論　文

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Physiological, morphological and growth responses of several tree species to flooding stress

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Abstract : The ecophysiological characteristics of *Alnus hirsuta* Trucz., *Populus alba* L., and *Taxodium distichum* L. saplings under flooding conditions were examined. Flooding caused reduction in gross photosynthetic rate (Pg_{max}) and chlorophyll fluorescence yield and reduced the growth of the *A. hirsuta* and *P. alba* saplings. On the other hand, the reductions in gross photosynthetic rate (Pg_{max}) and chlorophyll fluorescence yield were small, and the growth was not reduced during flooding period in the *T. distichum* saplings. The development of adventitious roots on the submerged stems was observed in the *P. alba* and *A. hirsuta* saplings. In the *T. distichum* saplings, diameter growth of the submerged stems was accelerated under the flooding condition without the development of adventitious roots on them.

Keywords : Adventitious roots, Chlorophyll fluorescence, Eco-physiology, Flooding stress, Photosynthesis.

ズルフィカル　ハリデ　ジャンダン・坂本圭児・吉川　賢：**冠水ストレスに対する数樹種の成長，形態，および生理的特性**

摘要:冠水条件におけるヌマスギ（*Taxodium distichum* L.），ギンドロ（*Populus alba* L.），およびケヤマハンノキ（*Alnus hirsuta* Trucz.）の生態生理的特性を比較検討した。これらの樹種のポット苗を冠水条件においたところ，ケヤマハンノキとギンドロでは，葉量の減少，光合成速度の低下，および葉の光阻害がみられ，成長が抑制された。その抑制の程度はケヤマハンノキの方が顕著であった。ヌマスギでは，葉量の低下がみられず，光合成速度の低下および葉の光阻害の程度もギンドロやケヤマハンノキに比べ小さく，成長が抑制されなかった。ギンドロとケヤマハンノキでは樹幹冠水部に不定根が発達した。ヌマスギでは樹幹冠水部に不定根がみられなかったが，冠水によって樹幹基部の形態が膨満となり，冠水によって材の組織構造が変化していることが示唆された。

キーワード：不定根，クロロフィル蛍光反応，生態生理，冠水ストレス，光合成

1. Introduction

There is a considerable problem in dam sites for the conservation of the environment. The water level in the dam sites fluctuates, depending on the balance of the rainfall and the water usage. These fluctuations cause soil erosion on the slopes of the dam site because of the repetition of bare conditions and flooding conditions on the slopes. The bare condition on the slopes is also a landscaping problem. Revegetation on the slopes in the dam sites is urgently needed for the resolution of these problems. However, due to the effects of the flooding stress on tree species, revegetation in these areas is difficult. Tree species distributed in flooded areas are tolerant to the flooding stress. Flood tolerance varies greatly among tree species and flood-tolerant tree species survive waterlogging by complex interactions of morphological, anatomical, and physiological adaptations[2,7]. It might be an appropriate way to apply these species for revegetation in the dam site slopes. However, it should be considered the possibilities from the aspect of revegetation practice. Environmental conditions in dam site slopes are different from those in the natural habitats. After planting tree species are exposed to both radical flooding stresses, and the moisture stresses fluctuating between flooding and drought.

The objective of the present study is to define the characteristics of the tolerance of *Alnus hirsuta* Trucz., *Populus alba* L., and *Taxodium distichum* L. under radical flooding conditions. *P.alba* and *T. distichum* were chosen as wetland tree species. *A. hirsuta* is frequently used for erosion control and the habitat is drier than that of other wetland *Alnus* spp. e.g. *Alnus japonica*. *A. hirsuta* was chosen as *Alnus* species with different ecological characteristics from wetland *Alnus* species. The saplings of these species were exposed to the flooding conditions at different water levels and oxygen concentrations. Then their physiological responses, growth characteristics and their tolerance to flooding stress were examined.

2. Materials and Methods

The saplings of *A. hirsuta* (53 cm-95 cm in height), *P.alba* (82 cm-129 cm in height), and *T. distichum* (48 cm-70 cm in height)

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were used for the experiments. Saplings were obtained from a local nursery in April 1999. Forty-two saplings of each species were transplanted into plastic pots in April 1999. The depth and diameter of the pots were 26 cm and 35 cm, respectively. Granite sand was supplied to the pots as the soil medium. After transplantation, the saplings were watered every day or every two days. All saplings were healthy at the beginning of the experiment.

The experiments were conducted in a green house and the saplings were exposed to flooding stress in plastic containers with different water levels and oxygen concentration. The saplings were divided into four groups as described below,

1. Six saplings were unflooded during the experiments. Abbreviation UF was assigned for this treatment. They were used as the control group. These saplings were watered every day or every two days for maintaining field capacity content in the soil.
2. Six saplings were flooded to the soil surface with non-aerated water in a plastic container. Abbreviation F_0 was assigned for this treatment. The container had the dimensions of 105 cm x 50 cm x 35 cm (depth).
3. Six saplings were flooded to the height of 20 cm above the soil surface with non-aerated water. Abbreviation F_{20} was assigned for this treatment. The container of this group had the dimensions of 90 cm x 50 cm x 52 cm (depth).
4. Six saplings were flooded to the soil surface with aerated water in a plastic container. Abbreviation F_{air} was assigned for this treatment. The container had the dimensions of 105 cm x 50 cm x 35 cm (depth). The air was supplied with an air pump (Hiblow air pump, 40/42 l/min, Shimadzu Co.).

The water levels of each treatment group were maintained constantly throughout the flooding period by adding water. During the experimental period, the water in the containers was regenerated twice.

Oxygen concentration of containers was measured with a portable dissolved oxygen meter (OM-14, Horiba) weekly from April 1999 to September 1999 (Table 1).

The heights and stem base diameters at the height of 3 cm and 20 cm above the soil surface were measured for all the saplings in April 1999. They were remeasured on September 13. The length of all branches of each sapling was measured once every two weeks from April to September 1999. The number of leaves of the saplings was counted once every two weeks from April 1999 to September 1999. In the *A. hirsuta* and *P. alba* saplings, the number of the leaves was counted in all the branches. In the *T. distichum* saplings, ten branches were selected from two years old branches. Number of the current year branches with leaves in the selected branches was counted as the number of the leaves.

Table 1 Oxygen concentration of treatment groups from April 1999 to September 1999. Mean±S.D.

Species	Oxygen concentration (mg/ l)		
	F0	F20	Fair
Alnus hirsuta	6.73±0.45	5.66±0.72	7.13±0.18
Populus alba	5.99±0.84	5.04±1.24	7.34±0.16
Taxodium distichum	6.29±0.43	5.64±0.24	7.43±0.19

Net photosynthetic rate (P_N) of leaves was measured with a portable photosynthesis measurement system (Li – 6400, Li-Cor). Three saplings of each treatment group were selected for the measurement. Net photosynthetic rate was measured for one leaf of the saplings at temperature of 20~25°C and relative humidity of 60~70%. Leaves were exposed to different light intensities to obtain light response curves. Variations in photolight intensities (PARi) were 0, 100, 300, 600, 1200 and 2000 μmol $m^{-2} s^{-1}$. Gross photosynthetic rates (Pg_{max}) at light saturated points were calculated from these measurements. The measurements were conducted once a month from April 1999 to September 1999.

The chlorophyll fluorescence of the leaves was measured with Mini-PAM (Mini - PAM, Walz), a photosynthesis yield analyzer. The same leaves selected for the photosynthesis measurement were used for the chlorphyll measurement also. Chlorophyll fluorescence was measured once a month from April 1999 to November 1999. It was measured at 1:00 a.m. to detect the characteristics of chlorophyll fluorescence during the dark period. Minimal fluorescence (F_0) and maximal fluorescence (F_m) of the leaves were measured in the dark period and optimal quantum yield (F_v/F_m) was calculated automatically using the formula $(F_m\text{-}F_0)/F_m$.

3. Results

3.1 Growth and Phenology

All the saplings of *P. alba* and *T. distichum* survived but five saplings of *A. hirsuta* died under the flooding conditions of the experiments (Table 2). Four saplings of *A. hirsuta* were dead within 3 weeks after beginning the flooding. Three of them were in the treatment group of water level at the soil surface (F_0), and the rest was in the treatment group of aerated water (F_{air}) on the soil surface. Another *A. hirsuta* saplings died during the 5th week of the experiment in the treatment group of water level at 20 cm above the soil surface (F_{20}).

Flooding induced reduction in height growth in all the treatment groups of the *A. hirsuta* saplings (Table 2). On the other hand, in the *P. alba* and *T. distichum* saplings, there were no significant differences between the treatment groups and the control group.

Table 2 Effects of flooding of soil on growth of *Alnus hirsuta, Populus alba* and *Taxodium distichum* saplings (n=6). Values with the same letters were not significantly different at 5% level by species (Tukey`s HSD Test). Mean±S.D.

	Alnus hirsuta				*Populus alba*				*Taxodium distichum*			
	UF	F0	F20	Fair	UF	F0	F20	Fair	UF	F0	F20	Fair
Number of samples	6	3	5	5	6	6	6	6	6	6	6	6
Height increment(cm)	58.4±7.8 a	9.3±2.2 b	8.8±4.5 b	11.1± 5.2 b	5.4±11.6 a	18.0±10.5 a	8.6 ±7.5 a	7.0 ±4.8 a	2.2±2.3 a	2.8±3.9 a	6.2±3.5 a	4.1±3.9 a
Branch length increment (cm)	228.8±51.9 a	19.5±9.9 b	19.6±5.4b	36.7±9.7 b	229.5±134.1 a	42.5±12.5 b	44.5±16.4 b	37.0±13.2 b	12.5±7.3 a	4.5±3.2 a	5.1±3.2 a	8.3±3.4 a
Diameter increment												
at 3cm height (mm)	6.8±1.0 a	1.8±1.2 b	1.0±0.3 b	5.3±2.5 a	5.3 ±1.1a	5.9± 0.9 a	3.2± 0.5 b	6.2 ±1.4 a	1.8±0.8a	3.7±1.2 a	3.6±1.2 a	2.7±1.6 a
at 20cm height (mm)	5.4±1.0 a	0.9±0.8 c	1.2±0.4 c	2.5±1.4 b	3.4±1.2 ab	2.5±0.7 a	3.8±1.3 b	2.6±0.4 a	1.7±0.7 a	1.4±0.8 a	2.5±0.9 a	1.7±1.0. a

Flooding greatly inhibited branch length increment of *A. hirsuta* and *P. alba* saplings in all the treatment groups compared with the control group (Table 2). *T. distichum* saplings exhibited no difference among all the treatment groups and the control group in the branch length increment. In the flooded *A. hirsuta* and *T. distichum* saplings, branch length increment was continuous as that of the control group. However, in the flooded *P. alba* saplings, branch length increment was not continuous and branch length growth was completed earlier than that of the control group (Fig.1). Responses of saplings to flooding in diameter increment varied with treatments and species (Table 2). Flooding induced reduction in diameter increment at 3 cm height in the F_0 and F_{20} treatment groups of the *A. hirsuta* saplings and F_{20} treatment group of *P. alba* saplings. *T. distichum* saplings exhibited no treatment differences between the treatment groups and the control group in diameter increment at 3 cm height. Flooding induced reduction in diameter increment at 20 cm height in the flooded *A. hirsuta* saplings (Table 2).

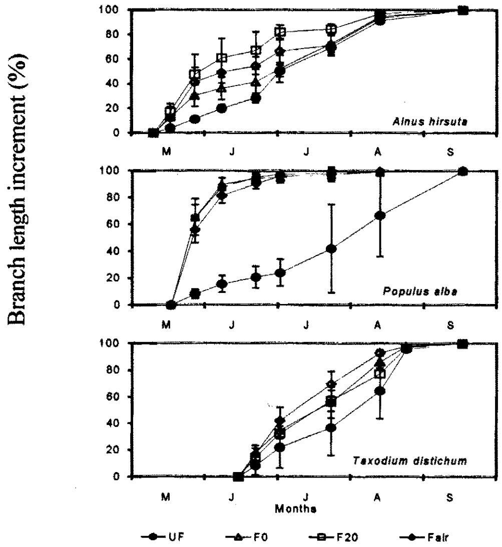

Fig. 1 Growth characteristics of branches. Cumulative increments were shown as the percentage to the height after the growth was completed. Vertical bars indicate standard deviations.

P. alba saplings exhibited no treatment differences between the treatment groups and the control group in diameter increment at 20 cm height. The F_{20} treatment group differed significantly from the F_0 and F_{air} treatment groups. The *T. distichum* saplings exhibited no differences among the treatments in diameter increment at 20 cm height. Number of leaves that emerged before treatment (old leaves) exhibited no difference between treatment groups and control group in all tree species. In the flooded *A. hirsuta* saplings, old leaves remained longer than control group (Fig. 2). Old leaves of the flooded *P. alba* and *T. distichum* saplings were remained as long as the control group. The number of the leaves that emerged after treatment (new leaves) in all the treatment groups of *A. hirsuta* and *P. alba* saplings was significantly smaller than that of the control group. In the flooded *T. distichum* saplings, number of the new leaves was not significantly smaller than that of the control group. The pattern of new leaves emergence in the flooded *A. hirsuta* saplings was similar to the control group. Emergence of the new leaves in the flooded *P. alba* and *T. distichum* saplings completed earlier than that of the control group. Total number of the leaves in the treatment groups of *A. hirsuta* and *P. alba* saplings was smaller than that of the control group.

3.2 Physiological Responses

Maximum gross photosynthetic rate (Pg_{max}) in light curve significantly decreased in the flooded saplings of three species in comparison with the unflooded control group (UF) (Fig. 3A). In the *A. hirsuta* saplings, reduction in Pg_{max} was greater in the F_0 and F_{20} treatment groups than that of the F_{air} treatment group. In all the treatment groups of *A. hirsuta* saplings and the F_0 and F_{20} treatment groups of *P. alba* saplings, Pg_{max} was significantly lower than that of the control group 10 days after the initiation of flooding. Pg_{max} in the F_{air} treatment groups of *P. alba* saplings was significantly lower than that of the control group 10 days after the initiation of flooding. Pg_{max} in the F_{air} treatment groups of *P. alba* saplings was significantly lower than that of the control group 3 weeks after the initiation of flooding. During the flooding period, reduction in Pg_{max} also was observed in the flooded *T. distichum* saplings; however, reduction in the Pg_{max} in the treatment groups

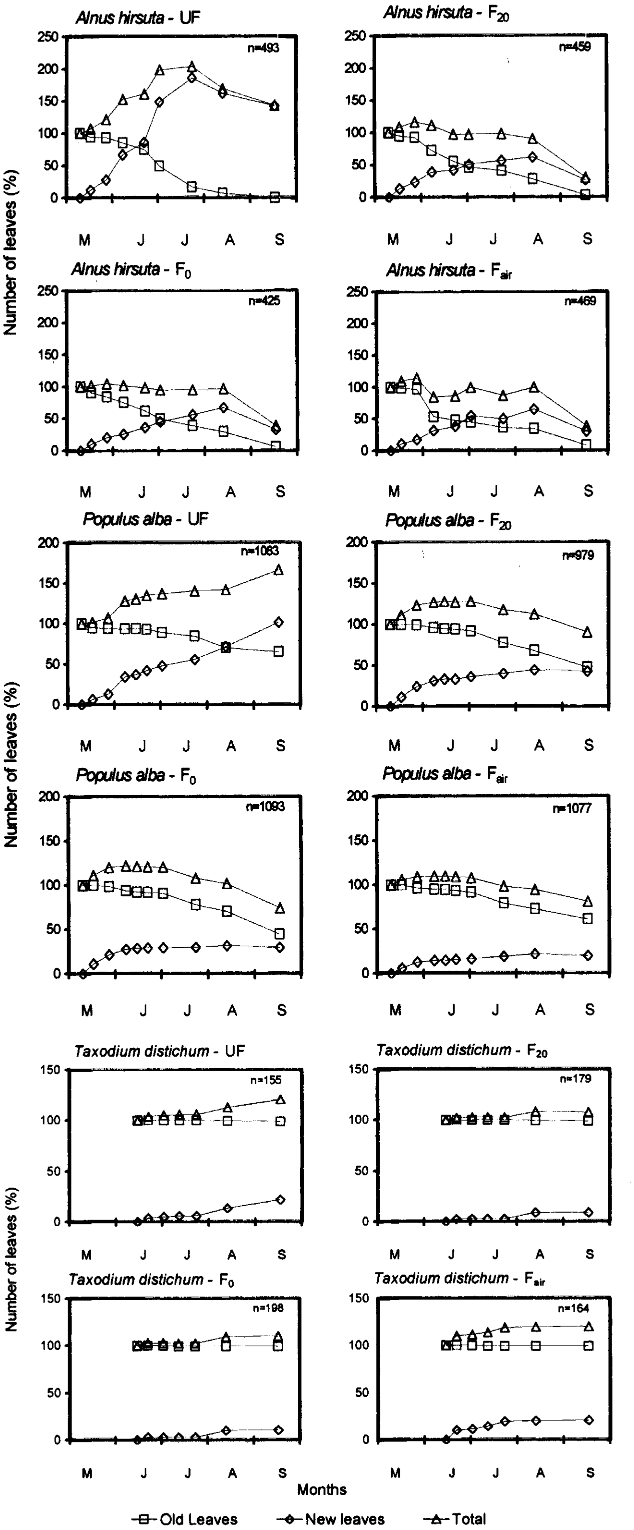

Fig. 2 Changes in number of the emerging leaves before and after treatment. The number of leaves before the flooding treatment was indicated as one hundred. Old leaves and new leaves meant the leaves emerging before flooding treatment and after flooding treatment, respectively.

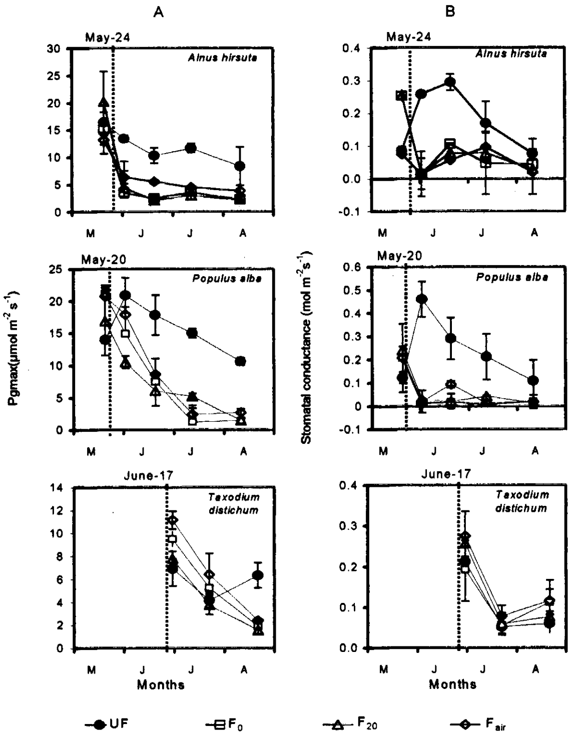

Fig. 3 Changes in relative value of gross photosynthetic rate (Pg_{max}) at saturated point (A). Changes in relative value of stomatal conductance (B). Dashed lines in the figures represent the start time of the experiment. Vertical bars indicate standard deviations

of *T. distichum* saplings was significantly lower than that of the control group 5 weeks after the initiation of flooding. During the flooding period, reduction in the stomatal conductance was also observed for *A. hirsuta* and *P. alba* saplings (Fig. 3B). The reduction in Pg_{max} was related to low stomatal conductunce.

Optimal quantum yield in the F_0 and F_{20} treatment groups of *A. hirsuta* saplings was significantly smaller than that of the control group (Fig. 4). In the F_{air} treatment group of *A. hirsuta* saplings, reduction in the optimal quantum yield was significantly lower than that of the control group.

Decrease in the optimal quantum yield began from June in the F_0 and F_{20} treatment groups of *A. hirsuta* saplings. In the *P. alba* saplings, decrease in the optimal quantum yield was observed in all the treatment groups. This decrease began from June in the F_0 and F_{20} treatment groups and from July in the F_{air} treatment group of *P. alba* saplings. In the flooded *T. distichum* saplings, optimal quantum yield was significantly lower than that of the control group in September.

3.3 Morphological Changes

Ratio of diameter increment at 20cm height to that of 3cm height ($\Delta D_{20}/\Delta D_3$) was significantly high in the F_{20} treatment group of *A. hirsuta* and *P. alba* saplings in comparison with the F_0 and F_{air} treatment groups and the control group (Fig. 5). The differences among the F_0, F_{air} and control group were not significant. In the *T. distichum* saplings, the ratio of $\Delta D_{20}/\Delta D_3$ in the F_0 treatment group was significantly smaller than that of the control group. In the F_{20} and F_{air} treatment groups, there were no significant differences from the control group.

Two weeks after the initiation of flooding, most of the flooded F_{20} treatment group of *P. alba* saplings had developed adventitious roots on the submerged portion of the stems (Fig. 6). F_0 and F_{air} treatment groups of *P. alba* saplings had developed adventitious roots in three or four weeks, of which number was smaller and length was shorter than that of the F_{20} treatment group. In the *A.* In the *A. hirsuta* saplings, adventitious roots were observed in the F_{20} treatment group. *T. distichum* saplings had not developed any adventitious roots on the submerged part of the stems.

4. Discussion

Flooding condition is a critical environmental factor affecting the survival, growth and distribution of plant species[2]. In the present experiment, some of the *A. hirsuta* saplings died while all saplings of *P. alba* and *T. distichum* survived. In the *A. hirsuta* saplings, survivorship was lower in the all treatments group than the control group. However, the survivorship in the F_0 treatment group was lower than that in the F_{20} treatment group. It is thought that it is necessary to examine the results in larger sample size to explain more clearly.

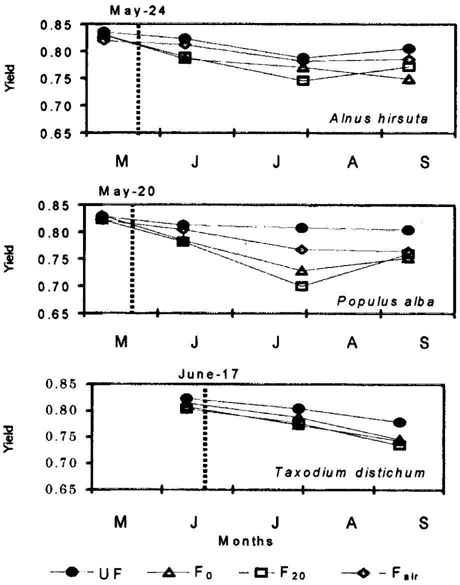

Fig. 4 Changes in the optimal quantum yield during the experiment. Dashed lines in the figures represent the start time of the experiment.

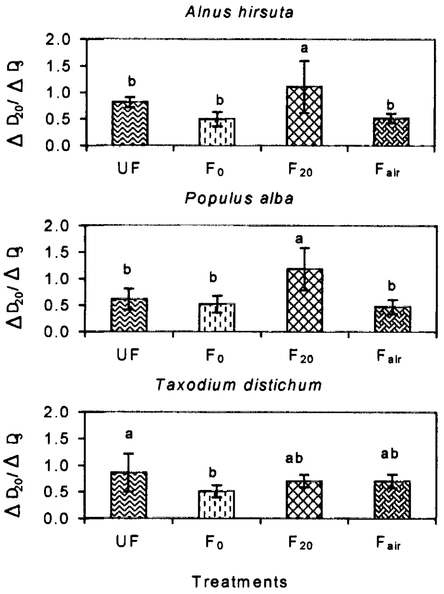

Fig. 5 Ratio of the $\Delta D_{20}/\Delta D_3$. ΔD_{20} diameter increment at 20cm height, ΔD_3 diameter increment at 3 cm height. Values with the same letters were not significantly different at 5% level by species (Tukey's HSD Test). Vertical bars indicate standard deviations.

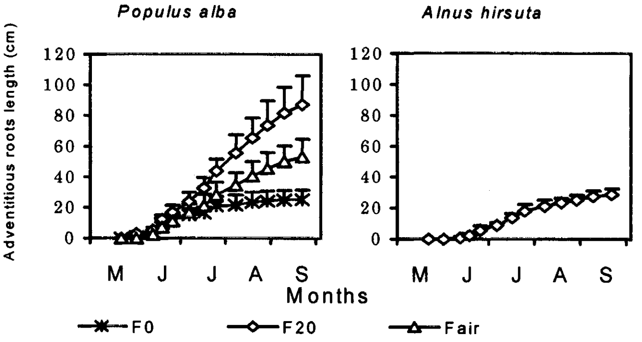

Fig. 6 Changes in length of the adventitious roots Vertical bars indicate standard deviations.

Flooding has effects on emergence and survivorship of leaves [7,8,12]. In the *A. hirsuta* and *P. alba* saplings, while emergence of new leaves of the flooded saplings was reduced, old leaves survived as long as the unflooded saplings. Especially, it is interesting that in the *A. hirsuta* saplings, survivorship of leaves emerged prior to flooding was higher in the flooded condition than that in the unflooded condition. The results might show a survival strategy of leaves in the *A. hirsuta* saplings under flooding stress. Flooding of soil is followed by a rapid decrease in the photosynthesis rate and stomatal closure in many species. [5,6,7,9]. According to the results, decreases in the gross photosynthetic rate (Pg_{max}) and decreases in the stomatal conductance of the *A. hirsuta* and *P. alba* saplings were observed under the flooding condition. The reduction occurred immediately after treatments in the A. *hirsuta,* and *P. alba* saplings. In the *T. distichum* saplings, the reduction also occurred in the gross photosynthetic rate (Pg_{max}). However, the reduction occurred one month after the treatment. Reduction in the gross of photosynthetic rate (Pg_{max}) means the decrease in CO_2 absorption by leaves, which is followed by the excessive irradiation of light energy in the leaves[1,5)]. It was thought that light inhibition in the flooded A. *hirsuta, P. alba* and *T. distichum* saplings was caused by the reduction in CO_2 absorption as a result of the stomatal closure. It was thought that the reduction of the photosynthesis rate in the flooded condition had relation to reduction in the height, branch length and diameter growth. However, in different species the growth reduction was detected in different parts of the saplings. Reduction was observed in the height growth in all the treatment groups of the *A. hirsuta* saplings and flooding significantly reduced the branch length increment of *A. hirsuta* and *P. alba* saplings. It was thought that in the flooded *A. hirsuta* saplings both apical and lateral growths were affected. In the flooded *P. alba* saplings only lateral growth was affected. In the *T. distichum* saplings, photosynthetic rate was not reduced as the flooded *A. hirsuta* and *P. alba* saplings, and flooding had not affected the growth.

Flood-tolerant plants survive waterlogging by complex interactions of morphological, anatomical and physiological adaptations [2,3,7,10,11,12)]. Important adaptations include production of hypertrophied lenticel, aerenchyma tissue and adventitious roots in the flooding condition[13)]. In the present experiment, some morphological changes that were formation of adventitious roots and alterations of diameter increment to flooding stress were observed. In the F_{20} treatment group of *A. hirsuta* and *P. alba* saplings, ratio of $\Delta D_{20}/\Delta D_3$ was significantly greater than that of the F_0, F_{air} and the control group. It was caused by large amounts of adventitious roots that were formed on submerged part of the stem in the F_{20} treatment group. *T. distichum* saplings had developed only a few adventitious roots under the pots and did not develop them on the submerged parts of the stems. However, morphological changes were also detected on submerged part of the stems. In the *T. distichum* saplings, flooding stimulated diameter increment on submerged part of the stem that was indicated by the ratio of $\Delta D_{20}/\Delta D_3$ in the F_0 treatment group. In the F_0 treatment group ratio of $\Delta D_{20}/\Delta D_3$ was significantly lower than that of the F_{20}, F_{air} and the control group. It was associated with stimulated diameter increment at 3 cm height that caused by production of hypertrophied lenticels and aerencyma tissue on submerged stem part. In the F_{20} and F_{air} treatment groups, the ratio of $\Delta D_{20}/\Delta D_3$ was not different from the control group. It was thought that flooding stimulated diameter increment at 3 cm and 20 cm height in F_{20} treatment group but in contrast, flooding did not stimulate diameter increment in F_{air} treatment group.

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