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# Adventitious root growth and relative physiological responses to waterlogging in the seedlings of seashore mallow (*Kosteletzkya virginica*), a biodiesel plant

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## Abstract

The biodiesel plant *Kosteletzkya virginica* has a high tolerance to salt, drought, and waterlogging. To determine the responses of *K. virginica* seedlings to waterlogging and subsequent recovery, the responses of three-month old plants during 35 d of waterlogging and another 14 d of recovery after drainage were investigated. Significant phenomena, such as adventitious root formation and increased root porosity in *K. virginica* seedlings after waterlogging, were observed. Serious stomata closure was also very serious. Waterlogging decreased the leaf chlorophyll concentration and weakened the photosynthetic capability of *K. virginica*. These phenomena were indicated by reduced maximum quantum yield of photosystem II ($F_v/F_m$), decreased preliminary efficiency of capturing photon (yield), and enhanced non-photochemical quenching (NPQ) in the treated seedlings. However, 14 d after drainage, these impaired characteristics gradually recovered. Statistical analyses indicated that the most significant adaptive mechanism was the generation of adventitious roots, followed by the increased aerenchyma. Stomata closure was the least significant mechanism for the survival of waterlogged *K. virginica* seedlings.

**Keywords:** adventitious root; growth; *Kosteletzkya virgini*ca; physiological response; seedlings; waterlogging.

**Abbreviations:** AR_adventitious root; DW_dry weight; ETR_electronic transport efficiency; Fv/Fm_ the maximum quantum yield of photosystem II; MR_main root; NPQ_non-photochemical quenching; Pn_photosynthetic rate; PS II_photosystem II; qP_photochemical quenching; SA_stomatal area; SD_standard deviation; Yield_the preliminary efficiency of capturing photon.

## Introduction

Soil water is a critical factor of plant distribution in the world, and even usually causes enviromental stress such as drought or waterlogging to affect plant characteristics (Iqbal et al., 2009; Ibraheem et al., 2011; Promkhambut et al., 2011). Waterlogging often occurs in many regions around the world because of excessive rainfall or poor drainage. By reason of hypoxia in the plant rhizosphere, waterlogging can severely impair the performance of terrestrial plants, whose morphological and physiological responses are prone to activation (Armstrong et al., 1994; Blom, 1999; Jackson and Armstrong, 1999; Kozlowski, 1997; Vartapetian and Jackson, 1997). Based on previous studies, most plant species are sensitive to waterlogging, and only some are able to highly adjust to waterlogging conditions, (e.g., adventitious roots and aerenchyma) (Jackson and Drew, 1984; Vartapetian and Jackson, 1997; Vignolio et al., 1999). Nevertheless, this adaptive capacity depends on the plant species (Laan et al., 1989; Poot and Lambers, 2003). The leaf is also very receptive to waterlogging; respiration changes in the leaf, leaf chlorophyll content, and photosynthetic assimilation have been detected during a waterlogging period (Parolin, 2000; Schlüter et al., 1993; Scholander and Perez, 1968; Waldhoff et al., 1998). In particular, chlorophyll fluorescence characteristics are usually influenced under waterlogging conditions. A reduction in the maximum quantum yield of photosystem II ($F_v/F_m$) after the onset of waterlogging has been reported in some plant species (Smethurst and Shabala, 2003; Smethurst et al., 2005). This decline may be induced by the lowered stomatal conductance (gs) (Lawlor and Cornic, 2002), altered hormonal status (Salisbury and Ross, 1992), and disordered mineral nutrient uptake (Castonguay et al., 1993). Furthermore, these factors can also change other indices of chlorophyll fluorescence, such as, electron transport, non-photochemical quenching (NPQ), and photochemical quenching (Maxwell and Johnson, 2000; Smethurst et al., 2005) in environmental stress.

After waterlogging, recovery is very critical to each plant species, especially to cultivars. An ideal waterlogging-tolerant species not only survives waterlogging, but also rapidly recovers after it. The recovering capabilities of some plant species are strikingly different from one another after waterlogging drainage. Some waterlogged species can rapidly recover to the control level after waterlogging, and others take a very long time (Parolin, 2001; Poot and Lambers, 2003; Smethurst et al., 2005). These previous studies are mainly limited to growth and photosynthetic characteristics; only a few papers briefly discuss special adaptative methods such as root porosity (Poot and Lambers, 2003). The focus is usually on woody plants, and there are limited reports on the adaptive characteristics of herbaceous species to waterlogging stress, special adjustments of adventitious bud and root (e.g., root porosity-related chlorophyll fluorescence characteristics), as well as restoration of waterlogged plants after drainage. *Kosteletzkya virginica* (L.) Presl, a perennial herbaceous plant, belongs to the Kosteletzkya genus of the family Malvaceae. *K. virginica* is native to the brackish portions of coastal tidal marshes in the mid-Atlantic and southeastern United States (Gallagher, 1985). As a potential biodiesel plant species, the use of *K. virginica* can improve coastal tidal flats and develop the biomass energy industry in China (Xu et al., 1996; Yin et al., 2000; Ruan et al., 2005). This plant has been introduced to China in 1992 and transplanted in the saline land along the coastal region of Jiangsu Province by the Halophyte Research Lab of Nanjing University in China. However, this plant often suffers from both drought and waterlogging stresses in North Jiangsu, given early drought periods, lack of rainfall (March to May), serious waterlogging from excessive rainfall (June and July), and lack of water drainage. Observations of this site have revealed that *K. virginica* did not tolerance such stresses. In one introduced site (Jinhai Farm), all crops and vegetation were waterlogged for approximately 40 d between June–July 2007. After this disastrous event, most plant species died, including Chinese tamarisks (*Tamarix chinensis* L.), which are widely mode plants that tolerate salt-alkali substances, waterlogging, and cold climate. However, seashore mallow survived this waterlogging and showed some visible adaptation characteristics, such as adventitious roots floating in water and adventitious buds of stem. Consequently, the current paper aimed to explore the adaptive mechanisms of *K. virginica* under waterlogging conditions. The relationship between the special mechanisms and survival of waterlogged seedlings was studied. The recovery capacity after drainage was also examined.

## Results

### *Adventitious root length, amount, and biomass*

During waterlogging, the adventitious root system of seashore mallow is an outstanding response and a critical adjustment for stress survival. In the present study, compared with the control seedlings that had no adventitious root, the mean length of the adventitious roots significantly increased at the early and middle stages of waterlogging. The lengths were 3.7, 6.9, and 12.5 cm when exposed to waterlogging for 7, 14, and 21 d, respectively. Afterwards, the root length was indistinctively enhanced at the late stage of flooding stress. The lengths were increased to 13.7 and 14.3 cm in the recovery stage at 28 and 35 d, respectively. The largest root-length increased with the duration of waterlogging, but its increasing mode was similar with that of mean root-length (Fig. 1-a).

Compared with the control seedling that had no adventitious root, the amount of adventitious root of stressed plants quickly increased with the duration of waterlogging. Five adventitious roots per plant formed in the 7 d-waterlogged seedlings, however this amount reached the maximum (11.5 roots per seedling) at the end of the stress (Fig. 1-b). Similarly, dry biomass of adventitious roots was constantly enhanced with the duration of waterlogging. The biomasses per seedling were 0.004 g and 0.017 g when exposed to waterlogging for 7 and 21 d, respectively (Fig. 1-c).

### *Root porosity*

Compared with the control in the adventitious root porosity trials, the values in the treated seedlings obviously increased during the first 14 d of waterlogging (approximately 11.6 %, 23.0% of the increase on days 7 and 14 of waterlogging compared with the corresponding control). The value reached the peak (43.3%) on day 21 of the stress. Afterward, this increase in treated porosity stabilized, but was still significantly different in the remaining time of the experiment, including in the recovery period (Fig. 2-a). Considering its similarity with the adventitious root porosity, the porosity of the main root increased compared with the control, and reached the maximum (27.5%, accounting for 1.54-fold of the control) on day 14 of waterlogging. However, the treated porosity constantly increased compared with the control, and the increase was 43.9% and 17.4% of the control, respectively, after 35 d of stress and 14 d of recovery (Fig. 2-b).

### *Leaf stomatal characteristics*

The stomatal lengths of the treated plants were lower than the control, except on days 14 and 28 of stress. The lengths were 86.6% and 96.5% of the control, respectively, at the end of the waterlogging and recovery (Fig. 3-a). The stomatal width significantly decreased (34.0% of control, $P \leq 0.05$) compared with the control on day 7 of waterlogging, and then increased to the maximum (12.1 μm, 83.1% of the control) on day 14 in the treated plants. Subsequently, the stomatal width constantly decreased with the waterlogging duration, reaching 65.1% of the control at the end of this stress. In the recovery period, the stomatal width obviously increased, and were 75.1%, and 89.9% of the control on days 7 and 14 of the recovery period (Fig. 3-b). Similar with the stomatal width, the stomatal area significantly decreased (37.7% of the control) on day 7 of the waterlogging, and its value on day 14 was the highest (494.4 $\mu m^2$ ) in all waterlogged seedlings. Nevertheless, from the days 14 to 35, the stomatal areas constantly decreased, and finally reached a minimum (301.9 $\mu m^2$, 61.2% of the control). After 7 and 14 d of recovery, the stomatal area enlarged by approximately 34.5% and 57.5%, respectively, compared with that in 35 d-waterlogged seedlings. At the end of recovery, the stomatal area was restored to an approximate control level (88.5 % of the control; Fig. 3-c).

### *Net photosynthetic rate and stomatal conductance*

The photosynthetic rate of the waterlogged plants was lower than that of the control seedlings (Fig. 4-a). On day 35 of waterlogging, the difference in the photosynthesis between the treatment and control was the highest at 42.5%, and was much significant ($P \leq 0.01$). When the waterlogging was withdrawn, the photosynthetic capacity gradually increased. After 7 and 14 d of recovery, the photosynthetic rates in the treated seedlings were 15.2% and 12.4% higher than that of the 35 d-waterlogged seedlings, but were still significantly lower than the corresponding control ($P \leq 0.05$). The values for stomatal conductance of the waterlogged seedlings were significantly lower than those of their corresponding controls, and occupied 53.9% of the control on day 35 of the waterlogging. However, after 14 d of recovery, the stomatal conductance was gradually restored to 72.6% of the control, but was still significantly lower than that in the controlled plants (Fig. 4-b). A correlation between the stomatal conductance and area was obtained and the model $y = 0.0003x - 0.0048$ was drawn with $R^2 = 0.422 > 0.396_{(0.001,\ 64)}$, where $y$ is the stomatal conductance, and $x$ is the stomatal area. This correlation coefficient indicated that the correlation between them was extremely significant ($P \leq 0.001$), and that the stomatal area showed the capacity of gas exchange via stomata.

**Table 1.** Net photosynthetic rate ($P_n$) of three kinds of waterlogged seedlings: *V. radiate*, *L. kaempferi and K. virginic*, in waterlogging stress and subsequent recovery. The number in bracket presents the corresponding treating time of seedlings, and the unit is 'day'.

| | *V. radiate** | *L. kaempferi* ** | *K. virginica* |
|---|---|---|---|
| Pn in waterlogging (% of the control) | 18.0–42.0 (8) | 40.0 (8) | 90.1 (7); 44.8 (35) |
| Decrease in Pn in waterlogging (% of the control) | 58.0–82.0 (8) | 60.0 (8) | 9.9 (7); 55.2 (35) |
| Pn in subsequent recovery (% of the control) | - | 46.0 (11) | 65.4 (7); 80.2 (14) |
| Decrease in Pn in recovery (% of the control) | - | 54.0 (11) | 34.6 (7);19.8 (14) |

* Data adopted from Ahmed et al (2002); ** data adopted from Terazawa et al (1992)

**Table 2.** Pearson correlation among the photosynthesis rate, chlorophyll concentration, stomatal area, and root parameters of *K. virginica* seedlings under waterlogging conditions. * and ** represent significant correlation ($\alpha = 0.05$) and very significant correlation ($\alpha = 0.01$), respectively. Pn_photosynthetic rate; CHC_chlorophyll concentration; STA_stomatal area; MRP_main root porosity; ARP_adventitious root porosity; ARA_adventitious root amount; ARB_adventitious root biomass (dry weight); ARL_adventitious root length.

| | Pn | STA | MRP | ARP | ARA | ARB | ARL |
|---|---|---|---|---|---|---|---|
| Pn | 1 | - | - | - | - | - | - |
| STA | 0.579** | 1 | - | - | - | - | - |
| MRP | -0.038 | 0.189 | 1 | - | - | - | - |
| ARP | 0.017 | 0.148 | 0.866** | 1 | - | - | - |
| ARA | -0.603** | -0.505* | 0.629** | 0.617** | 1 | - | - |
| ARB | -0.337 | -0.245 | 0.757** | 0.819** | 0.890** | 1 | - |
| ARL | -0.720** | -0.448 | 0.511* | 0.576** | 0.875** | 0.838** | 1 |

| | CHC | STA | MRP | ARP | ARA | ARB | ARL |
|---|---|---|---|---|---|---|---|
| CHC | 1 | - | - | - | - | - | - |
| STA | 0.804** | 1 | - | - | - | - | - |
| MRP | -0.124 | 0.189 | 1 | - | - | - | - |
| ARP | -0.002 | 0.148 | 0.866** | 1 | - | - | - |
| ARA | -0.757** | -0.505* | 0.629** | 0.617** | 1 | - | - |
| ARB | -0.461* | -0.245 | 0.757** | 0.819** | 0.890** | 1 | - |
| ARL | -0.697** | -0.448 | 0.511* | 0.576** | 0.875** | 0.838** | 1 |

### *Leaf chlorophyll concentration and chlorophyll fluorescence characteristics*

The chlorophyll in the waterlogged plants was obviously damaged. Except on day 21 of waterlogging, the concentration in the treated seedlings was significantly lower than that in the corresponding control (Fig. 5), and decreased with the duration of waterlogging. The minimum (1.41 mg $g^{-1}$ FW) was reached on day 35 of waterlogging. At the end of waterlogging, the difference between the treatment and control was the highest, and the concentration in the waterlogged seedlings was only 66.8% of the control. In the recovery, the treated chlorophyll concentration gradually increased, and was not significantly different with the control on day 14 of water drainage.

During waterlogging, $F_v/F_m$ and yield constantly decreased in the waterlogged seedlings, reaching the minimum ($F_v/F$m = 0.407; yield = 0.214) on day 35 of the stress, and were significantly lower than the corresponding control (Figs. 6-a and 6-b). Compared with the control, the NPQ in the treated plants increased with the duration of waterlogging, and peaked (1.042) on day 35 of stress. The NPQ was significantly higher than the control during waterlogging; for example, NPQ was 2.35-fold of the control on day 35 (Fig. 6-c). After drainage, the treated $F_v/F_m$ and yield gradually increased, but were still significantly different from the control. However, the NPQ in the waterlogged seedlings gradually decreased compared with those in the 35 d-treated seedlings, and occupied 1.62- and 1.39-fold of the corresponding control, respectively, on days 7 and 14 of recovery.

## Discussion

### *Special root structure for waterlogging resistance*

In the present study, an average of 11.5 adventitious roots per seedling of the mallow formed. The longest root grew to 23.6 cm in the 35 d-waterlogged seedlings, and all indices of the adventitious roots significantly increased (Figs.1 and 2) during waterlogging. The formation of adventitious roots was an emergent response at the early stress period, and the *K. virginica* seedlings had a strong ability of forming adventitious roots to sustain the survival of waterlogged plants. In Hakea species, adventitious roots are restricted to the surface layer of the cultivating medium (Poot and Lambers 2003), with root tips floating on the water surface. Presumably, this distribution mode facilitates aeration in the waterlogged plants. Hence, these roots are very critical to the survival of waterlogged *K. virginica* plants. Waterlogging causes oxygen deficiency. Consequently, the oxygen storage and transport functions of the aerenchyma become crucial to the survival of stressed plants. Primary aerenchyma is formed with the growth of adventitious root, and secondary aerenchyma is derived from programmed cell death as well as the subsequently connected internal spaces in damaged plant tissue (Kawase, 1981). In the current research, the main and adventitious roots reached a porosity of 24.4% and 42.9%, respectively, in the 35 d-treated *K. virginica* seedlings. These data revealed that the seedlings very readily formed aerenchyma to absorb and store oxygen under the waterlogging conditions. The increased root porosity was an immediate response and a critical adaptation strategy to the waterlogging.

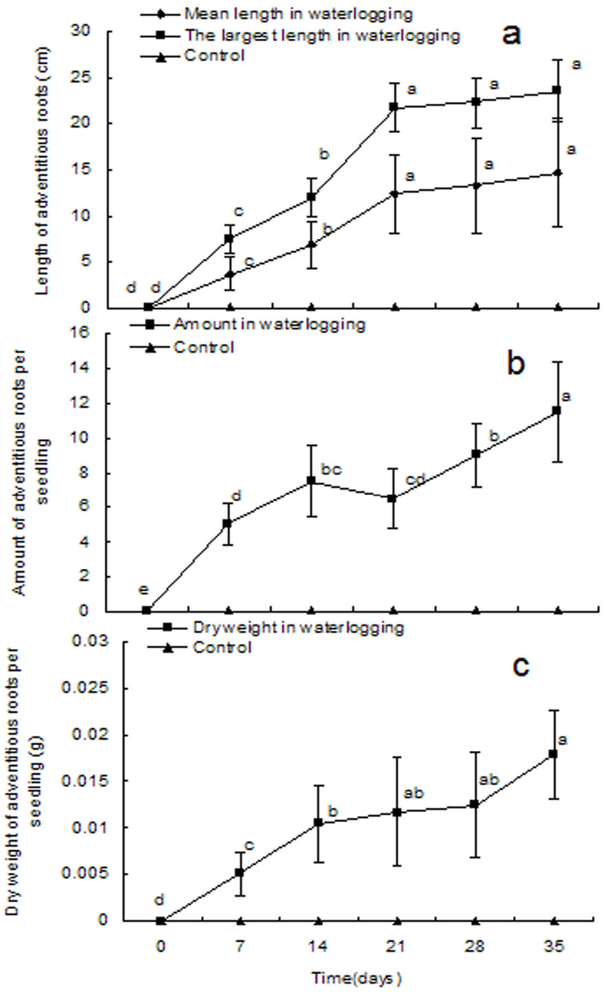

**Fig 1.** Changes of adventitious roots length (a), amount (b) and biomass (c) in *K. virginica* seedlings subjected to waterlogging for 0, 7, 14, 21, 28 and 35 days. Vertical bar represents mean ± SD of three replications. Values carrying different letters are significantly different at P≤0.05.

### *Emergent response of K. virginica to short-term waterlogging in terms of stomatal characteristics*

The stomatal size decreased to 64.4% of the control on day 7 of the waterlogging, and the stomatal conductance also significantly decreased. These results were similar with that of 3 d-waterlogged *Larix kaempferi* seedlings (Terazawa et al., 1992), and must be an emergent response of *K. virginica*-induced abscisic acid and ethylene (Blanke and Cooke, 2004) to the short-term waterlogging. Afterwards, the stomatal area increased to 89.2% of the control in the 14 d-treated plants. Such re-opening of stomata has been reported in seedlings of *Gmelina arborea*, *Tectona grandis* (Osonubi and Osundina, 1987), *Eucalyptus camaldulensis* (van der Moezel et al., 1989), and Japanese larch (Terazawa et al., 1992). This phenomenon is thought to be associated with the formation and growth of adventitious roots (Terazawa et al., 1992). However, the stomatal size gradually decreased after day 14 of waterlogging, suggesting decreased photosynthetic efficiency and damage to the photosynthetic system during the waterlogging. This suggestion was confirmed by the measured chlorophyll fluorescence indices $F_v/F_m$ and NPQ. After 14 d of recovery, the stomatal area of the waterlogged plants was restored to 88.5% of the control. This finding indicated that gas exchange was gradually restored, as confirmed by variations in stomatal conductance, but also showed that stomata guard cells were seriously affected by waterlogging.

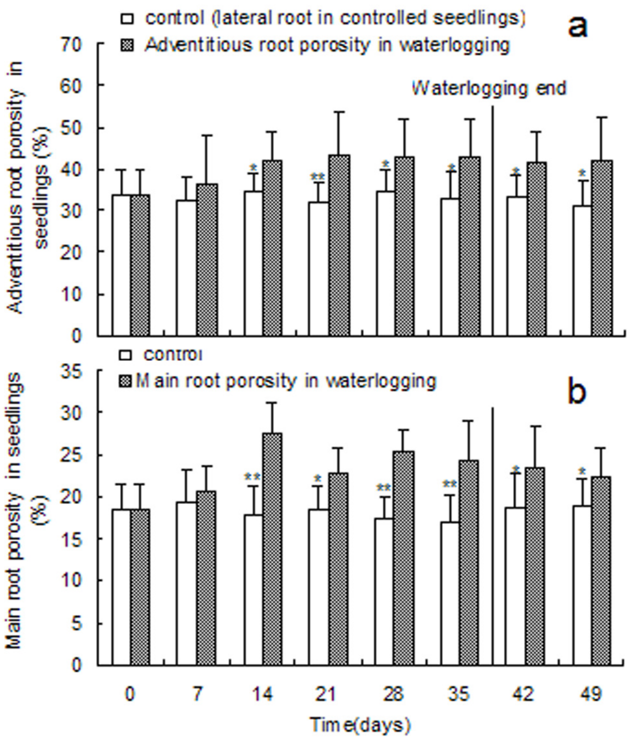

**Fig 2.** Changes of root porosity in *K. virginica* seedlings subjected to waterlogging for 0, 7, 14, 21, 28 and 35 days, and recovery for 7 and 14 days. Vertical bar represents mean ± SD of twenty four replications. ** indicates highly significantly different (P≤0.01) and * presents significantly different (P≤0.05).

### *Re-greening phenomenon in K. virginica after waterlogging stress*

As an injury symptom, chlorosis (or waterlogging spots) appeared in some leaves of *K. virginica* at the late stages of the waterlogging. This phenomenon indicated that the chlorophyll and chloroplasts in the treated plants were damaged by the waterlogging. This suggestion was confirmed by the decreased chlorophyll concentration in the waterlogged plants (Fig. 6). However, the chlorophyll concentration in the treated plants was restored gradually (86.40% of the control) during recovery. The stressed leaves began to re-green, and the chlorophyll was recovered after waterlogging drainage.

Photosynthesis was also negatively influenced by the waterlogging. Ahmed et al. (2002) have found that after 8 d of waterlogging with 2 cm of water level above soil, the photosynthetic rate of *Vigna radiata* (mungbean) decreased by 58.0% to 82.0%, respectively, at the vegetative stage. In another study, the photosynthetic rate of Japanese larch seedlings waterlogged to soil surface decreased by 60.0% after

**Table 3.** Multiple regression analysis of the yield and chlorophyll concentration in relation to six parameters of stomata and root properties for *K. virginica* seedlings under waterlogging conditions. $Y_1$_Pnis the $Y_2$_chlorophyll concentration; $x_1$, $x_2$, $x_3$, $x_4$, $x_5$, and $x_6$ are STA, MRP, ARP, ARA, ARB, and ARL, respectively; $b_1$, $b_2$, $b_3$, $b_4$, $b_5$, and $b_6$ are the standardized coefficients of $x_1$, $x_2$, $x_3$, $x_4$, $x_5$, and $x_6$, respectively.

| Equation of regression | *F* value | Sig. | Standardized coefficients | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | $b_1$ | $b_2$ | $b_3$ | $b_4$ | $b_5$ | $b_6$ |
| $Y_1 = 0.265 - 7.13E\text{-}05x_1 - 0.03x_2 + 0.343x_3 - 0.011x_4 + 7.295x_5 - 0.012x_6$<br>$R^2 = 0.874$ | 12.664 | 0.000 | -0.127 | -0.230 | 0.430 | -0.715 | 0.862 | -1.108 |
| $Y_2 = 0.001x_1 - 2.565x_2 + 3.799x_3 - 0.83x_4 + 13.725x_5 - 0.026x_6 + 1.178$<br>$R^2 = 0.969$ | 56.847 | 0.000 | 0.269 | 0.293 | 0.698 | -0.760 | 0.230 | -0.357 |

8 d of stress (Terazawa et al., 1992). In the current paper, the photosynthetic ability of waterlogged *K. virginica* seedlings as indicated by the yield decreased by 9.9 % and 44.8% of the control, respectively, on days 7 and 35 of stress (Table 1). These experimental data on photosynthetic capability also showed the strong tolerance of *K. virginica* to waterlogging stress.

The recovery capacity is very significant to waterlogged plant species after drainage. Terazawa et al. (1992) have found a photosynthetic recovery of 46% of the control in Japanese larch after 11 d of drainage. However, the photosynthetic rate in the stressed *K. virginica* seedlings in the present study was restored to 65.4% and 80.2% of the control, on days 7 and 14 of recovery, respectively (Table 1). These data showed that *K. virginica* plants had rapid and strong recovering abilities after drainage, but cannot completely recover to the control level after 14 d of drainage. The re-greening of leaves also reflected the strong recovering capacity of the seedlings after drainage.

### *Special integrated tolerance mechanism of K. virginica seedlings*

A correlation analysis among chlorophyll concentration, net photosynthetic rate, stomatal area, main root porosity, as well as porosity, amount, biomass and length of adventitious roots was conducted. Table 2 shows that the net photosynthetic rate and chlorophyll concentration were significantly correlated to the other parameters. The exceptions were the porosities of the main and adventitious roots, whose insignificant correlations with the photosynthetic rate and chlorophyll concentration may have been caused by the stabilization of the middle and late stages during waterlogging. The stomatal area was only significantly related to the adventitious root amount in all root parameters, whereas all other root parameters were significantly correlated to each other. Based on the analysis, the most measured parameters were highly correlated to one another. The strong tolerance of *K. virginica* seedlings to waterlogging, as indicated by photosynthesis, was obviously derived from such adaptive mechanisms as the formation of adventitious roots and aerenchymas, as well as leaf stomata closure. All these became components of a special integrated mechanism to waterlogging. The chlorophyll is the fundamental and essential substance for plant photosynthesis, and the photosynthetic rate directly reflects the actual photosynthetic capacity of plants. They can hence show the survival status of waterlogged seedlings. However, they can be affected by the stomatal area, porosity of main roots, as well as porosity, amount, dry biomass, and length of adventitious roots. To explore the degree of impact of each adaptive mechanism on the status of the waterlogged seedlings, a multivariate linear regression analysis of photosynthesis rates and chlorophyll concentrations was performed with the above six parameters. The absolute values of the standardized coefficients of the parameters were then compared to determine the extent of their impacts on the treated seedlings. The selected values for the correlation analysis, were used in this linear regression. Table 3 shows that the standardized coefficients associated with adventitious roots were all greater than those of main porosity. The stomatal area also had the lowest value among the six parameters for both photosynthetic rate and chlorophyll concentration. Hence, adventitious roots had the most significant effect on the survival of waterlogged *K. virginica* seedlings, followed by the main root porosity. The stomatal area had the least significant effect. Therefore, the most important adaptive mechanism for the survival of waterlogged *K.* seedlings was adventitious root generation for oxygen absorption, followed by increased aerenchyma for oxygen storage in the main roots. The least significant mechanism was that of stomata closure, which maintains the water potential and decreases oxygen release by stomata.

## Materials and methods

### *Experimental materials, treatment conditions, and experimental design*

The experimental materials (i.e., *K. virginica* seeds and cultivating soil) were obtained from the introduced sites in the north coastland of Jiangsu province. However, the soil contains the following physical and chemical properties such as 0.5% of salinity content, $1.34 g.cm^{-1}$ of bulk density, pH 8.5, $1.76 ds.m^{-1}$ of electrical conductivity, 0.66% of organic matter content. The selected plump seeds were soaked in 95% $H_2SO_4$ for 30 min, which eroded the hard seed capsule to improve seed germination, and were then soaked in water for 24 h. The treated seeds were sown in a mixed medium containing perlite and vermiculite (1:1), which had been disinfected at 120 °C for 30 min. After germination, the seedlings were grown in a greenhouse (25 °C/19 °C, day/night temperature), and irrigated with 1/4 strength Hoagland's solution each week. When the germinated seedlings were 45 d old, some uniform ones were transplanted to individual pots (8 cm in diameter, 8 cm deep; 2 plants per pot) filled with salt soil from the introduced coastland. Subsequently, the pots were placed in white plastic tanks (12 cm deep, 36 cm long, 26.5 cm wide; 12 pots per tank). Before the application of waterlogging, these seedlings were given a period of 45 d to adapt to the new growth conditions. At the beginning of the experiment, 5 plants (as 5 replicates) were sampled to measure the chlorophyll fluorescence and photosynthetic characteristics. Three plants were harvested to measure root characteristics such as the dry biomass (30 min at 105 °C and 48 h at 80 °C) of adventitious root, and to detect

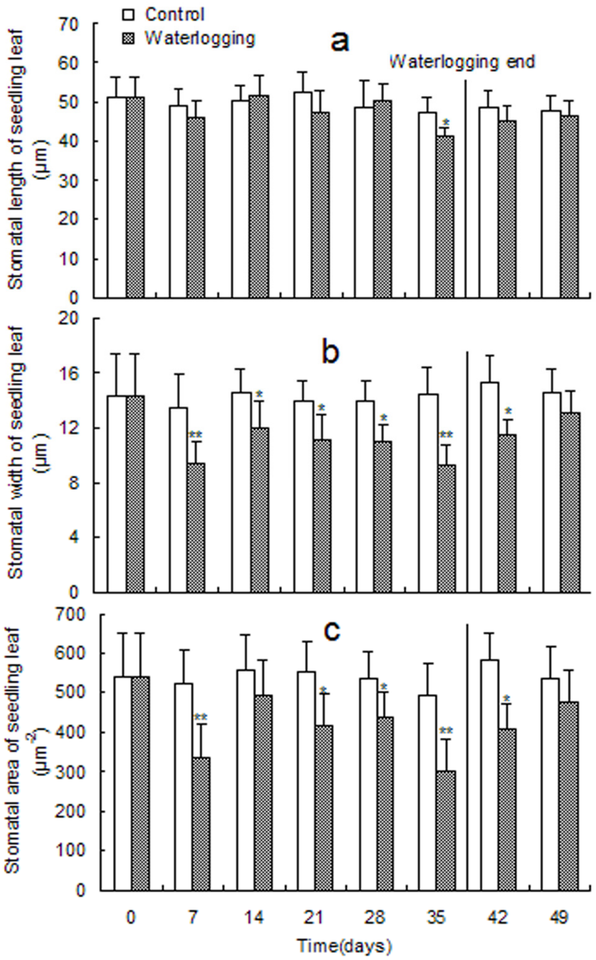

**Fig 3.** Changes of stomatal length (a), width (b) and area (c) of *K. virginica* seedlings subjected to waterlogging for 0, 7, 14, 21, 28 and 35 days and recovery for 7 and 14 days. Vertical bar represents mean ± SD of eight replications. ** indicates highly significantly different (P ≤0.01) and * presents significantly different (P ≤0.05).

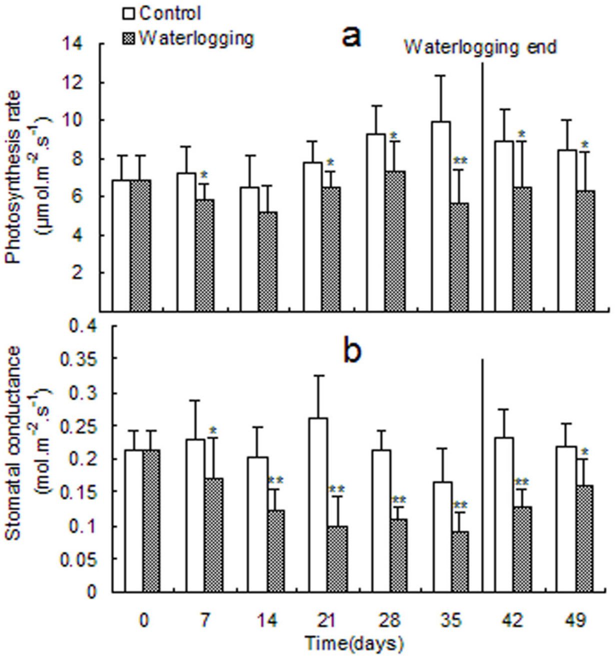

**Fig 4.** Change of photosythesis rate (a) and stomatal conductance (b) of *K. virginica* seedlings subjected to waterlogging for 0, 7, 14, 21, 28 and 35 days, and recovery for 7 and 14 days. Vertical bar represents mean ± SD of five replications. ** indicates highly significantly different (P≤0.01) and * presents significantly different (P≤0.05).

such physiological indices as chlorophyll content and stomatal characters. After the first harvest, the seedlings of the three tanks were waterlogged to 5 cm above the soil surface, and normal irrigation was used as the control. The samples were selected from three replicate tanks. Measurements were made after subjecting to waterlogging for 0, 7, 14, 21, 28, and 35 d, as well as recovery processes for 7 and 14 d. Given the absence of adventitious roots in the control plants, the lateral root porosity was used as the control of the adventitious root porosity during waterlogging.

### *Root porosity measurement*

Root porosity was measured by the microbalance method (Visser and Bögemann, 2003). Small segments were cut from a root using a razor blade, and gently rolled on tissue paper for 2 s to remove adherent water. The segments were transferred into a weighted micro-centrifugal tube ($W_0$) to prevent weight loss by evaporation. After closing the tube, the weight of the segments plus the tube was measured ($W_1$) on a microbalance (Sartorious BS 110S, Sartorious Company, Goettingen, Germany), transferred into a cuvette filled with water, and infiltrated for a maximum of 10 min under vacuum conditions. The infiltrated samples were rolled on tissue paper to remove surface water, and were weighed again in the same tube ($W_2$). The porosity was calculated from:

$$\text{Porosity (\%, v/v)} = 100 * \frac{(W_2 - W_1)}{(W_2 - W_0)} * SW \ ,$$

where $W_0$ is the weight of the micro-centrifugal tube, $W_1$ is the weight of both the non-infiltrated root and centrifugal tube, $W_2$ is the weight of both the water-infiltrated root and centrifugal tube, and SW, the density of the water-infiltrated roots, is 1.036 g $mL^{-1}$. In this measurement, the sample amount cannot be less than 24 replicates, and the weight of each sample must be less than 30 mg.

### *Measurement of stomatal characteristics*

By the same method, a leaf segment (2 × 2 $mm^2$) was immediately treated into a temporary section. Observation, measurement, and photomicrography on the section were performed using a light microscope and camera system (Nikon ECLIPSE 80i, Nikon Corporation, Tokyo, Japan). In the course of the observation, the stomatal length and width were measured using an NIS-Elements BR 2.30. Finally, the stomatal area was calculated by the following equation:

$$\text{SA } (\mu m^2) = \frac{\pi ab}{4} \ ,$$

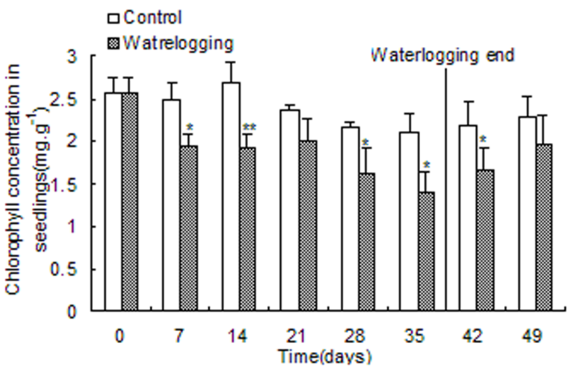

**Fig 5.** Change of leaf chlorophyll concentration of *K. virginica* seedlings subjected to waterlogging for 0, 7t, 14, 21, 28 and 35 days and recovery for 7 and 14 days. Vertical bar represents mean ± SD of three replications. ** indicates much significantly different (P≤0.01) and * presents significantly different (P≤0.05).

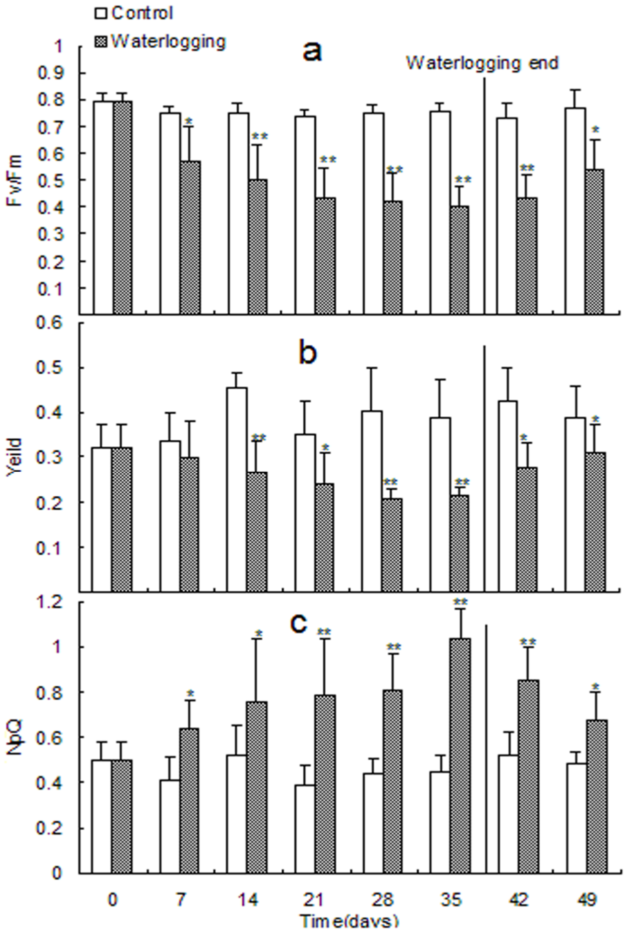

**Fig 6.** Changes of chlorophyll fluorescence in *K. virginica* seedlings subjected to waterlogging for 0, 7, 14, 21, 28 and 35 days and recovery for 7 and 14 days. Vertical bar represents mean ± SD of five replications. ** indicates highly significantly different (P≤0.01) and * presents significantly different (P≤0.05) between treatment and its corresponding control.

where *SA* is the stomatal area, *a* is the stomatal length, and *b* is the stomatal width.

### *Measurement of the net photosynthetic rate and stomatal Conductance*

The photosynthetic rate and stomatal conductance were measured by a portable photosynthesis system (LI-6400, LI-COR Co. Ltd., Nebraska, USA). Each treatment was designed to contain 5 plants, and the 5th leaf from the top was selected as the sample. The inner photo source was used to provide light energy, and stomatal conductance was determined by the light response curve method. The stomatal conductance at a light intensity of 1200 μmol $m^{-2}s^{-1}$ photosynthetic photon flux density was used as the reference value, because its corresponding photosynthetic rate was the highest in the series of light intensities in the light response curve of *K. virginica* seedlings.

### *Chlorophyll concentration measurement*

According to the acetone extraction method of Kirk (1968) with slight modifications, chlorophyll was extracted using a mixture of acetone and ethanol (1:1, v/v). About 0.2 g of leaf tissue was minced into small fragments. The fragments were soaked and extracted in the mixture solution for 12 h in the dark. When the leaf chips were entirely whitened, the extract was analyzed colorimetrically at 663 and 645 nm, respectively.

### *Chlorophyll fluorescence measurements*

The chlorophyll fluorescence characteristics of the leaves were determined by a chlorophyll fluorometer (MIN-PAM, WALZ Company Ltd., Effeltrich, Germany). Before analysis, the leaves were clipped with a special leaf clamp and darkened for 30 min. Some chlorophyll fluorescence indices such as $F_v/F_m$, yield, and NPQ were measured by the saturation pulse method described in the Mini-PAM manual.

### *Statistical analyses*

Duncan multiple comparison, multivariate linear regression, and correlation analysis were performed using SPSS 11.5. Comparisons with $P \le 0.05$ and $P \le 0.01$ were significantly different and highly significantly different, respectively. When the *P* value was less than 0.05 and 0.01 in the correlation analysis, the corresponding correlation was significant and highly significant, respectively.

## Conclusions

Under waterlogging conditions, the photosynthetic capability of *K. virginic*a seedlings was impaired, as indicated by chlorophyll fluorescence indices. However, the seedlings exhibited special survival strategies such as adventitious root growth, increased aerenchyma space in roots, and leaf stomata closure. Combined field observations and laboratory experimental data showed that *K. virginica* had a strong waterlogging tolerance. After waterlogging drainage, some impaired characteristics in treated seedlings quickly recovered to a large extent. Therefore, *K. virginica* may have a high potential as a biodiesel plant species to improve coastal tidal flats and help develop the biomass energy industry in China given its strong waterlogging tolerance and rapid recovery.

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