of $P < 0.05$. We also used hierarchical cluster analysis to classify the clones based on the results of our experiment.

Results

Morphological responses of different hybrid clones to flooding

The 14 hybrid clones exhibited different responses to 37 days of flooding. Although the

survival rate of all clones was 100% in each treatment, we observed clear symptoms of the effects of flooding in the flooded plants. The initiation and expansion of new leaves decreased in both treatments compared with the control. At the same time, the height, root-collar, and leaf growth were retarded. The number of leaves on the flooded plants were also somewhat smaller than in the control. After 14 days of flooding, 4–16 leaves of each cutting had abscised in the deep flooding treatment, but no leaves had been lost in the shallow flooding treatment. By day 37 (the first day after the flooding was ended), the number of shed leaves differed significantly among clones and treatments: Under shallow flooding, this number ranged from 8 for clone NM-6 to 21 for clones DN-14274 and 306-45; under deep flooding, this number ranged from 15 for clone DTAC-7 to 32 for clone DN-14274. In contrast with the shallowly flooded plants, the upper leaves of some submerged clones (15-29, 184-411, 328-162, and 306-45) exhibited chlorosis, whereas only the oldest leaves of the other clones appeared chlorotic. Adventitious roots developed from the bases of the stems of the shallowly flooded cuttings, and their numbers increased as the duration of the flooding increased; in contrast, no adventitious roots appeared in the deeply submerged plants. Some roots of the submerged plants became dark during destructive sampling of the cutting.

Growth and biomass

The height, root-collar, and leaf area growth were significantly inhibited by flooding (Fig. 1). With increasing duration of flooding, there was a significant reduction ($P < 0.001$) in height, root-collar, and leaf area growth in all 14 clones. However, the clones differed significantly ($P < 0.001$) in the magnitude of the response. There was also a significant interaction between flooding treatment and clone in terms of these growth variables ($P < 0.001$; Fig. 1). The increase in height growth of DN-2 and DN-17 was the smallest in both flooding treatments (1.53 for clone DN-2 and 0.71 for clone DN-17 in the deep flooding treatment, and 4 for both clones in the shallow flooding treatment) (data not shown on

figure). Clones 184-411, 59-289, and 15-29 had the highest growth in the deep flooding treatment and clones 328-162 and R-270 had the highest growth in the shallow flooding treatment. Under deep flooding, the height growth of some clones (DN-2, DN-17) ceased during the first 7 days of flooding (data not shown); other clones continued growing until day 37 (15-29, 195-522, 184-411, 328-162, and 306-45), and most clones ceased growing by day 17. As the flooding continued, the total leaf area decreased compared with the control due to a decrease in leaf area and a reduction in the expansion and initiation of new leaves (Fig. 1). The magnitude of the decrease in total biomass and canopy area differed among treatments and clones. The decreases were largest in clones 328-162 (for Low-pond type), DN-17, DN-14274, DN-2 and DN-182, whereas the decreases in clones NE-222, R-270, and 15-29 were small. Compared with the control, all 14 clones showed a significant decrease in canopy area in both flooding treatments ($P < 0.001$), and some of these decreases were large; for example, clones 15-29 and R-270 showed decreases of 36.99 and 12.66% in the shallow flooding treatment and 36.27 and 2.129% in the deep flooding treatment. Analysis of covariance indicated that both flooding treatment and clone had a significant effect on total biomass ($P < 0.001$). The interaction between flooding treatment and clone also significantly affected total biomass ($P < 0.001$). At the end of the experiment, the total biomass of the flooded cuttings had decreased compared with the control ($P < 0.001$). However, the total plant biomass of clone 195-522 increased in the shallow flooding treatment compared with the control, and clone 306-45 appeared to be unaffected by flooding. There were significant differences among clones in total biomass ($P < 0.001$).

Both flooding treatments significantly reduced shoot, root and leaf dry weights ($P < 0.001$). Clones differed significantly ($P < 0.001$) in their growth response to the flooding treatment, and the interaction between flooding treatment and clone significantly affected the growth parameters (Table 1). The shoot, stem, root, and leaf dry weights of the 14 hybrid clones were lower in the two flooding treatments than in the control (Table 1, $P < 0.001$). We also observed a

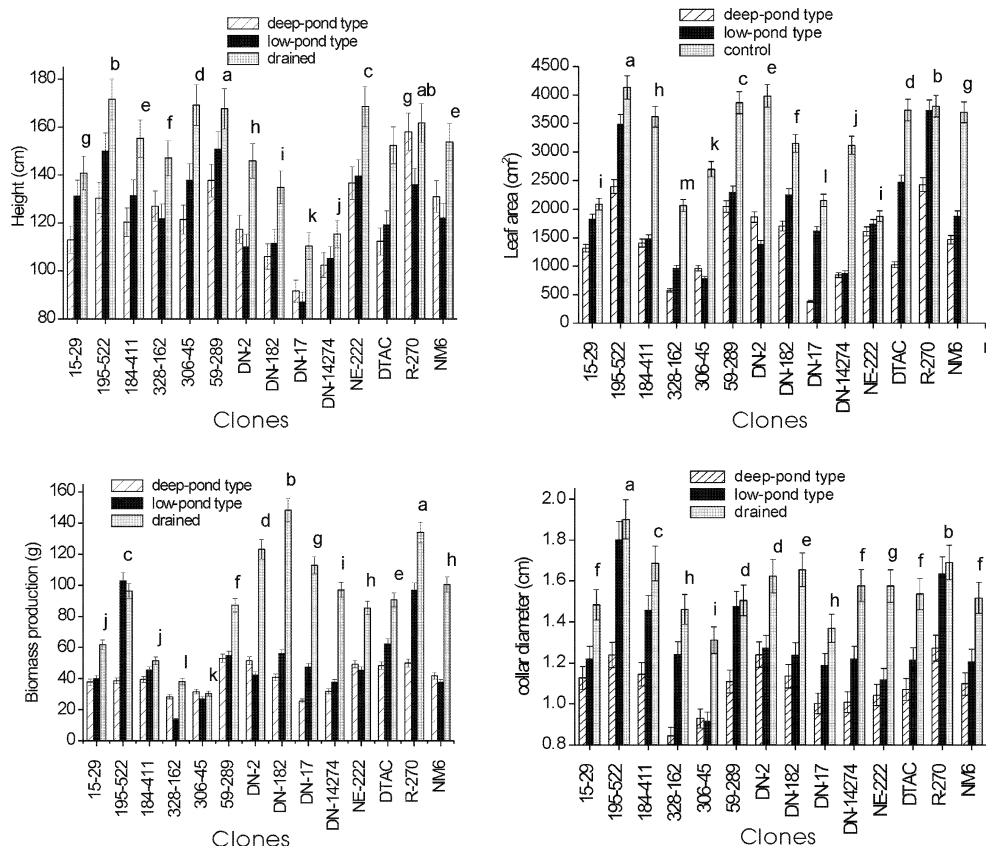


Fig. 1 The height, root-collar diameter, leaf area, and biomass production of 14 clones under different flooding conditions: control, shallow flooding, and deep flooding. Values were measured after 49 days, 13 days after the

flooding treatments were ended. For each of the four parameters, bars labeled with different letters differ significantly ($P < 0.001$)

significant difference in the photosynthate allocation pattern. Compared with the control, all flooded clones had a lower root weight ($P < 0.001$) and a lower leaf weight ($P < 0.001$). The stem weight was generally lower than that of the control ($P < 0.05$), with the exception of clones 184-411, 306-45, and 59-289. Therefore, the decrease in total biomass of the plants exposed to flooding was mainly due to a significant reduction in root and leaf weight. The root/shoot ratio was significantly affected by flood treatment, clone and the interaction between flooding treatment and clone ($P < 0.001$), most clones has a higher root/shoot ratio in the shallow than other treatment.

Gas exchange

We observed significant differences in net photosynthesis between the flooding treatments and the

control for all clones ($P < 0.001$, Fig. 2). The photosynthetic rate of all clones decreased significantly under flooding, and generally decreased more sharply with deeper flooding. Analysis of covariance indicated that flooding treatment and duration both had a significant effect on P_n ($P < 0.001$). The interaction between flooding treatment and duration also had a significant effect on P_n ($P < 0.001$). Compared with the control, the P_n values of DN-17, DN-14274, and NM-6 under deep flooding decreased by 76.54, 72.27, and 82.28%, respectively, by the third day. After 17 days of flooding, the P_n of some shallowly flooded clones increased, such as 15-29, 184-411, and 328-162. After the flooding was ended and cuttings were allowed to recover for 13 days, the P_n of most plants increased to a level similar to that in the control, with the exception of clones 15-29, 184-411, DN-17,

Table 1 Dry weights of shoots, stem, and roots and the root/shoot ratio of 14 poplar clones under three conditions (drained type, low-pond type, and deep-pond type) at the end of the study

Clone	Treatment	Shoot DW	Stem DW	Leaf DW	Root DW	Root/shoot ratio
15-29	Drained type	42.16 ± 1.32	21.08 ± 0.63	21.08 ± 0.23	19.65 ± 0.25	0.47 ± 0.07
	Low-pond type	26 ± 0.91	19.85 ± 0.52	6.153 ± 0.17	14.21 ± 0.14	0.55 ± 0.09
	Deep-pond type	25.54 ± 1.04	19.51 ± 0.56	6.03 ± 0.21	12.26 ± 0.09	0.48 ± 0.04
195-522	Drained type	70.99 ± 0.72	35.49 ± 0.71	35.49 ± 0.47	31.19 ± 0.21	0.48 ± 0.06
	Low-pond type	66.91 ± 1.21	48.11 ± 0.84	18.79 ± 0.26	35.87 ± 0.26	0.54 ± 0.07
	Deep-pond type	33.40 ± 0.63	21.05 ± 0.42	12.35 ± 0.13	13.15 ± 0.13	0.39 ± 0.02
184-411	Drained type	29.26 ± 0.72	14.63 ± 0.36	14.63 ± 0.24	19.02 ± 0.26	0.59 ± 0.04
	Low-pond type	30.25 ± 0.56	21.27 ± 0.43	8.98 ± 0.15	15.19 ± 0.14	0.51 ± 0.06
	Deep-pond type	27.72 ± 0.43	20.69 ± 0.52	7.03 ± 0.13	11.76 ± 0.13	0.42 ± 0.03
328-162	Drained type	27.36 ± 0.76	13.68 ± 0.64	13.68 ± 0.18	12.38 ± 0.25	0.48 ± 0.06
	Low-pond type	7.39 ± 0.43	5.24 ± 0.24	2.15 ± 0.09	6.34 ± 0.09	0.86 ± 0.11
	Deep-pond type	18.91 ± 0.62	16.75 ± 0.36	2.157 ± 0.07	9.28 ± 0.08	0.49 ± 0.06
306-45	Drained type	24.63 ± 0.65	12.31 ± 0.31	12.31 ± 0.32	8.28 ± 0.09	0.37 ± 0.05
	Low-pond type	14.40 ± 1.25	10.72 ± 0.21	3.68 ± 0.07	12.46 ± 0.11	0.87 ± 0.06
	Deep-pond type	19.16 ± 10.52	16.33 ± 0.32	2.83 ± 0.03	12.52 ± 0.16	0.65 ± 0.01
59-289	Drained type	62.22 ± 0.89	31.11 ± 0.65	31.11 ± 0.32	25.36 ± 0.34	0.41 ± 0.05
	Low-pond type	37.07 ± 0.72	26.05 ± 0.42	11.02 ± 0.21	17.71 ± 0.28	0.48 ± 0.06
	Deep-pond type	34.32 ± 0.94	26.86 ± 0.54	7.46 ± 0.12	18.75 ± 0.21	0.55 ± 0.02
DN-2	Drained type	98.85 ± 1.12	49.42 ± 0.73	49.42 ± 0.38	41.68 ± 0.52	0.51 ± 0.06
	Low-pond type	26.4 ± 0.54	18.92 ± 0.32	7.718 ± 0.24	15.87 ± 0.27	0.59 ± 0.07
	Deep-pond type	34.61 ± 0.67	30.40 ± 0.45	4.21 ± 0.18	17.01 ± 0.15	0.49 ± 0.03
DN-182	Drained type	98.34 ± 1.32	48.99 ± 0.68	49.34 ± 0.65	56.57 ± 0.67	0.69 ± 0.08
	Low-pond type	35.29 ± 0.94	24.94 ± 0.53	10.36 ± 0.26	20.79 ± 0.23	0.59 ± 0.07
	Deep-pond type	25.19 ± 0.82	19.143 ± 0.36	6.042 ± 0.14	15.72 ± 0.16	0.62 ± 0.09
DN-17	Drained type	73.91 ± 1.14	30.82 ± 0.62	28.96 ± 0.37	35.18 ± 0.34	0.83 ± 0.08
	Low-pond type	27.28 ± 0.58	21.11 ± 0.43	6.177 ± 0.26	19.99 ± 0.26	0.73 ± 0.06
	Deep-pond type	12.61 ± 0.51	11.07 ± 0.26	1.54 ± 0.04	13.05 ± 0.19	1.03 ± 0.05
DN-14274	Drained type	47.63 ± 0.74	35.34 ± 0.65	38.57 ± 0.89	48.13 ± 0.49	1.07 ± 0.07
	Low-pond type	16.9 ± 0.51	13.52 ± 0.27	3.39 ± 0.23	20.79 ± 0.16	1.23 ± 0.08
	Deep-pond type	18.39 ± 0.32	16.95 ± 0.41	1.41 ± 0.01	13.42 ± 0.09	0.73 ± 0.04
NE-222	Drained type	59.77 ± 0.72	28.84 ± 0.46	18.79 ± 0.13	37.55 ± 0.27	0.63 ± 0.03
	Low-pond type	29.23 ± 0.64	22.21 ± 0.32	7.01 ± 0.12	16.08 ± 0.13	0.55 ± 0.04
	Deep-pond type	35.4 ± 0.71	29.59 ± 0.45	5.81 ± 0.07	13.95 ± 0.08	0.39 ± 0.06
DTAC-7	Drained type	67.06 ± 1.13	29.92 ± 0.36	37.15 ± 0.23	37.04 ± 0.35	0.56 ± 0.07
	Low-pond type	43.09 ± 1.27	30.99 ± 0.72	12.09 ± 0.08	19.34 ± 0.28	0.45 ± 0.03
	Deep-pond type	31.32 ± 0.76	21.96 ± 0.23	9.35 ± 0.12	17.03 ± 0.21	0.54 ± 0.06
R-270	Drained type	117.67 ± 1.42	52.96 ± 0.86	64.71 ± 1.02	52.53 ± 0.79	0.48 ± 0.05
	Low-pond type	73.94 ± 0.87	48.41 ± 0.62	25.53 ± 0.34	22.94 ± 0.12	0.31 ± 0.04
	Deep-pond type	36.52 ± 0.53	24.64 ± 0.34	11.89 ± 0.12	13.47 ± 0.08	0.37 ± 0.06
NM-6	Drained type	84.93 ± 1.01	38.17 ± 0.43	46.76 ± 0.65	36.44 ± 0.34	0.44 ± 0.03
	Low-pond type	22.94 ± 0.43	16.91 ± 0.27	6.028 ± 0.14	14.74 ± 0.17	0.64 ± 0.05
	Deep-pond type	23.94 ± 0.56	19.09 ± 0.45	4.842 ± 0.09	17.84 ± 0.43	0.75 ± 0.07
Effect of water level		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Effect of clone		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Water × clone interaction		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

DN-14274, NE-222, R-270 and NM-6. And a number of the clones in deeper-flooded conditions recovered to higher P_n values than those that were shallow-flooded.

Water relations

There were significant differences in the stomatal conductance of all 14 hybrid clones

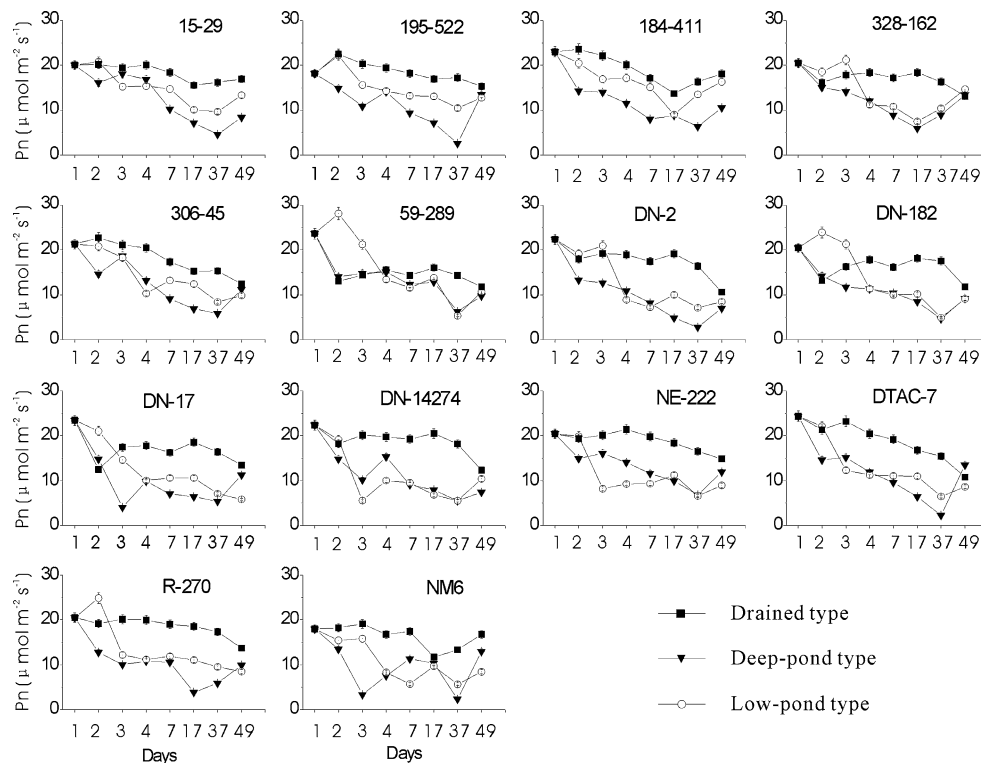


Fig. 2 Time course of net photosynthesis in the leaves of cuttings of 14 clones under three soil conditions: control (closed squares), shallow flooding (open circles), and deep flooding (closed triangles). Measurements were taken on days 2, 3, 4, 7, and 17 (during the flooding), day 37 (the first

day after flooding ended), and day 49 (13 days after flooding ended). ($P < 0.001$ for the effect of flooding, day after the start of the study, and the flooding day interaction)

($P < 0.001$, Fig. 3). As the treatments began, g_s decreased immediately. By day 7, g_s of most hybrids had decreased to the lowest values observed during the study period, but increased thereafter as the flood duration increased. After 14 days, the g_s of most deeply flooded plants tended to decrease again, whereas that of shallowly flooded plants rose continuously. When the flooding ended, g_s increased rapidly recovered to different degree. Analysis of covariance indicated that both flooding treatment and flood duration had a significant effect on g_s ($P < 0.001$). The interaction between the flooding treatment and flood duration was also significant ($P < 0.001$).

Flooding reduced the transpiration of all 14 clones. There were significant differences between treatments for all clones ($P < 0.01$, Fig. 4). The E value for the shallowly flooded plants declined more rapidly than that of the

deeply flooded plants during the first day. By day 7, E had reached its minimum value for all clones except DN-17 and NM-6, for which the minimum E occurred on days 3. After reaching its minimum value, there was an increase in some clones. When the flooding treatment ended, E generally recovered rapidly to reach a level similar to that of the control, though some clones (306-45, DN-17, DTAC-7, R-270, and NM-6) lagged behind others in their rate of recovery. Analysis of covariance indicated that both flooding treatment and flood duration had a significant effect on E ($P < 0.001$). The interaction of the flooding treatment and flood duration was also significant ($P < 0.001$).

There was an increase in the water potential of the deeply flooded plants compared with the shallowly flooded plants and the control (data not shown). However, the change became insignificant as flooding continued.

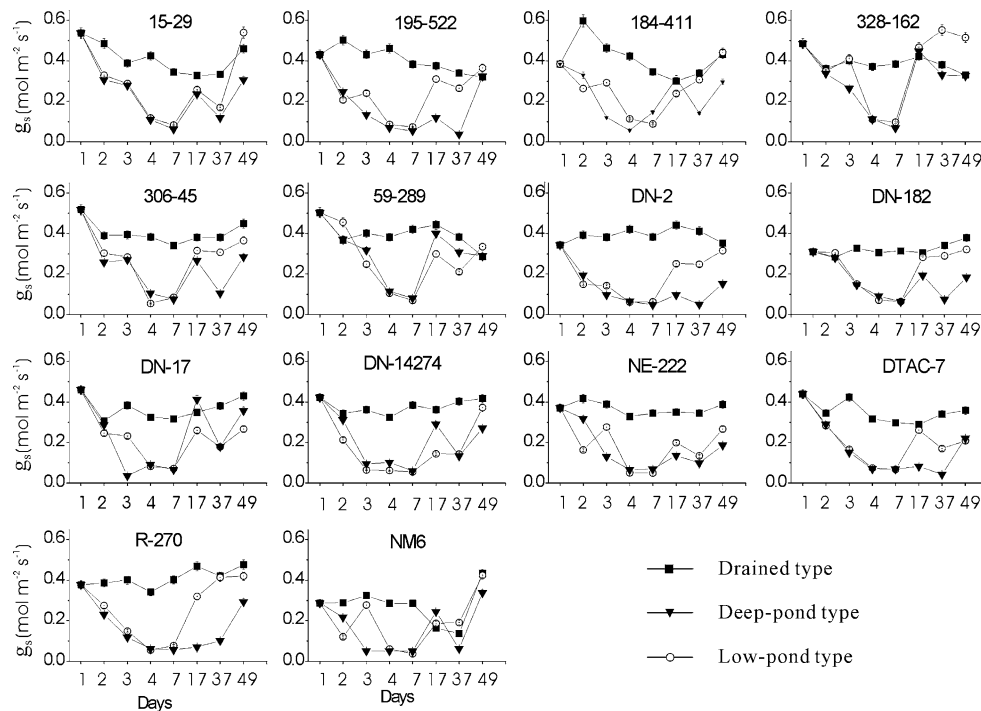


Fig. 3 Time course of stomatal conductance (g_s) in the leaves of cuttings of 14 clones under three soil conditions: drained (closed squares), low-pond type (open circles) and deep-pond type (closed triangles) of flood duration (2, 3, 4,

7, 17, 37 and 13 days recovery). (Fw: $P < 0.001$, Fd: $P < 0.001$, Fw \times d: $P < 0.001$. Fw: watering effect, Fd: flood duration effect, Fw \times p: Watering \times duration effect)

Flood tolerance

We classified the 14 hybrid clones into three groups by means of hierarchical cluster analysis based on a comparison of all the data obtained (for all parameters) during the flooding (Fig. 5). Based on this analysis, clones 15-29, 196-522, 184-411, 306-45, 59-289, DN-2, DN-182, DN-17, DN-14274, NE-222, DTAC-7, and R-270 appear to be flood tolerant, clone NM-6 is flood-susceptible, and clone 328-162 is moderately flood-tolerant. In the first group, clones 15-29, 184-411, and 306-45 tolerated flooding best and clone 59-289 tolerated flooding least.

Discussion

Survival of plants under adverse condition requires acclimatization and adaptation (Alscher and Cumming 1990). Even though we observed symptoms of flooding in our study, the survival of

all clones was 100% after 37 days of flooding and a 13-day recovery period. Shallowly flooded plants in all clones developed adventitious roots from the base of their stems, a phenomenon that has been described as an adaptive mechanism against flooding (Hook 1984; Kawase 1981). Many researchers have reported the formation of adventitious roots by flooded plants, particularly in flood-tolerant species (Gill 1970; Hook 1984; Kozłowski 1997), and that these roots play a positive role in supporting shoot growth during prolonged flooding (Armstrong et al. 1994; Jackson 1985). In our study, all 14 clones grew adventitious roots in the shallow flooding treatment, which allow oxygen to diffuse into their tissues, thereby enhancing water and nutrient uptake (Hook 1984). The formation of adventitious roots has been strongly correlated with increased water absorption, accompanied by an increase in damage to and death of the original roots (Chen et al. 2002; Sena Gomes and Kozłowski 1980).

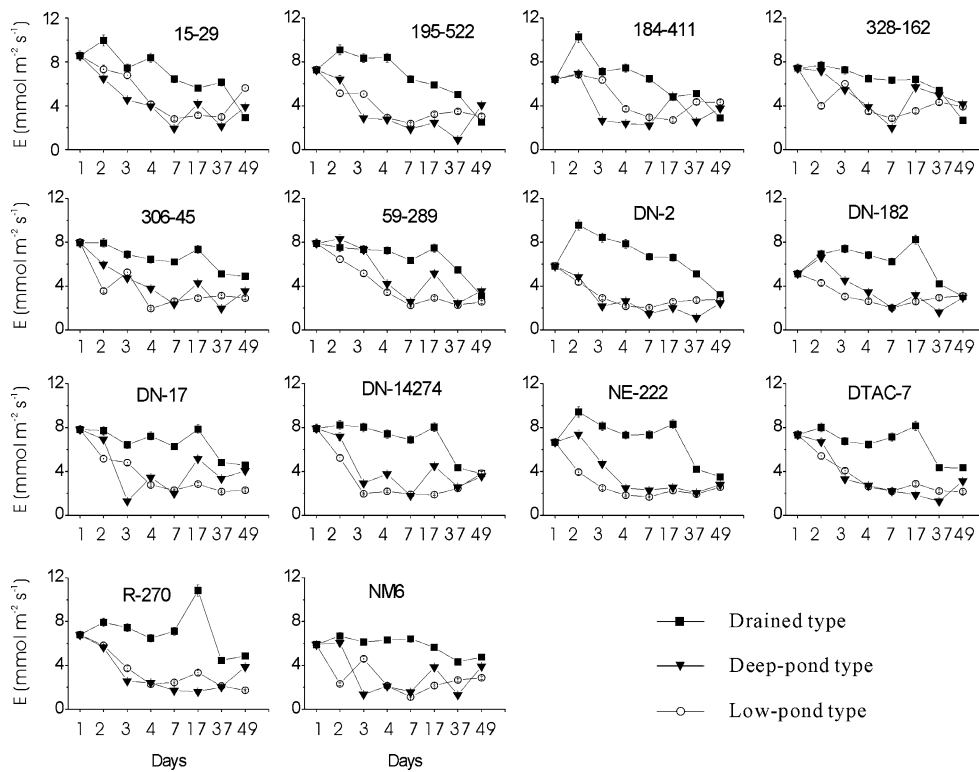
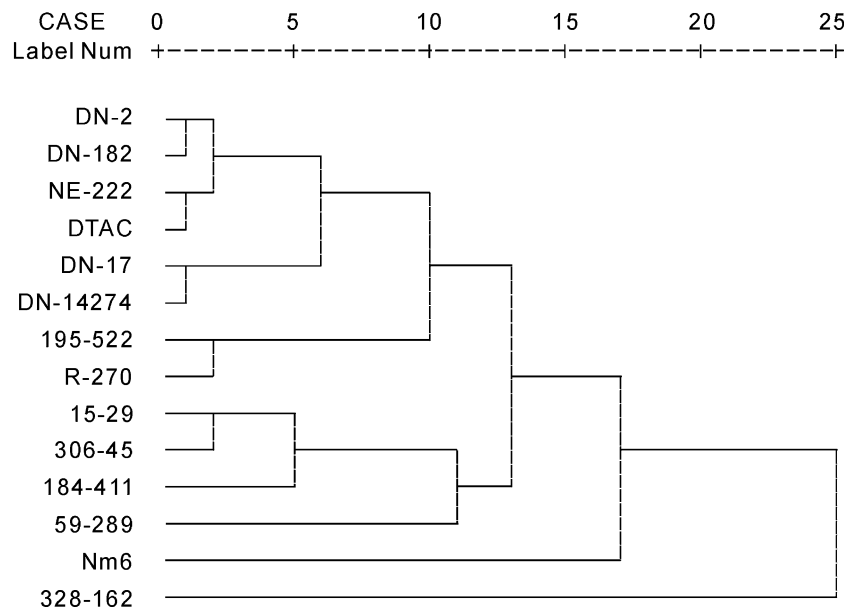


Fig. 4 Time course of transpiration (E) in the leaves of cuttings of 14 clones under three soil conditions: drained (closed squares), low-pond type (open circles) and deep-pond type (closed triangles) of flood duration (2, 3, 4, 7, 17,

37 and 13 days recovery). (Fw: $P < 0.001$, Fd: $P < 0.001$, Fw \times d: $P < 0.001$. Fw: watering effect, Fd: flood duration effect, Fw \times p: watering \times duration effect)

Fig. 5 Cluster analysis dendrogram of the performance of the 14 clones under flooding



Reductions in P_n and g_s under flooding have been observed in a number of different species, including temperate-zone woody species and crops such as wheat, pepper, and bean (Cao and Conner 1999; Marcelo et al. 2003; Pezeshki 1994; Pezeshki et al. 1996; Sun et al. 1995). In our study, P_n and g_s of all 14 clones immediately decreased in response to flooding compared with the control; values for some clones, such as DN-17, DN-14274, and NM-6, decreased and reached their lowest values of the whole treatment period by day 3. By day 7, the g_s of most hybrid clones had reached its lowest values. Species that are sensitive to flooding often exhibit severe reductions in CO_2 assimilation and stomatal conductance at low soil oxygen availability (Gravatt and Kirby 1998; Pezeshki 1993), so our results suggest that DN-17, DN-14274, and NM-6 are relatively sensitive to flooding. Irrespective of whether flooding is shallow or deep; the observed changes in our clones can be ascribed to the response of the shoots to root metabolic conditions. Under continued anaerobic conditions, root water uptake is inhibited; consequently, the permeability of roots and their hydraulic conductivity decrease, both of which decrease stomatal conductance (Jackson 1985; Pezeshki 2001). If stomatal conductance remains the same, but the roots cannot supply adequate amounts of water, the plant is likely to experience increasingly serious water stress; thus, stomatal closure prevents this problem by reducing the rate of water loss, thereby protecting the flooded plant against water stress. After 7 days of flooding, g_s generally started to increase again, especially in the shallow flooding treatment, where the increase was generally continuous. This response may be related to the development of adventitious roots in this treatment, since the cellular structure of these new roots may contain an extensive volume of aerenchyma that improves gas transport (Hook 1984). This phenomenon has been demonstrated in many species (Cao and Conner 1999; Pezeshki 1993; Sena Gomes and Kozlowski 1980). When the flooding ended, g_s began to rise rapidly until it reached the level of the control, though some clones lagged behind others in their recovery, indicating that flooding has greater effects on them than on others and the clones have different reaction to flood.

Before day 7, P_n and g_s of the treated plants decreased in parallel. The immediate decrease in P_n suggests that the rate of photosynthesis is controlled by stomatal factors during early flooding. After g_s started to increase again, P_n generally continued to decrease until the recovery period after flooding ended, therefore non-stomatal inhibition was also involved in the inhibition of P_n by flooding. Several studies have shown a close correlation between stomatal closure and the reduction of photosynthesis during flooding (Ismail and Noor 1996; Pezeshki 1993). The decrease in P_n resulted either from stomatal closure or nonstomatal inhibition (Pezeshki 1993). The mechanism of nonstomatal inhibition of P_n during flooding is not well understood. However, inhibition of photosynthetic enzymes has been reported during flooding (Liao and Liu 1994; Pezeshki 1994). We did not study this possibility during the present study.

The decreased E of the flooded plants implies that flooding also affects water relations. Since plants adjust E to maintain their water balance, decreased E means that the amount of water taken up by the roots decreases. This leads to stomatal closure, which is a protective mechanism that helps to resist flooding stress. In general, E reached its minimum value by day 7, and then started to increase except in clones DN-17 and NM-6; this is probably related to the development of adventitious roots, which were capable of taking up water. With the appearance of adventitious roots, stomatal conductance increased, root hydraulic conductance increased, and water uptake became more dependent on the adventitious roots than on the old roots. Higher levels of water potential in the deeply flooded plants compared with the shallowly flooded plants and the control suggest that the flooded plants did not experience significant differences in water stress as a result of flooding.

Flooding changes shoot and root growth and affects the whole plant biomass and the allocation of biomass. This has been demonstrated in many species, including both flood-tolerant and non-flood-tolerant species (Lopez and Kuesar 1999; Kozlowski 1997; Rubio et al. 1995). All 14 of the flooded clones had a lower leaf weight than the control plants, mainly as a result of decreases in

the size and numbers of leaves and the effects of leaf senescence and abscission. The decrease in canopy area during flooding has been considered to be an avoidance mechanism designed to maintain water balance by reducing water loss through decreased transpirational area (Sanchez-Blanco et al. 2002). In our study, the changes in stem weight among the 14 clones show that total biomass of plants exposed to the flood condition are mainly attributed to reductions in root and leaf weight. Therefore, the roots and leaves of the clones appear to be more sensitive to flooding than the stems. Compared with the control plants, flooded plants produced less total biomass. We observed differences among clones and treatments, with the largest decrease in clones 328-162, DN-17, DN-14274, DN-2, DN-182 and NM-6 and the lowest decrease in clones 15-29, 184-211, and 59-289. For clone 306-45, flooding appeared to have little effect on total biomass. During flooding, height growth stopped for clones DN-2, DN-17, and NM-6, but other clones began to increase their height between days 7 and 17, including clones 15-29, 195-522, 184-211, 328-162, and 306-45. Overall, clones 306-45, 15-29, 184-211, and 59-289 showed better flood tolerance than the other clones, and clones NM-6, DN-17, DN-14274, and 328-162 were most sensitive to flooding in terms of biomass accumulation and growth. The root/shoot ratio was significantly affected by flooding. Most clones have a higher root/shoot ratio in the shallow treatment than other treatment due to the formation of the adventitious roots.

Conclusions

We observed the responses to flooding of 14 American hybrid poplar clones near Beijing, China. The results indicate that these clones adapted to flooding by means of morphological and eco-physiological changes such as developing adventitious roots, reopening their stomata, reducing leaf number, and changing the allocation pattern of biomass. These changes suggest that some of the hybrids have good potential to adapt to the conditions encountered on flood plains such as those along the middle and lower reaches of the Yangtze River and the Yellow River.

Among the clones that we studied, 15-29, 184-411, 306-45, 59-289, 196-522, DN-2, DN-182, DN-17, DN-14274, NE-222, DTAC-7, and R-270 appear to be sufficiently flood-tolerant that they are suitable for use in reforestation; and clone 328-162 was moderately flood-tolerant; However, clone NM-6 appear to be poorly suited to wetland environments.

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