

## Waterlogging tolerance evaluation of fifteen poplar clones cultivated in the Jiangnan Plain of China

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### Abstract

To provide references for poplar cultivation in waterlogged prone area of Jiangnan Plain of China, the waterlogging tolerance of 15 poplar clones widely cultivated in these areas were evaluated based on their responses to 45-day waterlogging stress followed by 15-day drainage recovery in morphology, growth, biomass accumulation, leaf gas exchange and chlorophyll fluorescence parameters. The results showed that the normal watered seedlings (CK) of the 15 clones grew vigorously during the experiment, and no defoliation and death occurred. For the seedlings under waterlogging treatment (water 10 cm above the soil surface), its morphology changed markedly, including slowing growth, chlorosis and abscission of leaves, development of hypertrophied lenticels and adventitious roots etc. Waterlogging stress significantly inhibited the seedling growth of height and ground diameter, biomass accumulation, as well as leaf gas exchange and chlorophyll fluorescence parameters of the 15 clones with varying degrees. The net photosynthetic rate ( $P_n$ ), stomatal conductance ( $G_s$ ), transpiration rate ( $T_r$ ), intercellular CO<sub>2</sub> concentration/ environmental CO<sub>2</sub> concentration ( $C_i/C_a$ ), variable fluorescence ( $F_v$ ), variable fluorescence/ initial fluorescence ( $F_v/F_o$ ) and PS II primary light energy conversion efficiency ( $F_v/F_m$ ) decreased gradually with the prolonged waterlogging, and reached their bottom on day 45. During the terminal recovery stage, the leaf gas exchange and chlorophyll fluorescence parameters of the most clones increased, but their recovery abilities were significantly different. At the end of the experiment, the highest survival rates (100%) were observed in DHY, HS-1, HS-2, I-72, I-69, I-63 and NL-895, and the lowest (zero) occurred in XYY. Survival rates of the other clones ranged from 33.33% to 83.33%. Both results of cluster analysis and membership function analysis showed that HS-1, I-69, DHY, NL-895 and HS-2 had the strongest waterlogging tolerance, XYY and HBY were the worst, and the other clones were moderate. These results would provide guidance not only for the selection of cultivated varieties in Jiangnan Plain, but also for the selection of hybrid parents for waterlogging resistance breeding.

**Keywords:** poplar; waterlogging stress; waterlogging tolerance

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## Introduction

Jiangnan Plain is located in the central and southern part of Hubei Province of China. It is an important part of the plain in the middle and lower reaches of the Yangtze River, and is one of the lowest plains in China with average altitude of around 27 m. Jiangnan Plain has a huge area land prone to waterlogging. Due to the low-lying terrain and excessive seasonal rainfall, waterlogging has become one of the major natural disasters in these areas. Poplar (*Populus L.*) is one of the most important tree species for afforestation in these areas due to its characteristics of strong waterlogging resistance, rapid growth and broad applications. However, long-term waterlogging stress still adversely affects the growth and survival of poplar seriously (Peng *et al.*, 2018). Under waterlogging stress, due to the lack of oxygen supply to roots, plants often present the phenomenon of leaf chlorosis and abscission, root rotting, photosynthetic capacity decline and so on, which leads to slow growth and even death, coupled with a huge loss of wood yield and ecological function (He *et al.*, 2018; Zhao *et al.*, 2019; Yan *et al.*, 2019).

Previous studies have shown that different poplar clones always possessed significantly different waterlogging tolerance (Gong *et al.*, 2007; Azizi *et al.*, 2017; Sun *et al.*, 2020; Rodriguez *et al.*, 2020). For instance, *P. deltoides* 'Lux' is waterlogging-tolerant, while *P. simonii* is waterlogging-susceptible (Du *et al.*, 2010; Liu *et al.*, 2014; Chen *et al.*, 2015). Male poplar plants tend to have stronger waterlogging tolerance than female ones owing to its lower reproductive costs and energy requirements (Correia and Barradas, 2000; Jiang *et al.*, 2009; Yang *et al.*, 2012). At present, there are more than 15 poplar clones cultivated in Jiangnan Plain with different growth performance, which might be related to their different waterlogging tolerance. Therefore, it is of great significance to evaluate the waterlogging tolerance of these clones and select waterlogging-tolerant clones for afforestation and waterlogging resistance breeding in Jiangnan Plain. In this study, waterlogging tolerance of 15 poplar clones widely planted in Jiangnan Plain were evaluated by investigating their responses to waterlogging stress in morphology, growth, biomass accumulation, together with physiological and ecological characteristics, and waterlogging-tolerant clones were selected as well.

## Materials and Methods

### *Plant materials and experiments*

The experiment was performed in greenhouse of Huazhong Agricultural University located in Wuhan, Hubei Province (30°28' N, 114°21' E). The region belongs to Jiangnan Plain, and has a warm, temperate climate, with an annual average of 240 frost-free days, 1,269 mm of rainfall, and a mean yearly temperature of 16.3°C. Most rainfall occurs between June and August, accounting for about 40% of the annual rainfall. In a year, the lowest average temperature occurs in January (around 3.0 °C) and the highest average temperature occurs in July (around 29.3 °C) (Du *et al.*, 2008).

Fifteen poplar clones widely cultivated in Jiangnan Plain were adopted as the materials in the present study, including *P. simonii* (XYX), *P. deltoides* 50 × *P. deltoides* 36 (JBY), *P. canadensis* Moench (JNDY), *P. ningshanica* C. Wang et Tung (HBY), *P. deltoides* 'Danhong' (DHY), *P. Liaoningensis* Z. Wang et H. Z. Chen sp. nov (LNY), *P. deltoides* 'Huashi 1' (HS-1), *P. deltoides* 'Huashi 2' (HS-2), *P. × euramericana* 'San martino' (I-72), *P. deltoides* 'Lux' (I-69), *P. deltoides* 'Harvard' (I-63), *P. euramericana* 'I-45/51' (I-45/51), *P. euramericana* 'I-214' (I-214), *P. × euramericana* 'Nanlin-895' (NL-895), *P. deltoides* 'Lux' × *P. deltoides* 'Shanhaiguan' (ZL-2025).

In mid-March, one-year-old shoots of the 15 clones were selected and cut into around 15 cm cuttings with three to four buds. The cuttings were planted in 15 cm × 20 cm × 15 cm pots (one cutting per pot) containing mixed soil (natural light loam: sand: peat = 6:1:1, pH= 6.2) after soaking in tap water for 24 h, and grown in greenhouse. The soil consisted of 2–5% N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O, with more than 20% organic matter (dry weight). After the plants survived, tap water and 1/2 Hoagland nutrient solution were irrigated once a week,

respectively. The plants with a mean height of 30–40 cm were randomly assigned to one of two treatments for a 60-day study: (1) watered (CK); (2) partial submergence (PS). In both treatment groups, there were 12 cuttings of each clone (four blocks, three cuttings arranged randomly per block). The pots of the watered treatment group had drainage holes in the bottom, and the plants were watered using tap water every day as needed to maintain soil moisture at around 75% of the maximum field water capacity. The plants of the waterlogging treatment group were flooded to a depth of 10 cm above the soil surface in an artificial pond with a depth of 70 cm. To reduce the influence of sunlight on water temperature and to replace the water lost due to evaporation and transpiration, water was continuously supplied to maintain water temperature and waterlogging depth. After 45 days, the waterlogged plants were removed from the partial submergence treatment and allowed to recover for 15 days to simulate natural environmental conditions.

#### *Morphology and growth parameters*

During the waterlogging period, morphological changes of the plants were observed everyday including leaf chlorosis and abscission, development of hypertrophied lenticels and adventitious roots etc. At the end of the experiment, the survival rate of each clone was recorded.

On day zero, 45 and 60, the seedling height and ground diameter of all clones were measured to calculate its growth parameters during the experiment. At the end of recovery stage (60 d), all of the plants were harvested. Root, stem, and leaf components were dried and weighed. In addition, the stem and its leaves were combined as a total shoot value, that is shoot weight = leaf weight + stem weight, and total biomass = shoot weight + root weight (Rodriguez *et al.*, 2020).

#### *Measurement of leaf gas exchange and chlorophyll fluorescence*

On the 0, 15<sup>th</sup>, 30<sup>th</sup>, 45<sup>th</sup> and 60<sup>th</sup> day of waterlogging treatment, the 5<sup>th</sup> fully expanded and mature leaf from the top of the stem of each clone was chosen to measure leaf gas exchange and chlorophyll fluorescence (Peng *et al.*, 2018). For leaf gas exchange measurement, four plants per clone per treatment were measured between 9:00 am. and 11:30 am. using a LI-6400 photosynthesis system (LI-COR Inc., Lincoln, NE, USA) with a standard LI-COR gas exchange chamber (2 × 3 cm). A 1500  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  light intensity of illumination was provided by red diodes (6400-02 LED Source), and the gas flow rate was set as 500  $\mu\text{mol}\cdot\text{s}^{-1}$ . The gas exchange measurements included net photosynthetic rate ( $P_n$ ), transpiration rate ( $T_r$ ), stomatal conductance ( $G_s$ ), intercellular  $\text{CO}_2$  concentration/ environmental  $\text{CO}_2$  concentration ( $C_i/C_a$ ), and the corresponding ambient environmental conditions, such as leaf surface temperature, photosynthetically active radiation, relative atmospheric temperature and relative humidity. Chlorophyll fluorescence of leaves of four plants per treatment per clone was measured using a LI-6400 fluorescence system (LI-COR Inc., USA) after a 20-min dark adaptation under natural conditions, including variable fluorescence ( $F_v$ ), PSII primary light energy conversion efficiency ( $F_v/F_m$ ), variable fluorescence to initial fluorescence ratio ( $F_v/F_0$ ) and so on.

#### *Statistical analysis*

The data were analyzed by analysis of variance (ANOVA) and multiple comparisons (Duncan's) with SAS statistical software package version 9.0 (SAS Institute Inc., USA). The waterlogging tolerance of each clone was evaluated by the waterlogging tolerance indexes and membership function values. The waterlogging tolerance index (%) = average value of waterlogging treatment/ average value of watered treatment × 100% (Rodriguez *et al.*, 2020). The calculation formula of membership function value is  $U(x_i) = (x - x_{min}) / (x_{max} - x_{min})$  or  $U(x_i) = 1 - (x - x_{min}) / (x_{max} - x_{min})$  for the index which has a positive or negative correlation with waterlogging tolerance, respectively.  $U(x_i)$  represents the membership function value of an index of clone  $i$ ;  $x$  represents the mean value of an index of clone  $i$ ;  $x_{max}$  and  $x_{min}$  are, respectively, the maximum and minimum values of an index in all clones (Yan *et al.*, 2020). The cluster analysis of waterlogging tolerance of the 15 clones was carried out based on multivariate analysis (minimum distance method) of all data obtained during the study (Du *et al.*, 2008).

## Results

### *Plant morphology*

All watered plants (CK) grew vigorously, and no defoliation occurred. At the end of the experiment, the survival rates of all CK plants were 100%. In terms of waterlogged plants, obvious morphological changes were observed, including chlorosis and abscission of leaves, as well as development of hypertrophied lenticels and adventitious roots. Most clones firstly appeared hypertrophied lenticels on the 5<sup>th</sup>-6<sup>th</sup> day of waterlogging treatment, together with chlorosis leaves on the 10<sup>th</sup>-13<sup>th</sup> day, and defoliation on the 19<sup>th</sup>-20<sup>th</sup> day (Table 1). At the end of drainage recovery, DHY, HS-1, HS-2, I-72, I-69, I-63 and NL-895 displayed the highest survival rates (100%); XYY owned the lowest survival rate (0%), and that of the other clones ranged from 33.33% to 83.33%. Among the 15 clones, all plants of XYY and some plants of HBY and I-214 died during the waterlogging treatment, and XYY died earliest on day 32. Some plants of JBY, JNDY, LNY, I-45/51 and ZL-2025 died during drainage recovery.

**Table 1.** Morphological responses to waterlogging stress of the 15 poplar clones

Clone	Initial time of leaf chlorosis (d)	Initial time of defoliation (d)	Initial time of hypertrophied lenticels development (d)	Initial time of adventitious root formation (d)	Survival rate (%)
XYY	8	16	7	17	0
JBY	10	19	6	15	75
JNDY	8	16	6	15	83.33
HBY	9	17	6	17	33.33
DHY	11	19	5	10	100
LNY	10	20	6	16	75
HS-1	14	21	5	10	100
HS-2	15	22	5	10	100
I-72	10	18	6	12	100
I-69	14	21	5	10	100
I-63	13	20	6	11	100
I-45/51	12	19	6	16	83.33
I-214	12	19	6	16	66.67
NL-895	10	19	6	11	100
ZL-2025	13	21	7	15	83.33

Note:  $n=12$ .

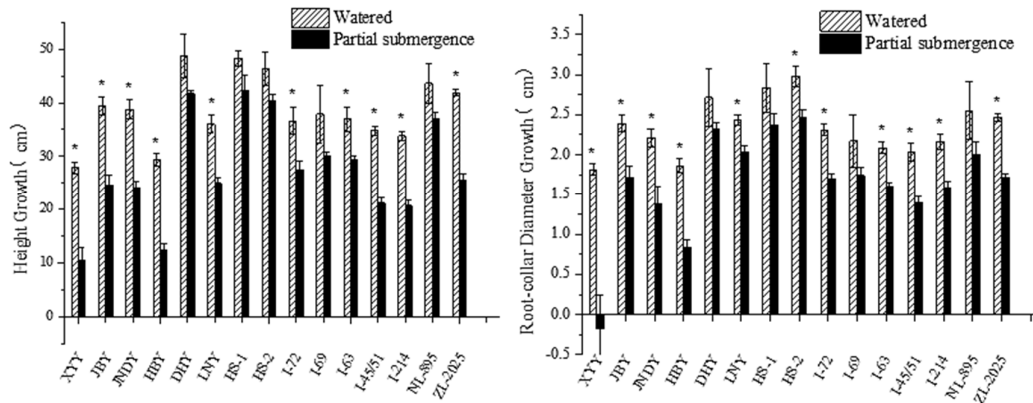
### *Plant growth*

#### Growth of height and ground diameter

Height growth of the 15 clones was significantly inhibited by waterlogging treatment to varying degrees ( $p < 0.05$ ; Figure 1). Among them, DHY, HS-1, HS-2 and NL-895 had better waterlogging tolerance indexes, which were 85.63%, 87.32%, 87.14% and 84.59%, individually; HBY and XYY showed the lowest waterlogging tolerance indexes, which were 42.06% and 37.56% respectively; while that of the other clones ranged from 60% to 80% (Supplementary Table 1). According to the waterlogging tolerance indexes, the slightest inhibition occurred in HS-1, and the most severe inhibition happened in XYY.

Waterlogging treatment significantly reduced ground diameter growth of the 15 clones with different degrees ( $p < 0.05$ ; Figure 1). Among them, waterlogging tolerance indexes in XYY and HBY were lower than 60%, and the lowest value occurred in XYY (-9.63%); DHY, LNY, HS-1, HS-2 and I-69 had higher

waterlogging tolerance indexes (>80%), and that of the other clones ranged from 60% to 80% (Supplementary Table 1). These waterlogging tolerance indexes indicated that the ground diameter growth of XYY was most affected by waterlogging stress, while that of DHY, LNY, HS-1, HS-2 and I-69 were least affected.



**Figure 1.** Height and ground diameter growth of the 15 poplar clones under waterlogging treatment  
 Note: Bars labeled with \* and \*\* indicated significant difference between watered and waterlogged seedlings of the same clone at  $p < 0.05$  and  $p < 0.01$ , respectively ( $t$  test; Mean  $\pm$  SE,  $n=4$ ; the same below)

**Biomass accumulation**

Waterlogging stress significantly reduced the biomass accumulation and root/shoot ratio in all of the 15 clones, and the reduction levels significantly varied between them ( $p < 0.05$ ; Table 2). The highest waterlogging tolerance indexes of root biomass were observed in DHY (73.60%), HS-1 (73.41%), HS-2 (70.59%), I-69 (73.23%) and NL-895 (71.02%), and the lowest were observed in XYY (7.25%) and HBY (16.50%), respectively (Supplementary Table 1). For stem biomass, the highest waterlogging tolerance index occurred in NL-895 (86.20%), and the lowest occurred in XYY (30.45%) and HBY (34.90%), respectively. In terms of leaf biomass, DHY (84.45%) owned the highest waterlogging tolerance index, and XYY (0) has the lowest value, followed by HBY (30.60%). The waterlogging tolerance indexes of root/shoot ratio mostly concentrated in 60% - 90%, with the exception of XYY (53.85%), HBY (50.00%) and HS-1 (88.37%). Among the 15 clones, I-69 (81.23%), DHY (80.42%) and HS-1 (80.69%) had more than 80% of waterlogging tolerance indexes in total biomass, and XYY was the lowest, only 12.54% (Supplementary Table 1).

**Table 2.** Biomass accumulation of the 15 poplar clones under waterlogging treatment

Clones	Treatments	Biomass (g)			Root/shoot ratios	Total biomass (g)
		Root	Stem	Leaf		
XYY	CK	1.38 $\pm$ 0.04	2.43 $\pm$ 0.08	2.89 $\pm$ 0.24	0.26 $\pm$ 0.07	6.70 $\pm$ 0.23
	PS	0.10 $\pm$ 0.03	0.74 $\pm$ 0.27	0.00 $\pm$ 0.00	0.14 $\pm$ 0.03	0.84 $\pm$ 0.14
JBY	CK	2.60 $\pm$ 0.27	3.56 $\pm$ 0.25	3.38 $\pm$ 0.14	0.37 $\pm$ 0.03	9.54 $\pm$ 0.39
	PS	1.19 $\pm$ 0.12	2.41 $\pm$ 0.25	2.61 $\pm$ 0.15	0.24 $\pm$ 0.02	6.21 $\pm$ 0.36
JNDY	CK	2.55 $\pm$ 0.26	3.97 $\pm$ 0.34	3.35 $\pm$ 0.15	0.35 $\pm$ 0.04	9.87 $\pm$ 0.51
	PS	1.21 $\pm$ 0.25	2.67 $\pm$ 0.35	2.63 $\pm$ 0.07	0.23 $\pm$ 0.04	6.51 $\pm$ 0.51
HBY	CK	2.06 $\pm$ 0.07	2.98 $\pm$ 0.24	2.68 $\pm$ 0.11	0.36 $\pm$ 0.07	7.72 $\pm$ 0.33
	PS	0.34 $\pm$ 0.25	1.04 $\pm$ 0.25	0.82 $\pm$ 0.04	0.18 $\pm$ 0.06	2.20 $\pm$ 0.45
DHY	CK	3.56 $\pm$ 0.24	4.05 $\pm$ 0.09	3.73 $\pm$ 0.10	0.46 $\pm$ 0.02	11.34 $\pm$ 0.27
	PS	2.62 $\pm$ 0.25	3.35 $\pm$ 0.39	3.15 $\pm$ 0.28	0.40 $\pm$ 0.02	9.12 $\pm$ 0.41

LNY	CK	2.54±0.20	3.68±0.15	3.37±0.14	0.36±0.02	9.59±0.36
	PS	1.17±0.12	2.76±0.24	2.29±0.08	0.23±0.02	6.22±0.38
HS-1	CK	3.61±0.21	4.76±0.11	3.54±0.17	0.43±0.02	11.91±0.27
	PS	2.65±0.17	3.98±0.25	2.98±0.19	0.38±0.04	9.61±0.45
HS-2	CK	4.08±0.09	4.37±0.41	3.62±0.30	0.51±0.05	12.07±0.78
	PS	2.88±0.20	3.66±0.15	2.75±0.12	0.45±0.06	9.29±0.11
I-72	CK	2.29±0.20	2.66±0.33	3.28±0.16	0.39±0.02	8.23±0.54
	PS	1.42±0.15	2.04±0.31	2.31±0.21	0.33±0.04	5.77±0.46
I-69	CK	2.54±0.06	4.58±0.20	2.79±0.10	0.34±0.02	9.91±0.23
	PS	1.86±0.03	3.91±0.05	2.28±0.13	0.30±0.02	8.05±0.17
I-63	CK	2.38±0.09	3.96±0.26	3.50±0.10	0.32±0.07	9.84±0.17
	PS	1.56±0.13	3.26±0.13	2.87±0.17	0.25±0.03	7.69±0.16
I-45/51	CK	2.92±0.12	3.27±0.18	2.64±0.08	0.49±0.03	8.83±0.21
	PS	1.31±0.20	2.06±0.29	1.66±0.15	0.35±0.03	5.03±0.26
I-214	CK	2.52±0.18	2.51±0.18	2.44±0.16	0.51±0.07	7.47±0.34
	PS	1.34±0.06	1.99±0.16	1.84±0.16	0.35±0.06	5.17±0.31
NL-895	CK	2.83±0.22	4.13±0.44	4.31±0.14	0.34±0.01	11.27±0.40
	PS	2.01±0.18	3.56±0.24	3.14±0.08	0.30±0.03	8.71±0.14
ZL-2025	CK	2.77±0.27	3.72±0.32	3.54±0.25	0.38±0.03	10.03±0.60
	PS	1.38±0.15	2.75±0.17	2.48±0.04	0.26±0.04	6.61±0.26

Note: Mean ± S.E.,  $n=4$ .

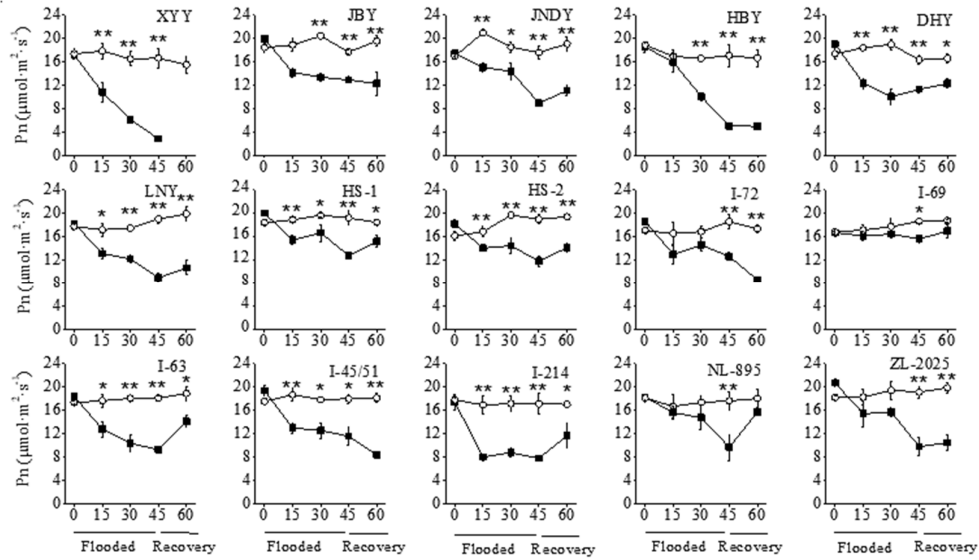
#### *Gas exchange of leaves*

##### Net photosynthetic rate

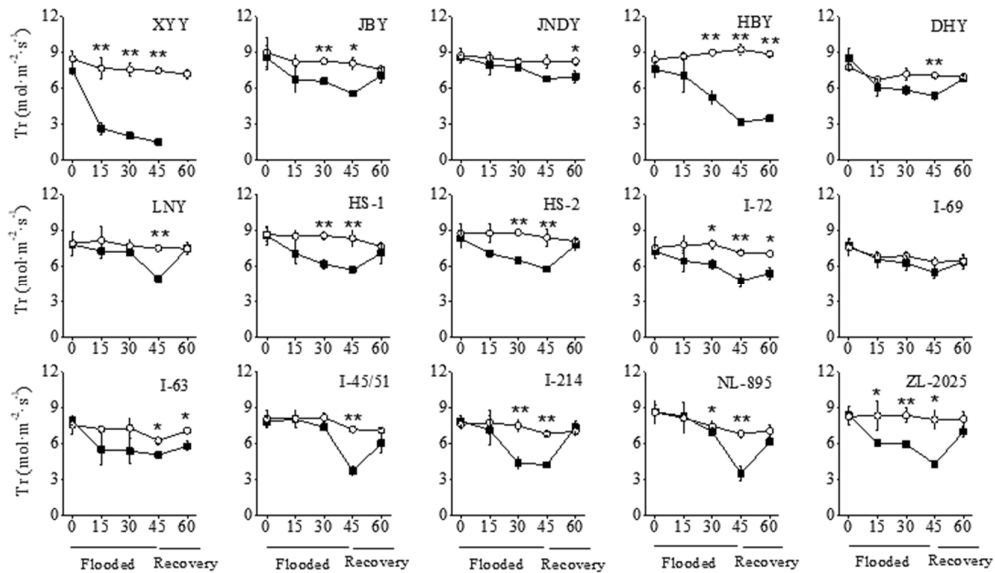
Waterlogging stress adversely affected net photosynthetic rates ( $P_n$ ) of the 15 clones with significantly different levels ( $p < 0.05$ ; Figure 2). The  $P_n$  values in the most plants decreased gradually with the prolonged waterlogging, and reached their minimum values on day 45. XYY and HBY decreased rapidly from the beginning of waterlogging, while the other clones decreased slower, and some clones even showed small fluctuations (Figure 2). On day 45, the lowest and the highest waterlogging tolerance indexes in  $P_n$  values were observed in XYY (17.49%) and I-69 (83.97%), respectively (Supplementary Table 2). During the terminal recovery stage,  $P_n$  values of I-63 and NL-895 increased obviously, while that of JBY, HBY and I-72 sustainedly declined. At the end of the experiment (60d),  $P_n$  values of all clones were still significantly lower than their CK values, except for I-69 and NL-895. Because all plants of XYY were dead during the waterlogging treatment (before recovery stage), it had no value on day 60 for all parameters of leaf gas exchange and chlorophyll fluorescence.

##### Transpiration rate

Similar with  $P_n$  values, waterlogging dramatically decreased values of transpiration rates ( $Tr$ ) in the 15 clones, and their responses differed significantly ( $p < 0.05$ ; Figure 3). During the experiment, the  $Tr$  values of all clones gradually decreased with the prolonged waterlogging, and reached their bottom on day 45. At that time, the lowest waterlogging tolerance index of  $Tr$  occurred in XYY (20.24%), and the highest occurred in I-69 (86.23%; Supplementary Table 2). After waterlogging removed,  $Tr$  values of the most clones increased in varying degrees. At the end of recovery stage,  $Tr$  values in the most clones almost recovered to the CK level, with the exception of JNDY, HBY, I-72 and I-63.



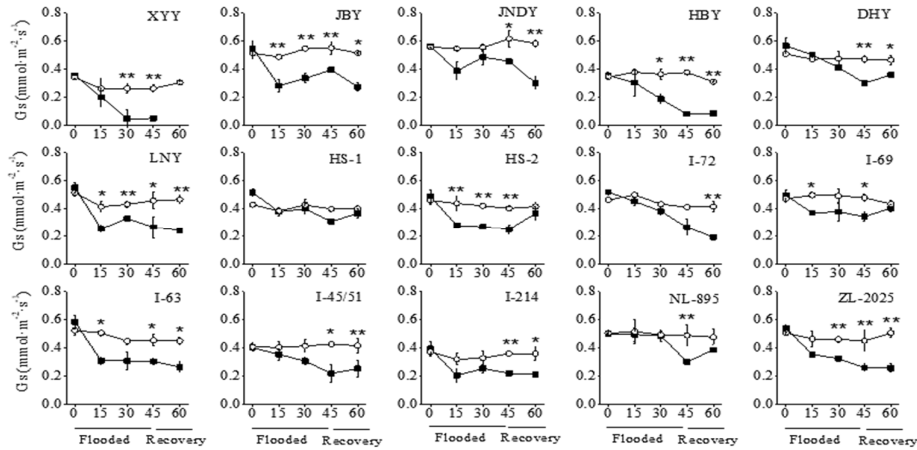
**Figure 2.** Responses of leaf net photosynthesis rates ( $P_n$ ) of the 15 poplar clones to waterlogging stress  
 Note: ○: Watered (CK); ■: Partial submergence treatment (PS); the same below



**Figure 3.** Responses of transpiration rate ( $T_r$ ) of the 15 poplar clones to waterlogging stress

Stomatal conductance

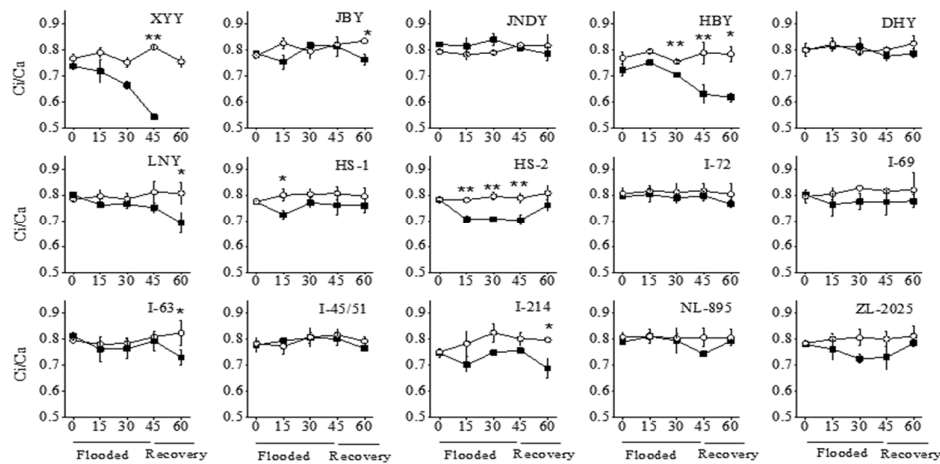
Stomatal conductance ( $G_s$ ) of the 15 clones was inhibited by waterlogging stress with significantly different degrees in the present study ( $p < 0.05$ ; Supplementary Table 1). The  $G_s$  values of the most waterlogged plants declined slowly with the prolonged waterlogging, except for slight fluctuation in a few clones, such as in JBY, JNDY and LNY on day 30. On the 45<sup>th</sup> day, the lowest waterlogging tolerance index of  $G_s$  value was observed in XYX (19.11%), and the highest happened in HS-1 (77.62%; Supplementary Table 2). During the terminal recovery stage, most of the clones did not recover obviously, even continuously decreased, in addition to DHY, HS-1, HS-2, I-69 and NL-895. At the end of the experiment,  $G_s$  values of the most clones were still dramatically lower than the CK level, except for HS-1, HS-2, I-69 and NL-895.



**Figure 4.** Responses of stomatal conductance ( $G_s$ ) of the 15 poplar clones to waterlogging stress

*Intercellular CO<sub>2</sub> concentration/ ambient CO<sub>2</sub> concentration*

Waterlogging stress significantly reduced the intercellular CO<sub>2</sub> concentration/ environmental CO<sub>2</sub> concentration ( $C_i/C_a$ ) of the 15 clones, and their responses differed significantly as well ( $p < 0.05$ ; Figure 5). Except for XXX, HBY, HS-2 and I-214,  $C_i/C_a$  values of the most clones changed slightly. On day 45, the lowest and the highest waterlogging tolerance indexes of  $C_i/C_a$  were observed in XXX (66.89%) and JBY (99.11%), respectively (Supplementary Table 2). At the end of the recovery stage,  $C_i/C_a$  values of the most clones recovered to their CK levels, with the exception of JBY, HBY, LNY, I-63 and I-214.

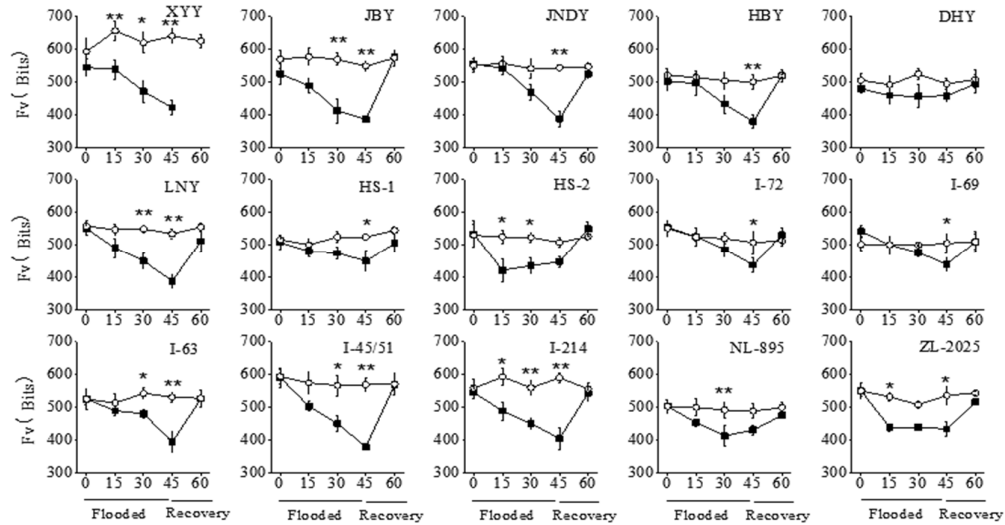


**Figure 5.** Responses of ratio of internal and atmospheric CO<sub>2</sub> concentrations ( $C_i/C_a$ ) of the 15 poplar clones to waterlogging stress

*Chlorophyll fluorescence of leaves*

Variable fluorescence

Similar with the gas exchange parameters, variable fluorescence ( $F_v$ ) of waterlogged plants of the 15 clones were dramatically reduced with the prolonged waterlogging ( $p < 0.05$ ), except for the slight decrease in DHY, HS-1, I-72, I-69 and NL-895 (Figure 6). On the 45<sup>th</sup> day, the lowest waterlogging tolerance index of  $F_v$  value happened in XXX (66.02%), and the highest occurred in DHY (92.61%; Supplementary Table 2). During the terminal recovery stage, most of the clones recovered their  $F_v$  values, and returned to the CK level on day 60.



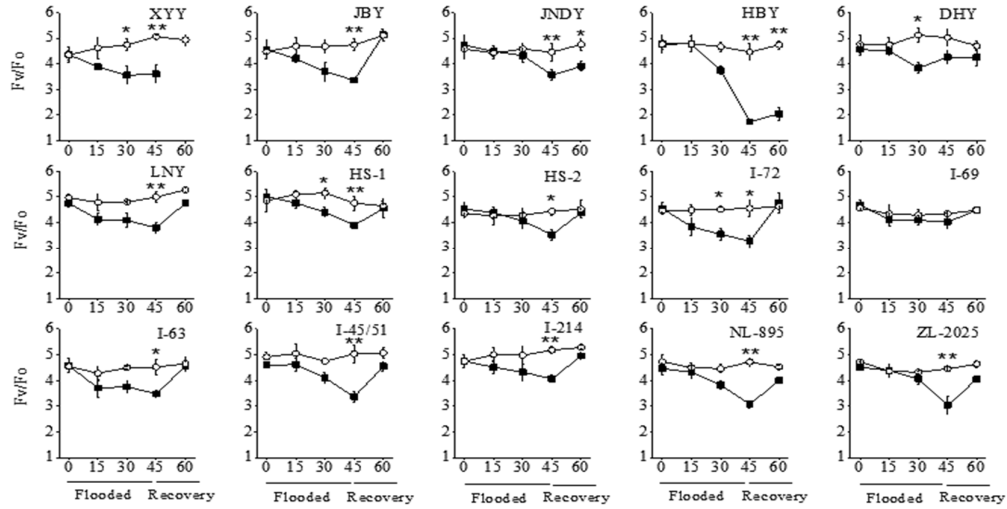
**Figure 6.** Responses of variable fluorescence ( $F_v$ ) of the 15 poplar clones to waterlogging stress

Variable fluorescence/ initial fluorescence

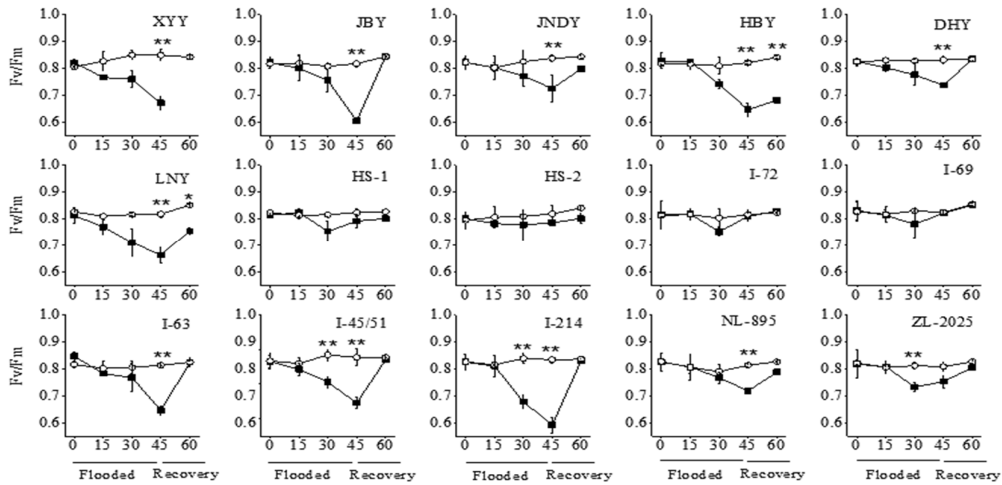
$F_v/F_o$  values of the 15 clones were significantly reduced under the waterlogging treatment by different degrees ( $p < 0.05$ ; Figure 7). Most of the clones slowly decreased their  $F_v/F_o$  with the prolonged waterlogging, and reached their minimum values on day 45. While, HBY declined sharply during 30–45d, and I-69 varied slightly throughout the experiment. On the 45<sup>th</sup> day, HBY (39.08%) and I-69 (92.51%) exhibited the lowest and the highest waterlogging tolerance index of  $F_v/F_o$ , respectively (Supplementary Table 2). At the end of the recovery stage, most of the clones recovered to their CK levels, with the exception of JNDY and HBY.

Primary conversion efficiency of PS II

In parallel with the changes of  $F_v$  values, PS II original light energy conversion efficiency ( $F_v/F_m$ ) of the 15 clones were adversely affected by waterlogging stress with significantly varied levels ( $p < 0.05$ ; Figure 8).  $F_v/F_m$  values of the most clones decreased severely with the prolonged waterlogging treatment, and reached the lowest values on day 45, with the exception of HS-1, HS-2, I-72 and I-69. On day 45, the lowest waterlogging tolerance index of  $F_v/F_m$  happened in I-214 (71.02%), and the highest happened in I-69 (99.73%; Supplementary Table 2). At the end of the recovery stage, most of the clones recovered to the CK level, except for HBY and LNY.



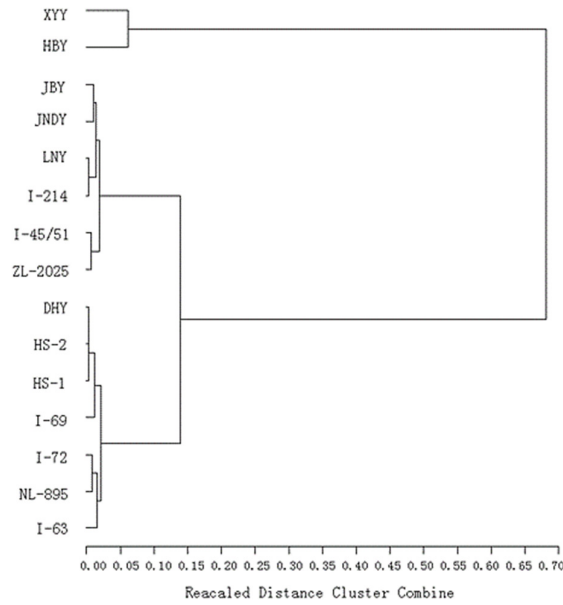
**Figure 7.** Responses of ratio of variable fluorescence to initial fluorescence ( $F_v/F_o$ ) of the 15 poplar clones under waterlogging treatment



**Figure 8.** Responses of efficiency of primary conversion of light energy of PS II ( $F_v/F_m$ ) of the 15 poplar clones to waterlogging stress

*Cluster analysis of waterlogging tolerance*

Based on the survival rates, coupled with the waterlogging tolerance indexes in height and ground diameter growth, biomass accumulation, as well as leaf gas exchange and chlorophyll fluorescence parameters on day 45, the 15 clones were classified into three groups by multivariate analysis (minimum distance method). XYY and HBY comprised the first group with susceptible waterlogging tolerance. JBY, JNDY, LNY, I-214, I-45/51, I-63 and ZL-2025 comprised the second group with moderate waterlogging tolerance. The third group was consisted of DHY, HS-2, HS-1, I-72, NL-895 and I-69, which has the strongest waterlogging tolerance (Figure 9).



**Figure 9.** Cluster analysis dendrogram of the performance of the 15 clones under waterlogging stress

*Membership function analysis*

According to the parameters used in cluster analysis of waterlogging tolerance, membership function values of the 15 poplar clones were calculated (Table 3). The results showed that HS-1 owned the highest average membership value (0.637), followed by NL-895 (0.620), and XYY had the lowest one (0.436). Identical to the results of cluster analysis, membership function analysis showed that waterlogging tolerance of HS-1, NL-895, I-69, HS-2 and DHY were the best, while XYY and HBY were the worst.

**Table 3.** Membership function values of the 15 poplar clones under waterlogging treatment

Clones	Membership function values											Average Membership function values
	Height	Ground diameter	Biomass	Root/shoot ratio	$P_n$	$G_s$	$T_r$	$C_i/C_a$	$F_v$	$F_v/F_o$	$F_v/F_m$	
XXY	0.344	0.377	0.464	0.275	0.472	0.337	0.486	0.534	0.476	0.391	0.643	0.436
HBY	0.372	0.375	0.445	0.370	0.479	0.494	0.510	0.538	0.485	0.552	0.511	0.467
I-214	0.444	0.500	0.400	0.654	0.404	0.433	0.348	0.620	0.517	0.529	0.465	0.483
LNY	0.402	0.506	0.466	0.581	0.592	0.383	0.651	0.410	0.536	0.626	0.478	0.512
I-45/51	0.389	0.421	0.445	0.515	0.564	0.550	0.617	0.551	0.525	0.526	0.541	0.513
JBY	0.563	0.550	0.319	0.538	0.455	0.491	0.624	0.639	0.418	0.470	0.590	0.514
ZL-2025	0.377	0.438	0.475	0.563	0.651	0.558	0.645	0.384	0.602	0.587	0.427	0.519
JNDY	0.640	0.448	0.537	0.525	0.628	0.562	0.610	0.404	0.508	0.607	0.532	0.546
I-72	0.657	0.617	0.613	0.615	0.460	0.542	0.606	0.538	0.516	0.496	0.623	0.571
I-63	0.650	0.610	0.550	0.618	0.466	0.620	0.559	0.362	0.636	0.596	0.630	0.572
HS-2	0.565	0.642	0.603	0.715	0.617	0.560	0.520	0.540	0.535	0.540	0.583	0.584
DHY	0.690	0.601	0.593	0.732	0.516	0.520	0.546	0.656	0.579	0.549	0.529	0.592
I-69	0.726	0.647	0.584	0.714	0.621	0.605	0.576	0.599	0.535	0.586	0.594	0.617
NL-895	0.745	0.664	0.727	0.719	0.616	0.651	0.576	0.588	0.479	0.533	0.519	0.620
HS-1	0.732	0.703	0.702	0.718	0.562	0.660	0.440	0.750	0.610	0.530	0.600	0.637

## Discussion

Lots of previous literatures reported that waterlogging stress would adversely affect morphology, anatomy, nutrient metabolism, hormone balance and photosynthetic performance in many plant species (Loreti *et al.*, 2016). Soil hypoxia is the main cause of waterlogging injury (Voeselek and Bailey-Serres, 2013). Survival and growth of plants under waterlogging stress are closely related to the function of leaves and roots (Kreuzwieser and Rennenberg, 2014; Striker and Colmer, 2017). Under waterlogging stress, roots of plants were firstly damaged, followed by inhibited growth of aboveground parts, such as retarded leaf initiation and development, decreased growth of height and ground diameter, chlorosis and abscission of leaves, as well as reduction of biomass accumulation (Chen *et al.*, 2010; Štícha *et al.*, 2016). Waterlogging-tolerant plants always can relieve injury by changing their morphology and physiological metabolism (Bailey Serres and Voeselek, 2008; Bejaoui *et al.*, 2012). It is generally believed that the characteristics of development of hypertrophied lenticels and adventitious roots, together with root aerenchyma formation are closely related to waterlogging tolerance of poplar (Kreuzwieser and Rennenberg, 2014; Peng *et al.*, 2017). In the present study, the waterlogging-tolerant clones generally developed hypertrophied lenticels and adventitious roots earlier than waterlogging-susceptible clones. For instance, DHY, HS-1, HS-2, I-69, NL-895, I-72 and I-63 hypertrophied lenticels on day 5-6 of waterlogging treatment, and induced their adventitious roots on day 10-11. While, the corresponding time in XYY, HBY, LNY, I-45/51 and I-214 were day 6-7 and day 16-17, respectively. In hypertrophied lenticels and adventitious roots, abundant aerenchyma is helpful for plants to relieve injury by enhancing absorption of O<sub>2</sub>, water and nutrient elements from water, accompanied with release of CO<sub>2</sub>, C<sub>2</sub>H<sub>2</sub>, ethanol and other harmful substances (Pedersen *et al.*, 2020). At the end of the experiment, all waterlogged plants of DHY, HS-1, HS-2, I-72, I-69, I-63 and NL-895 survived, and all waterlogged plants of XYY died, and survival rates of the other clones ranged from 33.33% to 83.33%. That means these seven clones could endure long-term waterlogging stress, and XYY was the most waterlogging-susceptible.

In addition to survival rate, growth and biomass accumulation are important indicators of plant waterlogging tolerance as well. Under waterlogging stress, waterlogging-tolerant plants can maintain superior growth and biomass accumulation to susceptible ones (Cao *et al.*, 1999; Gong *et al.*, 2007; Peng *et al.*, 2017). In our study, DHY, HS-1, HS-2, I-69 and NL-895 exhibited markedly higher waterlogging tolerance indexes compared to the other clones in growth and biomass accumulation, which indicated their superior vitality under waterlogging stress. The negative waterlogging tolerance index of ground diameter growth in XYY might due to the decay of stem base during waterlogging treatment, accompanied with the shrink during the recovery stage. A lower root/shoot ratio reflected the greater reduction in root than shoot growth, which mainly resulted from the decay of the original root system and the inhibition of new root growth (Liu and Dickmann, 1992; Zhou *et al.*, 2019). In our study, waterlogging-tolerant clones generally owned higher root/shoot ratios than waterlogging-sensitive clones, in accordance with those reported in the literatures (Rodriguez *et al.*, 2020).

Gas exchange reflects metabolism activities of plants (Chen *et al.*, 2005). Stomatal closure is one of the earliest responses of plants to waterlogging stress, which can reduce the rate of water loss and relieve waterlogging injury (Blanke and Cooke, 2004; Rood *et al.*, 2010). Waterlogging induced *Gs* decrease is usually associated with variation of hormone levels in plants, especially the increased concentration of abscisic acid (ABA) (Jackson *et al.*, 2003). Accompanied with the decrease of *Gs*, values of *Pn*, *Tr* and *Ci/ Ca* changed correspondingly as well (Mielke *et al.*, 2005). In the present study, the leaf gas exchange parameters of all clones decreased in varying levels with the prolonged waterlogging, which was not only related to the waterlogging induced damage of photosynthetic system, but also related to the reduced chlorophyll contents and premature leaf senescence (Sena and Kozłowski, 1980). Waterlogging-tolerant plants generally have higher leaf gas exchange parameters under waterlogging stress compared to waterlogging-sensitive plants (Du *et al.*, 2010; Liu *et al.*, 2011). In our study, the leaf gas exchange parameters of DHY, HS-1, HS-2, I-69 and NL-895 were remarkably less inhibited by waterlogging stress than those of XYY, HBY and the other clones. Therefore, they can survive after the long-term waterlogging stress, and maintain higher growth rate and biomass accumulation.

Chlorophyll fluorescence is the remission of energy absorbed by photosynthetic pigments, which was widely used as a sensitive measure of stress induced damage to PSII (Kozłowski *et al.*, 1984).  $F_v$ ,  $F_v/F_m$  and  $F_v/F_o$  are three important parameters to reveal the photochemical reactions of plants (Bilger *et al.*, 1995). Among them,  $F_v$  reflects the reduction of  $Q_A$  (primary quinone-type electron acceptor of PS II);  $F_v/F_o$  reflects the potential activity of PS II in leaves;  $F_v/F_m$  represents the maximum photochemical efficiency of PS II in leaves. It has been reported previously that  $F_v$ ,  $F_v/F_m$  and  $F_v/F_o$  decrease when plants are suffering from waterlogging stress (Smethurst *et al.*, 2005). The greater reduction levels usually reflect more severe damage to PS II (Du *et al.*, 2010; Peng *et al.*, 2017). In this study,  $F_v$ ,  $F_v/F_m$  and  $F_v/F_o$  values in all of the 15 clones pronouncedly decreased with the prolonged waterlogging. While, DHY, HS-1, HS-2, I-69 and NL-895 exhibited less decreased values and better recovery abilities than the other clones. That means DHY, HS-1, HS-2, I-69 and NL-895 suffered slighter waterlogging injury among the 15 clones, which was in accordance with their leaf gas exchange parameters.

### Conclusions

The waterlogging tolerance of plants is a complicated quantitative trait, which involved in characteristics of morphology, anatomy and physiology. Therefore, multi-indicator evaluation is necessary to accurately reflect waterlogging tolerance of plants (Zhou *et al.*, 2019). In this study, we evaluated waterlogging tolerance of the 15 clones by cluster analysis and membership function analysis, respectively, based on the parameters of their survival rate, growth of height and ground diameter, biomass accumulation, leaf gas exchange and chlorophyll fluorescence. Results of the both analysis methods showed that HS-1, I-69, DHY, NL-895 and HS-2 had the strongest waterlogging tolerance, XYY and HBY were the worst, and the other clones were moderate. Therefore, these waterlogging-tolerant clones should be selected preferentially for afforestation and breeding for waterlogging resistance in Jiangnan Plain.

### Authors' Contributions

Implementation of the experiment: RN G, Q H and TH N; Provision of experimental materials: XY Z and XP Z; Writing - original draft: RN G and XY Z; Writing - review and editing: KB D; Methodology: KB D; Data curation: RN G; Formal analysis: XY Z.

All authors read and approved the final manuscript.

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### Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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## Supplementary Files

**Supplementary Table 1.** The waterlogging tolerance indexes of the 15 poplar clones (%)

Clone	Seedling height	Ground diameter	Biomass				Total biomass
			Root	Stem	Leaf	Root/shoot ratio	
XYY	37.56	-9.63	7.25	30.45	0	53.85	12.54
JBY	62.27	71.68	45.77	67.70	77.22	64.86	65.09
JNDY	62.11	62.67	47.45	67.25	78.51	65.71	65.96
HBY	42.06	45.10	16.50	34.90	30.60	50.00	28.50
DHY	85.63	85.36	73.60	82.72	84.45	86.96	80.42
LNY	69.26	67.04	46.06	75.00	67.95	63.89	64.86
HS-1	87.32	83.63	73.41	83.61	84.18	88.37	80.69
HS-2	87.14	82.94	70.59	83.75	75.97	88.24	76.97
I-72	74.83	73.41	62.01	76.69	70.43	84.62	70.11
I-69	79.66	80.07	73.23	85.37	81.72	88.24	81.23
I-63	79.63	76.77	65.55	82.32	82.00	78.12	78.15
I-45/51	60.86	68.84	44.86	63.00	62.88	71.43	56.96
I-214	61.17	73.23	53.17	79.28	75.41	68.63	69.21
NL-895	84.59	78.55	71.02	86.20	72.85	88.24	77.28
ZL2025	60.86	69.43	49.82	73.92	70.06	68.42	65.90

**Supplementary Table 2.** The waterlogging tolerance indexes of leaf gas exchange and chlorophyll fluorescence parameters in the 15 poplar clones after 45-day waterlogging treatment (%)

Clones	$P_n$	$G_s$	$T_r$	$C_i/C_a$	$F_v$	$F_v/F_o$	$F_v/F_m$
XYY	17.49	19.11	20.24	66.89	66.02	71.34	79.10
JBY	73.09	71.76	68.67	99.11	70.39	70.77	74.00
JNDY	51.21	73.89	82.10	98.69	71.61	79.94	86.61
HBY	29.73	21.74	34.55	79.89	73.99	39.08	78.76
DHY	69.41	63.88	76.00	96.96	92.61	84.78	88.67
LNY	47.44	58.48	66.12	92.60	72.74	76.08	81.06
HS-1	66.91	77.62	68.73	93.86	85.83	81.72	96.21
HS-2	62.31	62.55	69.67	89.18	88.11	79.51	95.93
I-72	68.03	64.70	67.15	97.67	86.29	71.80	99.13
I-69	83.97	72.01	86.23	94.76	87.09	92.51	99.73
I-63	51.21	67.24	81.00	98.11	74.19	76.99	79.36
I-45/51	64.38	51.61	51.77	98.17	66.81	66.88	79.95
I-214	45.70	61.30	62.32	94.39	68.73	78.39	71.02
NL-895	55.07	62.04	51.55	92.17	88.11	64.99	88.36
ZL-2025	51.45	57.99	54.01	91.21	80.94	68.14	93.17