SHORT COMMUNICATION

Growth, morphology and photosynthetic activity in flooded *Alnus japonica* seedlings

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Abstract This study was conducted on *Alnus japonica* seedlings subjected to flooding for 2, 4, and 6 weeks to examine responses in growth, morphology, and photosynthesis to different periods of flooding. Seedlings subjected to flooding for 2 and 4 weeks were drained after flooding then watered daily. Increases in biomass of leaves, roots, and whole plants were less for 6-week-flooded seedlings. Rate of photosynthesis and stomatal conductance of flooded seedlings decreased within 2 weeks. For 2-week-flooded seedlings recovery from reduced stomatal conductance and recovery of photosynthetic activity occurred after drainage. For the 6-week-flooded seedlings stomatal conductance recovered by the end of the experiment. Adventitious root formation by the 4 and 6-week-flooded seedlings was observed from the third week of flooding. These results suggest that recovery of reduced function in leaves may progress with development of adventitious roots during the period of flooding.

Keywords Adventitious roots · *Alnus japonica* · Drainage · Flooding · Photosynthesis

Introduction

Alnus japonica (Thunb.) Steud. is native to swamp areas along rivers and lakes and is widely distributed in northeast Asia (Fujita and Kikuchi 1986). Typical habitats of this species are constantly or periodically flooded areas with

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poorly drained soil (Fujita 2002). In the Kushiro Mire located in Hokkaido Island, Japan, this species is commonly observed and is one of the representative species of wetland forests.

Depth and duration of flooding in the Kushiro Mire vary depending on site and season. The different sizes and forms of *A. japonica* trees observed in the Mire may be affected by this variation of the flooding. It is important to investigate the effects of flooding conditions on the growth, morphology, and physiology of *A. japonica* for analysis of the characteristics of the distribution and survival strategy of this species. Most previous investigations of *A. japonica* have focused on growth responses to continuous flooding, however (Terazawa and Kikuzawa 1994; Yamamoto et al. 1995), and very little information has been obtained about responses to environmental differences in flooding conditions.

Soil flooding is usually followed by rapid stomatal closure that causes severe restriction of CO_2 diffusion in the leaves of the flooded plants (Jackson and Colmer 2005; Kozlowski (1997). In addition to stomatal closure, oxygen deficiency in the roots causes physiological disruption, including inhibition of photosynthetic activity under conditions of prolonged flooding (Bradford 1983; Pezeshki (2001). It is well known that during flooding many flood-tolerant species undergo a variety of morphological and physiological changes to maintain more normal conditions. For example, under conditions of continuous flooding a variety of morphological and anatomical changes occur in the flooded portions of the stems of *A. japonica* seedlings (Terazawa and Kikuzawa 1994; Yamamoto et al. 1995). These changes, which include adventitious root formation and aerenchyma development, are regarded as facilitating oxygen transport to anaerobic tissues. According to Yamamoto et al. (1995), a large number of adventitious

roots with abundant aerenchyma appeared in *A. japonica* seedlings after flooding for 2 weeks or more. These results suggest that establishment of an effective ventilation system, including formation of adventitious roots, seems to be important in the acquisition of flood tolerance in this species.

This study was conducted to examine the responses of photosynthetic activity to morphological changes in *A. japonica* seedlings under different flooding conditions.

Materials and methods

The experiment was conducted on 1-year-old *A. japonica* seedlings. Seeds of *A. japonica* were obtained from the Botanical Garden of Hokkaido University in October, 2001 and kept under room-temperature conditions. On March 20, 2002, the seeds were soaked in water for 24 h and germinated on damp vermiculite. On May 30, 2002, the seedlings with active apical meristems were planted in plastic pots (ϕ10.5 × 11.5 cm) containing one part vermiculite, one part bark compost, and three parts sandy loam. On March 26, 2003, 1-year-old seedlings were transplanted to plastic pots (ϕ19.5 × 14.5 cm) containing the same soil components and kept under outdoor conditions. Each pot was watered daily with tap water and fertilized every 2 weeks with 200 ml 1/500 nutrient solution (Hyponex 70781; Hyponex Japan, Osaka, Japan).

On May 6, 2003, the seedlings were selected for uniformity of size and development: mean heights ± standard error (SE), 96.1 ± 1.6 cm; mean stem diameters 1 cm above the ground level (GL) ± SE, 11.6 ± 0.2 mm. The seedlings were then transferred to the greenhouse of the Arid Land Research Center, Tottori University. Average temperature in the greenhouse throughout the study period ranged from 21 to 30°C, and average relative humidity ranged from 67 to 98%.

Twenty seedlings were divided into four groups:

1. five seedlings unflooded and watered daily, as control;
2. five seedlings flooded for 2 weeks then drained condition for 4 weeks;
3. five seedlings flooded for 4 weeks then drained conditions for 2 weeks; and
4. five seedlings flooded for 6 weeks.

Water levels during flooding were maintained at 30 cm above the GL.

At the end of the experiment, growth in height and diameter were measured with a rule and a microcaliper, respectively. All the seedlings were then harvested, separated into leaves, stems, and roots, and dry weights were determined separately after drying at 80°C for 48 h. The number of adventitious roots on stems was counted every week.

Rates of photosynthesis and stomatal conductance were determined 0, 1, 7, 14, 24, 27, 31, 36, and 42 days after the initiation of the treatments by means of a portable photosynthesis system (Li-Cor LI-6400). Measurement of rates of photosynthesis and stomatal conductance were made at a photosynthesis photon flux density (PPFD) of 1,100 μmol m^{-2} s^{-1}, determined from light-saturated rates of photosynthesis using the photosynthesis–PPFD response curve. To obtain photosynthesis-PPFD response curve, rates of photosynthesis at different PPFD were measured on three leaves from different seedlings prior to the initiation of treatments. The CO_2 concentration, temperature, and vapor pressure deficit in leaf surface were not controlled. Measurements of photosynthesis were conducted from 8:00 to 9:30 a.m. to avoid midday depression of photosynthesis. The fourth or fifth leaves from shoot apices (one leaf per plant) were selected for these measurements. Air temperature (AT), relative humidity (RH), and CO_2 concentration in the chamber during photosynthesis measurements were 30.2 ± 0.5°C; 44.8 ± 2.8%, 381.2 ± 3.0 μmol m^{-2} s^{-1}, respectively.

The significance of the difference between any two mean values for growth, dry weight of plant parts, rates of photosynthesis, and stomatal conductance was analyzed by use of Scheffe's multiple comparison test at the level of 0.05 and was expressed by use of different letters. Statistical differences between data for the number of adventitious roots were determined by means of the two-sided Mann–Whitney's U test at $P < 0.05$, with the Bonferroni correction.

Results

Flooding did not significantly inhibit growth in the height of the seedlings (Table 1). For the 4 and 6-week-flooded seedlings increases in diameter at approximately water level (30 cm above GL) were enhanced (Table 1) whereas dry weights of roots and total plants were reduced (Table 2). The *T/R* ratio for flooded seedlings was not changed by flooding treatments (Table 2).

Flooding for 1 or 2 weeks reduced the rate of photosynthesis (Fig. 1). Drainage after 2-week-flooding enabled rates of photosynthesis and stomatal conductance to recover by the 24th day (Fig. 1). For 4-week-flooded seedlings the reduced rate of photosynthesis and stomatal conductance recovered after drainage (Fig. 1). Recovery of stomatal conductance for the 6-week-flooded seedlings was similar to that for seedlings drained after 4-week-flooding; recovery of rates of photosynthesis was not similar, however (Fig. 1). Differences between the rates of photosynthesis of control and the flooded seedlings decreased gradually with time (Fig. 1). Rates of photosynthesis for

6-week-flooded seedlings decreased to 23.6% of those for controls on the 14th day and recovered to 71.0% on the 42nd day.

Within 3–4 weeks of initiation of flooding many adventitious roots appeared on submerged portions of the stems of most of the flooded seedlings (Fig. 2). Hypertrophied lenticels developed on submerged stems within a few days of flooding (data not shown). The adventitious roots and hypertrophied lenticels of the 4-week-flooded seedlings withered a few days after the end of the flooding (Fig. 2).

Discussion

In this study, flooding for 4 and 6 weeks reduced root biomass and induced adventitious root development (Table 2, Fig. 2). Many woody species exposed to flooding lose part of their original root system as a result of decay (Kozlowski 1997). Adventitious rooting is a mechanism for replacing roots that existed before flooding, which have been killed, or whose function is impaired by oxygen deficiency (Vartapetian and Jackson 1997). The function of adventitious roots is to support shoot growth during prolonged soil submergence, by supplying water, minerals, and hormones (Islam and MacDonald 2004; Jackson and Drew 1984; Kozlowski 1997; SenaGomes and Kozlowski 1980; Tsukahara and Kozlowski 1985; Vartapetian and Jackson 1997). As some reports have indicated (Kozlowski

1984 Kozlowski 1997; Pezeshki et al 1996), the initial reduction in rates of photosynthesis of flooded plants is a consequence of stomatal closure, whereas rates of photosynthesis are progressively reduced by other inhibitory effects (Bradford 1983; Kozlowski 1997; Pezeshki 2001).
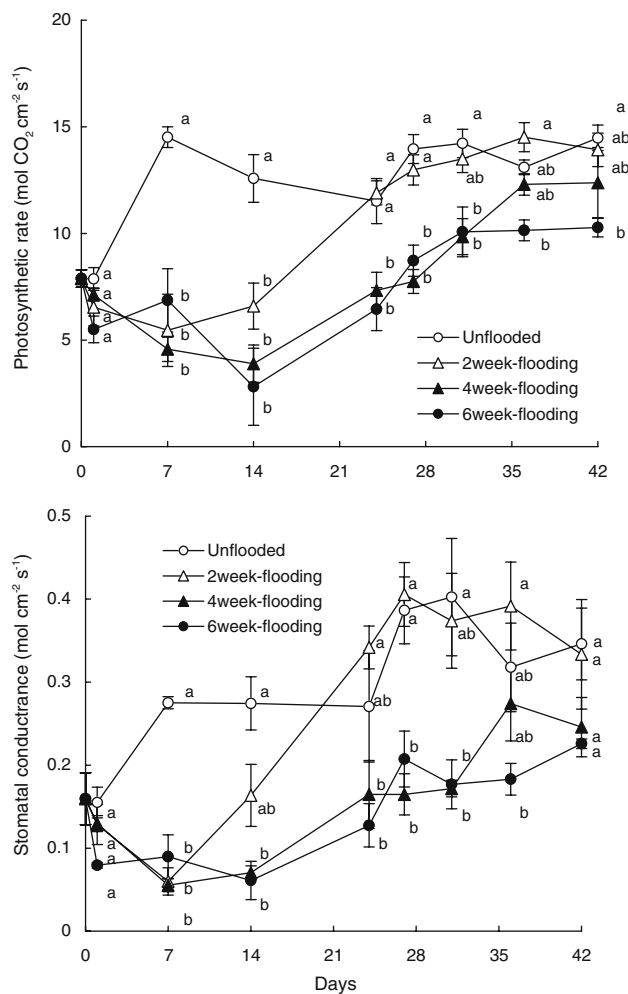

Fig. 1 Effects of duration of flooding on rates of photosynthesis (*upper*) and stomatal conductance (*lower*) of flooded seedlings: *open triangles*, seedlings flooded for 2 weeks; *filled triangles*, seedlings flooded for 4 weeks; *filled circles*, seedlings flooded for 6 weeks; *open circles*, unflooded seedlings. Values are means ($n = 5$) with standard error. *Different letters* for the same day indicate significant differences at $P < 0.05$ using Scheffe's test

Table 1 Effects of flooding duration and drainage on increases in the height and diameter of flooded seedlings

	Height increase (cm) [a]	Diameter increase (mm)[a]	
		1 cm above GL	30 cm above GL
Unflooded	18.3 ± 4.3a	1.6 ± 0.3a	1.8 ± 0.1a
2-week-flooded	14.7 ± 1.3a	1.1 ± 0.3a	2.0 ± 0.2ab
4-week-flooded	14.3 ± 2.6a	1.4 ± 0.3a	3.1 ± 0.3b
6-week-flooded	9.5 ± 0.2a	0.2 ± 0.5a	3.0 ± 0.5b

[a] Result are given as mean ± SE. Values without the same letter in each column are significantly different at $P < 0.05$ using Sheffe's test ($n = 5$)

Table 2 Effects of flooding duration and drainage on dry weights of the seedlings

	Leaves (g)[a]	Stems (g)[a]	Roots (g)[a]	Total plants (g)[a]	T/R[a]
Unflooded	17.3 ± 0.9a	26.2 ± 2.2a	46.2 ± 4.5a	90.9 ± 5.0a	1.0 ± 0.1a
2-week-flooded	14.0 ± 0.7a	25.4 ± 2.3a	42.2 ± 3.6ab	84.6 ± 5.2ab	1.0 ± 0.1a
4-week-flooded	12.0 ± 1.8ab	24.7 ± 3.8a	20.8 ± 2.7c	56.9 ± 3.9b	1.9 ± 0.3a
6-week-flooded	7.9 ± 1.2b	24.6 ± 4.0a	25.1 ± 5.0bc	57.7 ± 8.3b	1.5 ± 0.4a

[a] Results given as mean ± SE. Values without the same letter in each column are significantly different at $P < 0.05$ using Sheffe's test ($n = 5$)
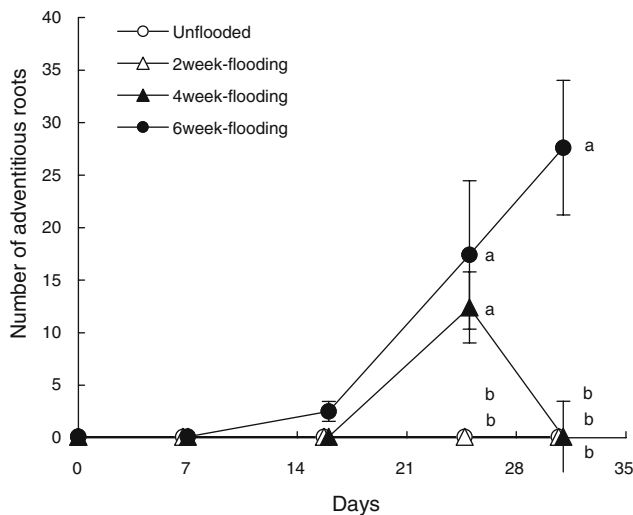
Fig. 2 Number of adventitious roots on stems: *open triangles*, seedlings flooded for 2 weeks; *filled triangles*, seedlings flooded for 4 weeks; *filled circles*, seedlings flooded for 6 weeks; *open circles*, unflooded. Values are means ($n = 5$) with standard error. *Different letters* for the same day indicate significant differences at $P < 0.05$ using Mann–Whitney's U test with Bonferroni correction ($n = 5$)

Under conditions of prolonged flooding, morphological and structural modifications are important in the maintenance of more normal physiological conditions in plants. Close association of adventitious root formation with stomatal reopening in some species have suggested that adventitious roots may be very important in the recovery of gas exchange during flooding stress (Tang and Kozlowski 1984; Kozlowski and Pallardy 1984). Increased stomatal conductance in flooded *A. japonica* seedlings with adventitious roots (Figs. 1, 2) also suggests that formation of adventitious roots contributes to recovery of photosynthetic activity, in conjunction with stomatal reopening, in this species under continuous flooding conditions.

Rates of photosynthesis and stomatal conductance of *A. japonica* seedlings reduced by 2-week-flooding rapidly recovered to the same level as for the controls in response to drainage. The photosynthetic response of flooded plants to drainage varies with species and with the intensity and duration of flooding (Kozlowski 1984; Pezeshki and Chambers 1985). Rapid recovery of photosynthesis during and after the flooding is important for survival of flooded woody plants in wetland forests (Pezeshki 1994; Pezeshki and Chambers 1985). The ability of this species to recover swiftly from reduced photosynthetic activity may enable it to grow and to survive in periodically flooded areas such as the Kushiro Mire.

It is concluded that both the ability to recover the rate of photosynthesis with stomatal re-opening after drainage and to improve the rate of photosynthesis by formation of adventitious roots during flooding may contribute to adaptation of *A. japonica* to wetland.

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