

海面上昇にともなう陸域の塩濃度変化が低地湿原の森林植生に及ぼす影響

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目的: 地球温暖化による海面の上昇や台風の増加、時には津波の襲来などによって生じる陸域の塩濃度上昇は、低地湿原の植生に極めて大きな影響を及ぼす。このような湿原は、日本の釧路湿原、アメリカのエバークレイズ湿原、ルーマニアのドナウデルタなど、世界各地に存在する。これらの多くはさまざまな動植物からなる生物多様性の高い貴重な生態系を持つことから、ラムサール条約による保全の対象となっている。わが国の釧路湿原では、ヨシ・スゲ類やミズゴケなどの植生とともに、過湿環境に耐性を持つハンノキを中心とした樹木が湿地林を形成しており、野鳥などの野生動物のシェルターや繁殖の場所として重要な役割を果たしている。海面の上昇にともなう塩濃度の上昇は、湿原の植生に影響を及ぼすと同時にこれら湿地林の衰退や消滅を引き起こす恐れがあり、野生動物の生存に大きな危機をもたらす可能性がある。

本研究では、湿地林構成樹種に及ぼす塩濃度増加および水位上昇の影響を生理的に解析するとともに、湿原の植生分布と地下水位との関係、土壌中の塩分濃度の現状などについての生理・生態的な調査を行い、海面上昇が湿地林に及ぼす影響を明らかにした。

研究課題は以下の2課題である。

1. 釧路湿原における湿地林の構造および生育状況と地下水位との関係、および苗木の生理・成長に及ぼす水位の影響

報告: Iwanaga F. and Yamamoto F.: Effects of flooding depth on growth, morphology and photosynthesis in *Alnus japonica* species. *New Forests*. (2007 印刷中)

2. 塩水中に一定期間水没したヌマスギ苗木の生存と成長

報告: Iwanaga F. and Yamamoto F.: Effects of complete submergence with saline water on growth and survival of *Taxodium distichum* seedlings. 第54回日本生態学会大会講演要旨集 P1-221. (2007)

研究1: 釧路湿原における湿地林の構造および生育状況と地下水位との関係、および苗木の生理・成長に及ぼす水位の影響

Effects of flooding depth on growth, morphology and photosynthesis
in *Alnus japonica* species

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要旨: 生態的調査および実験によって釧路湿原に分布するハンノキの生理・成長と形態的变化との関係を解析した。釧路湿原における調査では、地下水位の上昇にともない、土壌中の酸化還元電位の低下を確認するとともに、同時に複数樹幹の個体が増加することを明らかにした。これに対して苗木を使ったモデル実験では、冠水位の上昇とともに幹からの不定根および萌芽シュートの形成発達が顕著となること、その位置は水位の上昇とともに上昇することを認めた。さらに苗木の光合成および気孔コンダクタンスは、冠水後に一時的に低下するが、冠水後の時間が経過するにつれて形成さ

れる不定根増加によって回復することが明らかになった。

ABSTRACT

The present study deals with effects of flooding depth on growth, morphology and photosynthesis in *Alnus japonica* species thorough one field study and two controlled experiments. In the field study performed in Kushiro Mire, Hokkaido Island, Japan, tree heights and stem diameters decreased with an increase in water depth accompanied with the reduction of soil redox potential. In contrast, the rate of multiple stems per individual tree increased. In the controlled experiments for seedlings flooding suppressed the shoot elongation and biomass increment in roots. However, diameter increment around water levels, epicormic shoot development and adventitious root formation were enhanced in flooded seedlings. The photosynthetic rate and stomatal conductance of flooded seedlings also were lowered with an increase in flooding depth. The recovery of the reduced photosynthetic rate and stomatal conductance occurred simultaneously with the advancement of adventitious root formation in the flooded seedlings. These results indicate the importance of a series of morphological changes occurring on stems around water levels in flood tolerance in *A. japonica* species.

INTRODUCTION

Flooding results in poor soil aeration and depletion of oxygen in rhizosphere of plants (Kozlowski et al. 1991). The declined gas diffusion by flooding induces chemical reduction. Decrease in soil Eh under flooding condition indicates progressive increase in demand for oxygen and accumulation of phytotoxic compounds in soil (Ponnamperuma 1984, Pezeshki 2001). Those compounds including reduced form of Fe and Mn, ethanol, lactic acid, acetaldehyde, aliphatic acids, and cyanogenic compounds cause injury in root metabolism and inhibition of shoot and root growth in plants (Jackson and Drew 1984, Armstrong and Armstrong 1999, Armstrong *et al.* 1996, Pezeshki 2001). Such declined root system by low soil Eh condition affects shoot growth and physiological activity through inhibition of water and mineral uptake, and their transport (Pezeshki 2001).

There are many examples for effects of flooding such as inhibition of shoot elongation, leaf expansion, senescence and abscission of leaves and lowered photosynthetic activity (Kozlowski et al. 1991). Flood-tolerant species respond to flooding by morphological changes such as the development of intercellular spaces in lenticels, increase in stem growth, formation of adventitious roots and aerenchyma development (Yamamoto *et al.* 1995a,b, Kozlowski et al. 1996, Pezeshki 2001, Dat et al. 2004).

Alnus japonica (Thunb.) Steud. is native to swamp area along rivers and lakes and widely distributed in northeast Asia (Fujita and Kikuchi 1986). This species has high flood-tolerance and a dominant species in swamp forests of Kushiro Mire, Hokkaido, Japan (Grosse et al. 1993, Nakamura et al. 2002). In swamp forest of Kushiro Mire, A.

japonica trees assume the form of shrubs consisting of multiple stems derived from regeneration system by coppicing in deeply flooded areas, in contrast, they can grow up to 20-15 m in shallowly flooded areas (Shinshoh 1985). Such phenomenon suggests that changeable flooding depth may affect expression of stress adaptability relating to modification of various physiological processes in plants. However, there is little information about effects of depth of flooding on growth, morphology and physiology of this species.

In the present report we examined development and distribution of *A. japonica* trees in wetland forests at Kushiro Mire in relation to environmental factors including soil redox potentials. We also investigated the effect of flooding depth on size, growth and morphology in controlled experiments using *A. japonica* seedlings. On the results of these two different studies, the stress adaptability of this species to flooding was discussed.

MATERIALS AND METHODS

Field studies

Field studies were performed in a swamp forest of *Alnus japonica* species in Kushiro Mire, Hokkaido Island, Japan. A 10m wide and 200m long rectangular plot extended from the edge of Lake Takkobu, a lagoon of the mire, toward a deep area of it was made at 43°04'N and 144°29'W. Locations of *A. japonica* trees in the plot were recorded and their heights and stem diameters at 130cm above the ground level (DBH) were measured with a measuring stick and a diameter tape, respectively. The number of stems including epicormic shoots was counted in each *A. japonica* tree. Ground heights from the water level of Lake Takkobu were surveyed with a leveling instrument (Automatic level 10316, Sokkisha Co. Ltd.) from the shore edge to another edge in the plot at 10m intervals. Values of soil redox potential (Eh) were determined with a portable pH/conductivity meter (D-24S, Horiba, Ltd.) in the plot at 20m intervals. The values were taken for three times at each measuring point.

Experimental studies

Plant materials

Two different experiments were performed using 3-year-old *Alnus japonica* seedlings. About 2 months before the beginning of experiments, the seedlings grown from seeds obtained from the Botanical Garden of Hokkaido University were transplanted in plastic pots (φ19.5cm x 14.5cm) containing 1 part vermiculite, 1 part bark compost and 3 parts sandy loam and grown under outdoor condition. The seedlings were watered daily with tap water and fertilized every 2 weeks with 200ml of a 1/500 Hyponex solution (Hyponex: 10N-3P-3K-0.05Mg-0.001Mn-0.005B, HYPONeX Japan, Co. Ltd.) until the initiation of treatments.

Experiment 1

Eighteen seedlings were selected for uniformity in size and development. Average

seedling heights and stem diameters at 1cm above the ground level (GL) were 134.1 ± 2.9 cm and 14.6 ± 0.6 mm, respectively. The seedlings were submitted to three treatments: 1) 6 seedlings unflooded and watered daily as control, 2) 6 seedlings flooded at 1cm above the ground level (GL) and 3) 6 seedlings flooded at 30cm above the GL. In flooding treatments water was periodically added to keep each water level. Those flooding conditions were maintained until September 4 for 80 days.

During the experimental period, terminal shoot elongation and diameter growth at 1cm and 30cm above the ground level were measured once a week with a ruler and a microcaliper, respectively. The number of adventitious roots and epicormic shoots developing on stems and stocks were counted every week. At the end of experiments, heights of developing positions and lengths of epicormic shoots were measured with a ruler. Then, the seedlings were harvested, separated into leaves, stems, epicormic shoots, roots and adventitious roots and their dry weights were determined separately after drying at 80°C for 48 h.

Experiment 2

On the basis of the results in the field study and the experimental study 1, photosynthetic rates of flooded *A. japonica* seedlings were determined in this experiment. The seedlings were selected for uniformity in size and development as: heights, 96.1 ± 1.6 cm; stem diameters at the 1cm above the GL, 11.6 ± 0.2 mm. The seedlings were transferred to the greenhouse of Arid Land Research Center, Tottori University. At the beginning of the experiment, five seedlings were harvested, separated into leaves, branches, stems and roots and their dry weights were determined separately after drying at 80°C for 48 h.

In this experiment fifteen seedlings were submitted to 3 treatments. Five seedlings unflooded were watered daily, as control. The remaining seedlings were divided into two groups: (1) five seedlings flooded at 1cm at the GL and (2) five seedlings flooded at 30cm above the GL. Water was periodically added to keep each water level, but the water was not changed. These flooding treatments were performed for 42 days.

Measurements of photosynthetic rates and stomatal conductance were made at a photosynthesis photon flux density (PPFD) of $1100 \mu\text{mol m}^{-2} \text{s}^{-1}$ determined from light-saturated photosynthetic rates using the photosynthesis-PPFD response curve. To obtain photosynthesis-PPFD response curve, photosynthetic rates at different PPFD were measured on 3 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. The photosynthesis measurements were conducted from 8:00 to 9:30 a.m. to avoid midday depression of photosynthesis and made on well expanded, matured and developed leaves positioned on the fourth or fifth node from shoot apices. Air temperature (AT), relative humidity (RH) and CO_2 concentration during photosynthesis measurements were recorded as follows: $\text{AT} \pm \text{SE}$, $30.2 \pm 0.5^{\circ}\text{C}$; $\text{RH} \pm \text{SE}$, $44.8 \pm 2.8\%$; CO_2 concentration $\pm \text{SE}$, $381.2 \pm 3.0 \mu\text{mol m}^{-2} \text{s}^{-1}$. Every week during the experimental period,

terminal shoot elongation, stem diameters, number of adventitious roots and epicormic shoots and biomass were measured as described above.

The statistical analysis of variance was applied to each data set. To compare mean values of treatments, *t*-test was used. Multiple comparisons among treatments were performed by the method of Scheffe's test.

RESULTS AND DISCUSSION

Field studies

Figure 1 indicates ground heights, soil redox potential (Eh), tree heights, stem diameters (DBH) and number of stems and epicormic shoots in *A. japonica* trees growing at the study site. The total of *A. japonica* trees surveyed was 1495 individuals. The ground height above the water level descended slowly toward Lake Takkobu. Values of soil Eh decreased as a decrease in ground heights. The values gradually reduced and reached about 150mV at the point of 120m from the shore edge of the plot. The heights and stem diameters of *A. japonica* trees indicated the same tendency to increase as the changes in the values of soil Eh. Meanwhile, the total number of stems and epicormic shoots per tree (Fig.1) increased with the decrease of soil Eh.

Experimental studies

Experiment 1: development of epicormic shoots

Flooding at 30cm above the GL suppressed shoot elongation in the seedlings (Tab. 1). In the flooded seedlings diameter increments were enhanced around the water levels. Flooding at 1cm above the GL caused rapid diameter increment at the stem base, whereas 30cm-flooding increased stem diameter at 30cm above the GL (Tab. 1). Flooding at 30cm reduced the dry weight increment of roots and total plants. The 1cm-flooding reduced dry weights of roots only. Flooding did not change the dry weights of stems and leaves (Tab. 1).

Formation of adventitious roots was observed in the flooded seedlings (Tab. 1). The number of adventitious roots in the 30cm-flooded seedlings was greater than those of the 1cm-flooded seedlings. Epicormic shoot development also was observed on the stems of the flooded and unflooded seedlings (Fig. 2, Tab. 1), however, more epicormic shoots in the flooded seedlings were observed than those of unflooded seedlings. The number of epicormic shoots in the flooded seedlings kept increasing during the experimental term (Fig. 3). The dry weights of epicormic shoots in the 1cm-flooded seedlings were greater than those of the unflooded and the 30cm-flooded seedlings as well as the number of epicormic shoots (Tab. 1). Developing positions of epicormic shoots as well as adventitious roots were heightened as water levels increased (Fig. 4 and 5). The epicormic shoots of the 1cm-flooded seedlings were comparatively larger than those of other seedlings (Fig. 4). The average lengths of epicormic shoots in the 1cm-flooded, 30cm-flooded and the unflooded seedlings were $8.75\pm 0.83\text{cm}$, $1.76\pm 0.26\text{cm}$ and $3.10\pm 0.89\text{cm}$, respectively (Tab. 1).

Experiment 2: photosynthesis and adventitious root formation

Table 2 indicates effects of flooding depth on growth, biomass and morphology of the flooded seedlings in Experiment 2. The same tendency was observed in this experiment as the data obtained in Experiment 1 (Tab. 1). Dry weights of leaves in the flooded seedlings were significantly lower than those of the unflooded seedlings (Tab. 2), whereas flooding did not change them in Experiment 1 (Tab. 1). Although flooding enhanced the formation of adventitious roots, there was no significant difference in the number of epicormic shoots between the flooded and the unflooded seedlings (Tab. 2).

The rates of photosynthesis in the unflooded seedlings were continuously higher than those of the flooded seedlings (Fig. 6). Within 25 days after flooding initiated, the photosynthetic rates of the 30cm-flooded seedlings were lower than those of the unflooded seedlings. On the 56th day after flooding was initiated, there was no significant difference in photosynthetic rate among the treatments although flooding treatment caused consistently lower photosynthetic rates (Fig. 6). The tendency of changes in stomatal conductance was similar to that of photosynthetic rate. The recovering process of photosynthesis was highly related to changes in the number of adventitious roots (Fig.7). In the 30cm-flooded seedlings the decreased rates of photosynthesis recovered with an increase in the number of adventitious roots (Fig.7 and 8). The dry weights of adventitious roots in the 30cm-flooded seedlings were less than those of the 1cm-flooded seedlings whereas more adventitious roots were observed in the 30cm-flooded seedlings (Tab. 2).

Under flooding condition in Kushiro Mire, distribution and development of *A. japonica* trees declined with decrease in the value of soil Eh. This phenomenon may be explained by the negative effects of the low soil Eh condition on growth and physiology. For instance, soil Eh significantly affects energy condition through inhibitory effects on various woody plants (DeLaune et al. 1998, Anderson and Pezeshki 2001, Pezeshki 2002). Flooded *Quercus nuttallii*, *Q. michauxii* and *Taxodium distichum* seedlings showed decrease in photosynthetic rates and stomatal conductance under reduced condition with about -200 mV soil Eh (Anderson and Pezeshki 2001). According to Pennington and Walters (2006), inhibitory effects of low soil Eh condition are greater than those of hydrology on growth and photosynthesis of several woody species.

In general, flooding suppresses height growth, formation and expansion of leaves and root growth in many upland species (Kozlowski 1984a). Our controlled experiments indicated that deep flooding apparently inhibits shoot growth, total biomass increment and photosynthetic activity of *A. japonica* seedlings, as well as declined development and distribution of this species in Kushiro Mire. In contrast, flooding stimulated many kinds of visible change in stem portions of flooded seedlings around water levels. In a few days after initiation of flooding, stem lenticels on submerged portions showed hypertrophic development. Lenticels are the important pathway of gas exchange between atmosphere and internal tissues in stems and roots (Kozlowski 1984b, Buchel

and Grosse 1990, Schaffer 1998). Hypertrophied lenticels having abundant intercellular spaces in flooded plants enhance gas diffusion by thermo-osmotic activity (Buchel and Grosse 1990) and contribute oxygen supply to submerged roots (Kozłowski and Pallardy 1996, Grosse et al. 1992, Yamamoto et al. 1995a, b, Angelov et al. 1996).

Numerous adventitious roots began to develop on submerged stems about 3 weeks after the initiation of flooding. The root formation occurred coincidentally with the increment of stomatal conductance and photosynthetic rates. Several investigations reported the close relationships between adventitious root formation and stomatal reopening in flooded plants (SenaGomes and Kozłowski 1980, Kozłowski 1984b, Topa and Cheeseman 1992). In flooded *Fraxinus pennsylvanica*, for instance, adventitious root formation lowered leaf diffusion resistance (SenaGomes and Kozłowski 1980). The function of adventitious roots is regarded as the support for shoot growth during prolonged soil waterlogging by supplying water, minerals and hormones (SenaGomes and Kozłowski 1980, Jackson and Drew 1984, Tsukahara and Kozłowski 1985, Vartapetian and Jackson 1997, Islam and MacDonald 2004). Furthermore, Terazawa et al. (1989) found the oxidization of rhizosphere occurred around adventitious roots of flooded *A. japonica* seedlings.

The importance of adventitious rooting on growth in flooded woody plants would vary with species. Removing adventitious roots from submerged portions of stems reduced height and diameter growth in flooded *Platanus occidentalis* seedlings (Tsukahara and Kozłowski 1985). In contrast, there was no significant effect on height growth in flooded *A. glutinosa* seedlings (Gill 1975). However, the present experiments on *A. japonica* species indicated the coincidental relationship between adventitious root formation and recovery of reduced photosynthetic rate, suggesting the importance of this phenomenon for the survival of this species in wetland.

Most of flood-tolerant woody plants showed reduced diameter growth by prolonged flooding (Kozłowski 1997), whereas the occurrence of stem hyperplasia also was observed in flooded *Nyssa aquatica* (Hook 1984), *Fraxinus mandshurica* (Yamamoto et al. 1995b) and *A. japonica* (Terazawa and Kikuzawa 1994, Yamamoto et al. 1995a). Such enlarged stems had lower density and abundant intercellular spaces in woody plants (Hook 1984, Yamamoto 1987, 1995a, b), although the increase in stem diameter of *A. japonica* resulted largely from both increased number and size of xylem cells consisting mainly of libriform wood fibers (Yamamoto et al. 1995a).

In our field studies, most of the flooded *A. japonica* trees at Kushiro Mire had multiple stems presumably derived from epicormic shoots (Shinshoh 1985). Flooded *A. japonica* seedlings also showed acceleration of epicormic shoot development in controlled experiments. Under anaerobic condition causing decline of shoot and root growth, regeneration system by coppicing is efficient for the maintenance, existence and development of individual tree. Rapid development of epicormic shoots in flooding environment would give a substantial advantage to *A. japonica* species for survival.

The epicormic shoots are produced from dormant buds on the main stems or branches of the trees (Kozlowski and Pallardy 1997). The dormant bud is dominated by the terminal shoot and released by thinning or partial cutting of the shoot (Zimmermann and Brown 1971, Kozlowski and Pallardy 1997). Suppressed growth in roots and shoots of flooded plants may affect the balance of plant hormones in relation to apical dominance. In our previous experiments, the flooded *A. japonica* seedlings girdled or applied with NPA (1-naphthylphthalamic acid), an auxin transport inhibitor, indicated more epicormic shoot developments than those of untreated seedlings, suggesting that the reduced shoot growth caused by flooding condition is one of the direct factors for promoting epicormic shoot formation in *A. japonica* growing in mires (Iwanaga and Yamamoto, unpublished data).

In the experimental studies the depth of flooding controlled positions of epicormic shoot development, adventitious root formation and stem hyperplasia. Grinchko and Glick (2001) mentioned regulatory roles of plant hormones including auxin and ethylene in both stem hyperplasia and adventitious root formation. Yamamoto et al. (1995a) also suggested that such morphological changes in flooded *A. japonica* seedling were highly related to flood-induced ethylene. Elevated levels of ethylene in some portions of stems in flooded plants may cause auxin accumulation at the same point. For instance, Eklund and Little (1996) reported that auxin accumulation in stems was enhanced by the application of ethrel, an ethylene-generating compound, in *Abies balsamea* seedlings. In woody plants auxin is regarded as a promoter of both xylem production (Little and Pharis 1995, Eklund and Little 1996) and adventitious root formation (Kozlowski et al. 1996). The present results suggest that changes in water levels may shift positions of both rapid ethylene production and auxin accumulation in stems, causing hypertrophic stem growth and adventitious root formation. However, more direct information about the relationship between roles of plant hormones and morphological changes is needed to clarify physiological mechanisms and function of morphological changes in *A. japonica* under flooding condition.

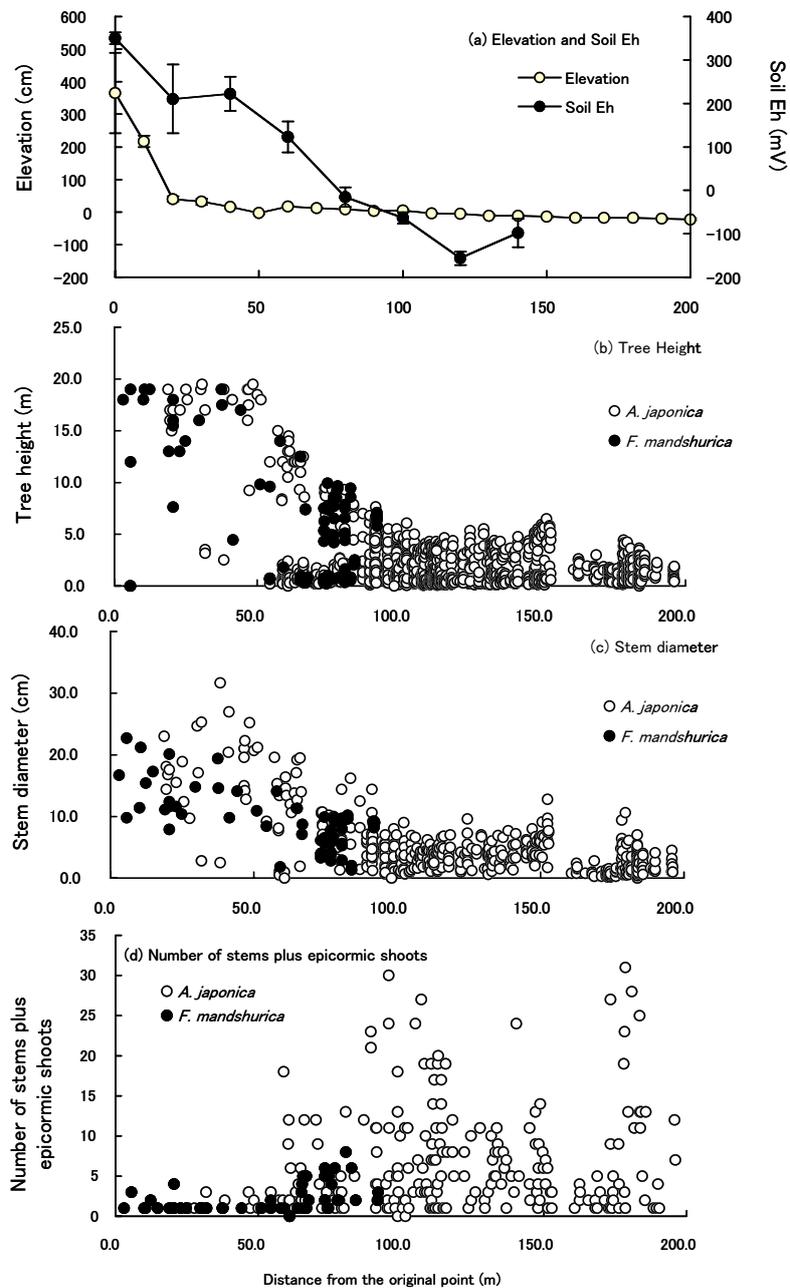


Fig.1 Ground heights, soil redox potential (Eh), tree heights, stem diameters (DBH) and total number of stems and epicormic shoots in *Alnus japonica* trees growing at Kushiro Mire.



Fig.2 Multiple stems and epicormic shoots appearing on flooded *A. japonica* saplings. The arrow shows the water level.

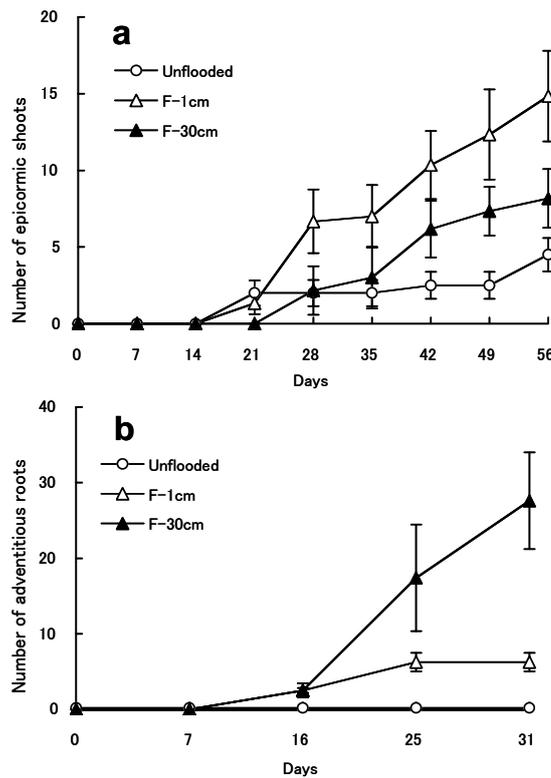


Fig.3 Effects of flooding depth on changes in number of epicormic shoots (a) in Experiment 1 and adventitious roots (b) in Experiment 2: Δ , flooded at 1cm above the GL; \blacktriangle , flooded at 30cm; \circ , unflooded. Values without common superscripts are significantly different at $P < 0.05$ using Scheffe's test.

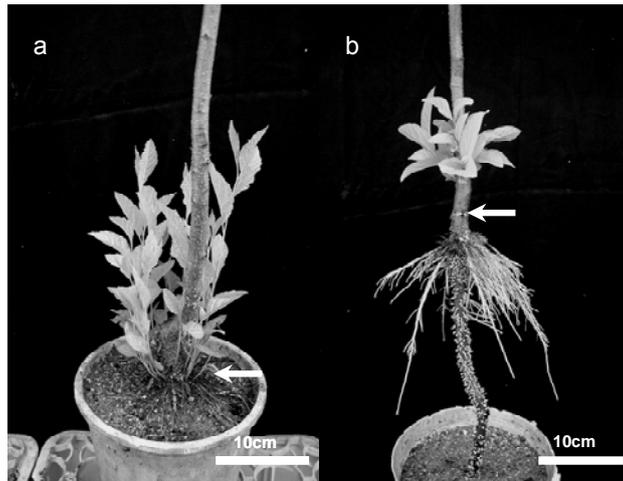


Fig.4 *A. japonica* seedlings flooded at 1cm (a) and 30cm (b) above the GL. Arrows in each figure indicate water levels.

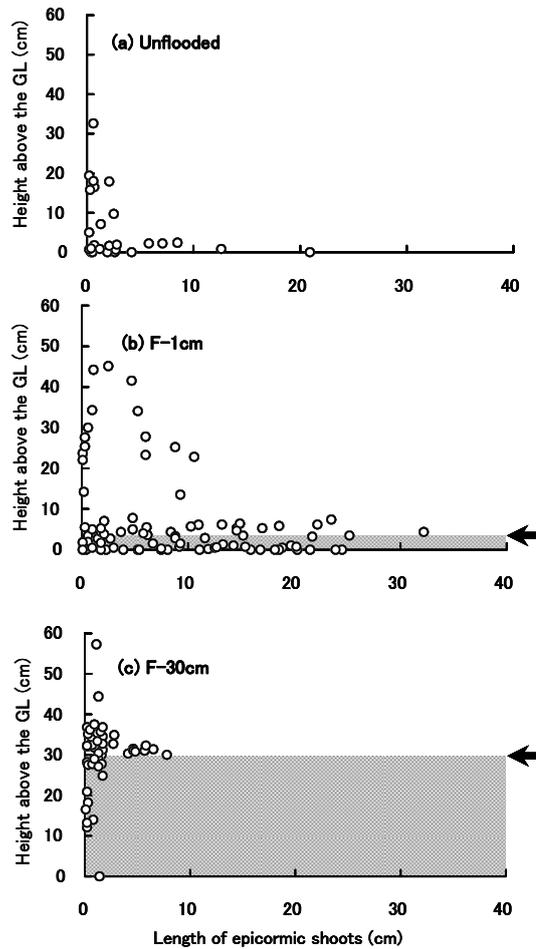


Fig.5 Positions of epicormic shoot development and shoot lengths in the seedlings unflooded (a), flooded at 1cm (b) and 30cm (c) above the GL. Arrows indicate each water level.

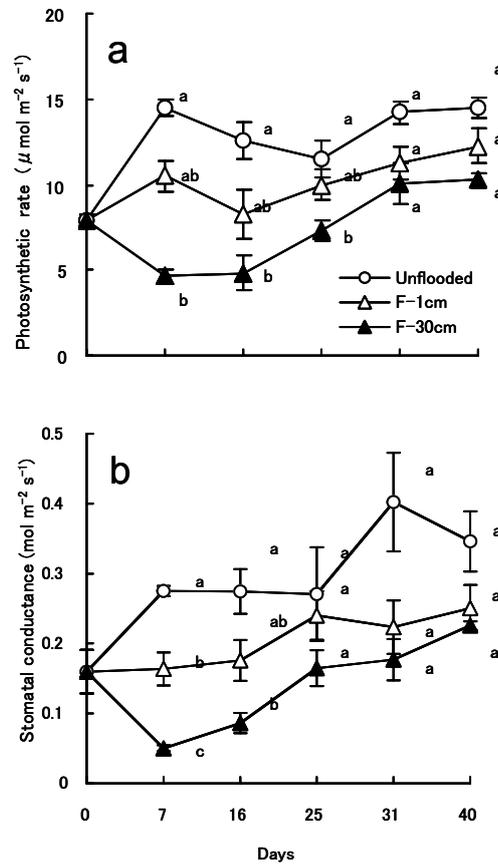


Fig.6 Changes in photosynthetic rate (a) and stomatal conductance (b) in leaves of the seedlings. Symbols with common superscripts on the same day are not different at the 5% level using Scheffe's test.

Fig.7 Linear regression relationships between photosynthetic rates and number of adventitious roots in flooded *A. japonica* seedlings of Experiment 2 ($P < 0.05$).

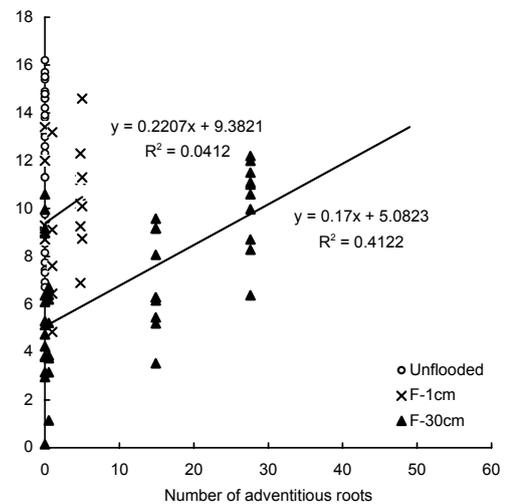


Table 1 Effects of flooding depth on the growth, biomass and morphology of *A. japonica* seedlings in Experiment 1.

| | Treatments | Unflooded | F-1cm | F-30cm |
|-------------------|------------------------------|--------------|---------------|--------------|
| <i>Growth</i> | Shoot elongation (cm) | 32.33±4.07 a | 14.07±1.73 ab | 8.55±1.81 b |
| | Diameter increment (cm) | | | |
| | at 1cm | 2.72±0.37 a | 6.91±1.13 b | 1.51±0.71 a |
| | at 30cm | 2.92±0.34 a | 3.27±0.50 a | 6.18±0.66 b |
| <i>Biomass</i> | Total plants (g) | 68.01±7.79 a | 47.60±5.25 ab | 36.62±5.35 b |
| | Leaves (g) | 23.42±1.73 a | 19.41±2.95 a | 14.79±2.54 a |
| | Stems (g) | 5.92±0.69 a | 6.57±0.80 a | 4.90±0.74 a |
| | Roots (g) | 38.68±6.15 a | 21.62±2.81 b | 16.93±2.41 b |
| | T/R | 0.81±0.09 a | 1.28±0.17 a | 1.17±0.10 a |
| <i>Morphology</i> | Number of adventitious roots | 0.0±0.0 a | 5.0±2.0 b | 20.7±7.3 b |
| | Number of epicormic shoots | 4.5±1.1a | 14.8±3.0 b | 8.2±1.9 ab |

Table 2 Effects of flooding depth on the growth, biomass and morphology of the seedlings in Experiment 2.

| | Treatments | Unflooded | F-1cm | F-30cm |
|-------------------|------------------------------|--------------|---------------|--------------|
| <i>Growth</i> | Shoot elongation (cm) | 30.23±6.79 a | 12.80±3.26 ab | 8.00±2.01 b |
| | Diameter increment (cm) | | | |
| | at 1cm | 2.34±0.80 a | 7.51±1.03 b | 0.66±0.11 a |
| | at 30cm | 1.80±0.14 a | 2.60±0.37 ab | 3.20±0.17 b |
| <i>Biomass</i> | Total plants (g) | 90.92±5.00 a | 75.80±6.30 ab | 57.69±8.32 b |
| | Leaves (g) | 17.45±0.92 a | 12.94±0.52 b | 7.91±1.21 c |
| | Stems (g) | 26.23±2.22 a | 26.25±5.96 a | 24.55±4.00 a |
| | Roots (g) | 46.17±4.50 a | 32.46±3.77 ab | 25.10±5.02 b |
| | T/R | 1.02±0.14 a | 1.43±0.27 a | 1.51±0.39 a |
| <i>Morphology</i> | Number of adventitious roots | 0.0±0.0 a | 6.3±1.2 b | 27.6±6.4 b |
| | Number of epicormic shoots | 4.2±1.0 a | 5.0±1.5 a | 2.4±0.9 a |

研究2: 塩水中に一定期間水没したヌマスギ苗木の生存と成長
Effects of complete submergence with salt water on growth and survival
of *Taxodium distichum* seedlings

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要旨:ハリケーン、台風、あるいは地球温暖化などともなう海面上昇は、海浜や内水面湿地の植生に大きな影響を及ぼす。本研究では、アメリカにおけるハリケーンの来襲がヌマスギ(*Taxodium distichum*) 湿地林に及ぼした生態的な影響を明らかにするために、塩水による冠水がヌマスギ苗木の生存や成長に及ぼす影響を解析した。実験には2年生のヌマスギ苗木を用いた。苗木は 0, 4000, および 8000ppm の塩水中に2週間水没させ、排水後の成長回復を調べた。淡水中に水没させた苗木は、一時的に成長が停止したが、排水後、速やかに回復した。4000ppm の塩水に水没させた苗木は、排水後、光合成及び気孔コンダクタンスの顕著な低下とともに成長の遅滞が認められた。しかしながら2週間後にはほぼ回復した。淡水および 4000ppm の処理区では、排水後の幹の成長にも回復が認められた。8000ppm の塩水に水没させた苗木は、排水後、葉の壊死・脱落が生じ、成長は著しく減退した。しかしながら排水 48 日後には幹から多数の萌芽が認められ、枯死することはなかった。これらの結果から、ヌマスギは 8000ppm 程度の一過的な塩水によるストレス環境にさらされても、一時的に成長は減退するものの、生存は可能であることがわかった。

ABSTRACT

Sea-level rising derived from hurricanes frequently brings saline water into inland vegetations. Such multiple stresses including salt and flooding stress disrupt wetland forests consisting of glycophytic woody plants. We investigated effects of rapid salt stress in combination with flooding on growth, photosynthetic activity and stem anatomy of 2-year-old *T. distichum* seedlings that dominant species in wetland forests. Seedlings were submerged with water having three different salt concentrations: 0, 4000, and 8000ppm. All seedlings in each treatment were submerged completely in one tank for 14 days. As control, seedlings were not submerged, watered daily and well drained. After submergence, seedlings were taken out from water and flooded with tap water at the soil surface. All seedlings survived complete submergence with saline water in every salt concentration. The complete submergence with/without salt temporally inhibited shoot elongation. Seedlings submerged with 8000ppm NaCl shed all leaves and showed dieback phenomena. However, many coppice shoots came out from stem surface at the 48th day after drainage. In treatments with 0 and 4000ppm NaCl, photosynthetic rates and stomatal conductance temporary decreased, and recovered after the 15th day. Anatomical observations of stems at the water level showed increased xylem production and bark thickness in the seedlings treated with 0 and 4000ppm NaCl.

INTRODUCTION

Bottomland and coastal swamp forests are important components of forest systems in the southeastern United States (Dickson et al. 1965). In recent years, wetland forests face to serious problems accompanied with environmental changes including sea-level rising. Salt intrusion is one of the major effects of sea level rising on wetland forests. Several researches reported that the consequences of rising water level along the Gulf Coast caused rapid deterioration and disappearances of coastal forests (Conner & Inabinette 2005).

In addition to moderate increase of salinity by sea level rising, wetland forests in southern U.S. are abruptly exposed to high saline stress brought with hurricanes. Such a sudden elevation in salt concentration and water level would cause flooding and submergence with salt water, and disrupt wetland forests consisting of glycophytic woody plants.

Forested wetlands in which *Taxodium distichum* is a dominant overstory tree species are common in the coastal plains and river floodplains (Allen et al. 1996). While *T. distichum* species is considered highly tolerant of flooding and soil waterlogging, growth and development of *T. distichum* trees decline in the region flooded by brackish water. Conner (1995) reported effects of a 3-m storm surge associated with Hurricane Hugo on *Taxodium /Nyssa* forests. *Taxodium / Nyssa* forests which affected saltwater indicated high mortality and dramatic changes in forest structure related to lack of seedlings of canopy species. Although those results suggests the severe impact of high tide and submergence with saltwater on growth and survival of seedlings, less understood and little reported is the effects of submergence with saltwater storm-caused saline stress on *T. distichum* seedlings. This study was designed to determine the effects of submergence with saltwater on the growth and survival of *T. distichum* seedlings.

MATERIALS AND METHODS

Plant materials

This experiment was conducted at the nursery of Tottori University. Two-year-old *T. distichum* seedlings grown under outdoor condition were transplanted into plastic pots (17 cm×φ19 cm) containing 1 part bark compost and 3 part sandy loam. The seedlings were watered daily with tap water and fertilized every 2 weeks with 200 ml of a 1/500 nutrient solution (Hyponex70781, Hyponex Japan, Co. Ltd.) until the initiation of treatments. Seedlings were selected for their uniformity of size and development. The average seedling height and stem diameter were 103.8 ± 0.6 cm (mean \pm SE) and 12.35 ± 0.13 mm, respectively.

Submerging treatments were held at three different concentrations: 1) 7 seedlings submerged with non-saline water (S-0), 2) 7 seedlings submerged with 4000ppm salt water (S-4000), 3) 7 seedlings with 8000ppm (S-8000) and 4) 7 seedlings well drained and watered daily as control (US). All seedlings of each submerging treatment were put

in a plastic tank ($\phi 120\text{cm} \times \text{H}120\text{cm}$) filled with water adjusted to each salt level. After submergence, seedlings were put out from water and grown under soil flooding condition.

On the 1st and 15th day after finishing submergence, photosynthetic activity and stomatal conductance measurements were taken with portable photosynthesis system (Li-6400, Li-cor). Measurements were made at a photosynthesis photon flux density (PPFD) of $1300\mu\text{mol m}^{-2} \text{ s}^{-1}$ determined from light-saturated photosynthetic rates using the photosynthesis-PPFD response curve. To obtain photosynthesis-PPFD response curve, photosynthetic rates at different PPFD were measured on 3 leaves from different seedlings prior to the initiation of experiments. The CO_2 concentration, temperature and vapor pressure deficit in leaf surface were not controlled. The photosynthesis measurements were conducted from 8:30 to 9:30 a.m. to avoid midday depression of photosynthesis and made on well expanded, matured and developed leaves positioned on the fourth or fifth node from shoot apices. Air temperature (AT), relative humidity (RH) and CO_2 concentration during photosynthesis measurements were recorded as follows: $\text{AT} \pm \text{SE}$, 35.5 ± 0.1 ; $\text{RH} \pm \text{SE}$, $41.4 \pm 2.2\%$; CO_2 concentration $\pm \text{SE}$, $380.1 \pm 1.9 \text{ mol m}^{-2} \text{ s}^{-1}$.

To identify the amount of xylem produced prior to treatment, the pin-marking method of Wolter (1968) was used. After submerging treatments, a small rust-resistant insect-mounting needle was horizontally inserted into stems at the water level in the flooded seedlings and at equivalent stem location in unflooded seedlings and withdrawn after a few seconds, penetrating the bark and cambial zone. The resultant injury to the dividing and differentiating cells caused formation of abnormal cambial derivatives that later could be identified microscopically, thereby delineating the xylem produced before flooding from that produced after initiation of flooding. At the end of each treatment, stem portion around the water level was collected and fixed in FAA solution (ethanol-water-formalin-acetic acid, 60: 30: 5: 5, v/v). Transverse sections of stems were made at the needle marked location. For each section, xylem growth, number of libriform wood fibers, and bark thickness were studied using light microscopy.

Shoot and stem growth in the seedlings were measured on days 1 and 15 after the beginning of the submerging treatment. After 48 days of submerging treatment, seedlings in each treatment were harvested, separated into leaves, stems, and roots. The dry weights were then determined after drying at 80°C for 48 h.

RESULTS AND DISCUSSION

Shoot growth and biomass

Complete submergence with non-saline water didn't affect shoot development and biomass increment of *T. distichum* seedlings (Table 1). Although there was no significant effect in shoot growth and biomass, submergence with 4000ppm NaCl caused necrosis on the cusp and verge of leaves. Seedlings in S-8000 shed all leaves and

showed dieback in a few days after treatments. Dry weights of leaves, stems, roots and complete plants decreased in seedlings of S-8000. After 48 days from the end of submergence, however, many leaves were observed on the lower portion of stems in S-8000 (Fig. 1).

Photosynthetic activity

Photosynthetic rates were inhibited by complete submergence with both saline and non-saline water on the 1st day of recovery term (Fig. 2). Stomatal conductance decreased only in seedlings of S-4000 and S-8000. The inhibitory effects of saline submergence on photosynthesis and stomata were maintained 15 days after submergence. However, the same degree of photosynthetic rates was showed in un-submerged seedlings and seedlings submerged with non-saline water.

Anatomical observations

In submerging treatments, seedlings were kept under flooding condition at soil surface with non-saline water. Accelerated stem growth of submerged portion was observed in flooded seedlings of S-0 and S-4000, but not in S-8000 (Fig. 3). However, number of tracheid cells in S-4000 was fewer than that of S-0. Bark thickness also increased in flooded seedlings of S-0 and S-4000, as shown in the increments of xylem width and number of tracheids.

Our results indicated that the negative effects of saline submergence on *T. distichum* were greater than those of submergence alone. When submergence and salt treatment were combined, growth in shoot and stem diameter, dry weight of shoots, and photosynthesis were significantly reduced (Table 1 & Fig. 2), although the seedlings submerged with fresh water indicated high photosynthetic rates and growth as same as those in control seedlings.

Growth reduction due to submergence with salt water is generally greater than that with fresh water for plants. For instance, Allen et al. (1996) reported reduction in net photosynthesis and growth of *T. distichum* seedlings flooded with 3ppt salt water, whereas there was no significant decrease in each group of seedlings subjected soil flooding and salinity.

There are several kinds of adaptation in plants growing in a saline environment such as ion exclusion, compartmentation within vacuoles, and accumulation of some organic compounds including osmolites. These mechanisms for salinity tolerance

T. distichum species have high tolerance to flooding and waterlogging with fresh water and their high tolerance have been demonstrated in various studies. According to Pezeshki (1991), the soil-flooded seedlings showed initially the decline in physiological performance including photosynthetic rate, stomatal conductance and some enzyme activities in photosynthetic pathway. However, both photosynthetic rates and stomatal conductance began to recover within 2 weeks under continuous shallow flooding condition (Pezeshki et al. 1987, Pezeshki 1993).

Most researches about complete submergence have carried out on herbaceous plants.

The effects of complete submergence on growth and survival of woody plants is still obscure. However, several researches using herbaceous plants reported that re-aeration following complete submergence impaired photosynthetic activity.

Present results indicate that *T. distichum* seedlings have high tolerance to complete submergence with fresh water and even with salt water containing 4000ppm NaCl or more. The maximum salt concentration for these seedlings to survive may close to 8000ppm NaCl.

Table 1 Height growth and dry weight of plant parts in flooded *T. distichum* seedlings.

| Treatments | Shoot elongation (cm) ^a | Total plants (g) ^a | Leaves (g) ^a | Stems (g) ^a | Roots (g) ^a | T/R |
|------------|------------------------------------|-------------------------------|-------------------------|------------------------|------------------------|--------------|
| Control | 0.3±0.1 a | 43.68±2.90 a | 5.54±0.67 a | 17.52±1.17 a | 20.62±1.26 a | 1.12±0.03 b |
| S-0 | 0.9±0.4 a | 40.75±2.76 a | 6.46±0.41 a | 19.10±0.61 a | 17.91±0.78 a | 1.30±0.18 a |
| S-4000 | 0.6±0.1 a | 42.17±2.12 a | 6.06±0.38 a | 17.53±0.88 a | 18.58±1.18 a | 1.28±0.06 ab |
| S-8000 | — | 26.76±1.89 b | 0.65±0.30 b | 12.93±0.88 b | 13.17±1.05 b | 1.05±0.09 b |

^a Data values given as mean ± SE. Values without the same letter in each column are significantly different at $P < 0.05$ using Sheffe's-test (n=7).

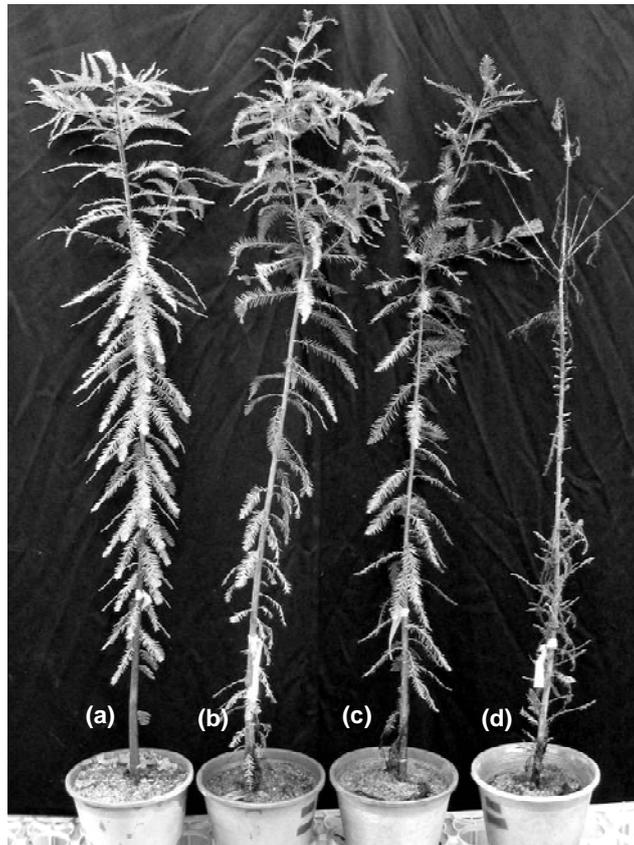


Fig. 1 Seedlings submerged with saline water; a) unflooded seedlings (Control), b) submerged with 0 ppm NaCl water (S-0), c) 4000 ppm (S-4000), and d) 8000 ppm (S-8000).

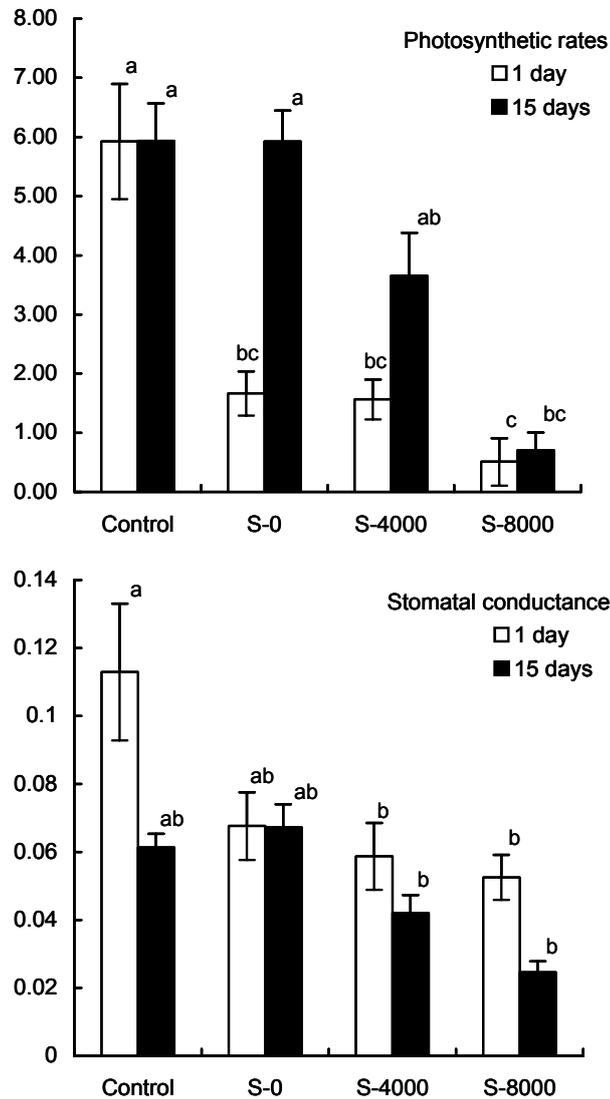


Fig. 2 Effects of submergence with saline water on photosynthetic rate (upper) and stomatal conductance (lower) of seedlings 1(□) and 15 (■) days after the end of treatments. Values are means (n=7) with standard error. Different letters at the same day indicate significant difference at P<0.05 using Scheffe's test.

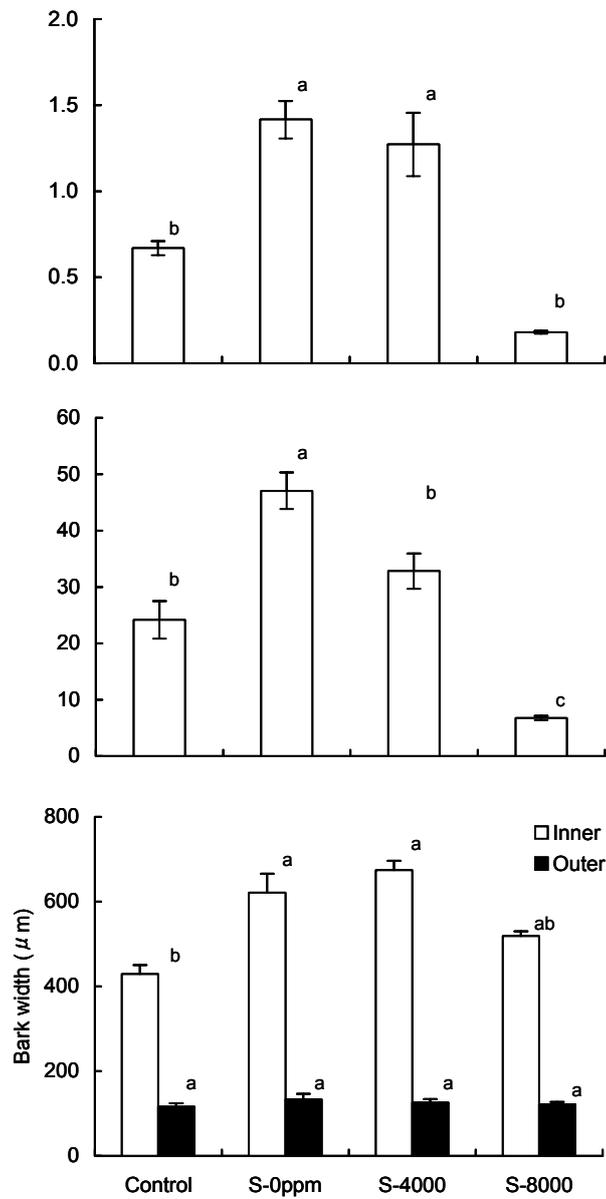


Fig. 3 Effects of salt concentration on increments in xylem width (upper) and number of tracheids (middle), and bark width (lower) of the submerged seedlings: Values are means (n=7) with standard error. Different letters in each figure indicate significant difference at P<0.05 using Scheffe's test.