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ORIGINAL RESEARCH

Effects of Light, Temperature, and Water Depth on Growth of a Rare Aquatic Plant, *Ranunculus kadzusensis*

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Abstract *Ranunculus kadzusensis* is an endangered aquatic plant species that commonly reproduces in the rice paddies of Korea and Japan during winter and early spring. Here, we investigated the effects of main aquatic environmental factors—light, temperature, and water depth—on its growth, with the goal of seeking information that will contribute to its in situ conservation. As the amount of shading increased, biomass, maximum shoot length, number of branches, flowers, and fruits, main stem diameter, and maximum leaf length decreased. Although seed germination occurred under a 12-h photoperiod and at either 30/20°C (day/night) or 20/15°C, most plants died at the higher temperature. Survival was 0% for surface-grown plant sets when tested in a wintertime pond experiment. The rate of maximum shoot extension was greatest for plants grown at depths of 50 and 100 cm versus those at 20 cm. Thus, we demonstrated that *R. kadzusensis* is intolerant of high temperatures and shade, which may explain why its growth is limited to paddies with no shading and where temperatures are low early in the year, before rice cultivation begins.

Keywords Endangered plant · Growth · Light · *Ranunculus kadzusensis* · Temperature · Water depth

Aquatic environments are not suitable habitats for most plant species because water blocks gas transport and reduce light transmission, both of which are essential for their survival. Consequently, <1% of the earth's angiosperms have adapted to such locations (Wetzel 1988). Aquatic plants are, therefore, strongly influenced by water-induced factors, such as light, temperature, and water depth. Garbey et al. (2006) have reported that regeneration of *Ranunculus peltatus* is strongly dependent on light availability in the water, and Strand and Weisner (2001) have found that *Myriophyllum spicatum* that grows in deep water, where less light is transmitted, has increased vertical growth and fewer branches. The morphology of macrophytes is related to their depth distribution in water (Middelboe and Markager 1997). Madsen and Brix (1997) also have shown that the local distribution and seasonal growth patterns of *Elodea canadensis* and *Ranunculus aquatilis* are strongly influenced by temperature. These study results indicate that light, temperature, and water depth are important determinants of aquatic plant growth.

Ranunculus kadzusensis Makino is a rare submerged macrophyte that lives mainly in Korean rice paddies. Although it is an endangered species there and in Japan (Oitaken Seikatsukankyobu 2001; Suh et al. 2002; Lee et al. 2005; Ku et al. 2007; Ministry of Environment of Korea 2007), few studies have focused on how it is influenced by various aquatic environmental factors. Because of its restricted habitat and timing of growth, examining such patterns in response to light, temperature, and water depth is requisite for developing a plan for its in situ conservation. We believe that the research described here will help to clarify these questions, and will contribute to the formation of a strategy for its perpetuation in Korea.

Materials and Methods

Effect of Shading on the Growth of *Ranunculus* in the Greenhouse

Mature seeds of *R. kadzusensis* were harvested in April 2008 from its native habitat at Ganghwa Island, South Korea. After being dried at room temperature (20°C), they

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were stored in a cold room (4°C). Before being sowed, they were surface-sterilized to prevent decay by soaking in 5% Na-hypochlorite for 5 min, then washed five times with distilled water (Benvenuti et al. 2001; Ren et al. 2002). In all, 150 two-leaf seedlings were obtained after seed germination, and were reared for 5 weeks in a growth chamber (HB-301 L Hanbaek Scientific Co., Korea) at 20/15°C (day/night), under a 14-h photoperiod and a light intensity of 100-110 μmol $m^{-2}s^{-1}$. The seedlings were then transplanted into three 50-well pots containing bed soil (60% vermiculite, 20% cocopeat, and 20% of other additives such as zeolite, loess, and peat moss), and were placed, in the greenhouse, into a stainless steel tank (83 cm long, 166 cm wide, 35 cm deep) that was filled with tap water. One pot was covered with a single layer of shade netting, another with two layers; the third was left uncovered to create a light gradient. Their transmission rates, as measured with a light meter (Model LI-250; Li-Cor, USA) were 41±1.90%, 10±0.68%, and 100% (±SE), respectively. The water level was maintained at 20 cm, with half being replaced with tap water every 2 weeks to reduce algal blooms. During this greenhouse experiment (9 May-27 June 2008), the maximum and minimum mean water temperatures were 23.1°C and 19.5°C, respectively. Flowers and fruits were counted every other day from the time the first flower was detected. At 50 days after transplantation, all plants were harvested and their maximum shoot lengths (MSLs), root and shoot biomasses, and branch numbers were recorded. The longest leaf among those attached to the main stem was measured (maximum leaf length, MLL) and the main stem diameter (MSD) was defined as the greatest circumference among all internodes along that main stem. The relative growth rates (RGRs) for shoot and root biomass (RGR-biomass) and maximum shoot length (RGR-MSL) from each plant were calculated according to the equation of Hunt et al. (2002):

$$RGR = (\ln W_2 - \ln W_1) / (t_2 - t_1)$$

where, W is the value of a particular parameter measured per plant, and $t_2 - t_1$ is the harvest interval.

Effect of Temperature on Plant Growth in the Greenhouse

Soil for these greenhouse trials was obtained from the native habitat of *Ranunculus* (described above), and was dried and autoclaved at 121°C for 1 h to eliminate microbial activity and weed seed viability (Trevors 1996). After being sieved to 2 mm, it was spread evenly to a depth of 1 cm in ten square plastic pots (125×125×20 mm), which were then soaked in water to saturate the soil. Afterward, 30 seeds were planted on the soil surface of each pot. Half of these pots were placed randomly on shelves in a growth chamber maintained at 20/15°C, under a 12-h photoperiod at a light intensity of 100-110 μmol $m^{-2}s^{-1}$. The other pots were placed on shelves in a chamber set at 30/20°C (12-h photoperiod, 100-110 μmol $m^{-2}s^{-1}$.

Germination was tallied every 2 days for the first 25 days; surviving plants were counted every 2 days for another 25 days. Seeds were considered to have germinated when their cotyledons appeared, and a plant was considered dead when its bud decayed.

Effect of Water Depth on Plant Growth in the Field

Shoot samples were removed at about 5 cm from the top of the plants, then placed in plastic pots (45×45 mm) containing sand autoclaved at 121°C for 30 min. They were grown for 4 weeks in a 4°C cold room (11-h photoperiod, 30-50 μmol $m^{-2}s^{-1}$) to prevent decay. In an earlier pre-test, we had discovered that *Ranunculus* survives and roots well even at a low temperature. In December 2007, eight pots for each treatment were put into plastic boxes (20×20 cm), which were then covered with a net to prevent the plants from being foraged by fish. Three boxes were set at each of four water depths—0, 20, 50, or 100 cm. Corresponding light transmission rates were 100%, 60±0.08%, 59±0.12%, or 42 ± 0.10% (±SE). The temperature was relatively constant at 50 and 100 cm, but ground temperature and that of the water at 20 cm fluctuated during this experiment (Table 1). Plants were harvested after 106 days, and their maximum shoot length (MSL), number of branches, and newly formed shoot biomass

Table 1 Water temperatures at different pond depths

Temperature (°C)	Water depth (cm)			
	0	20	50	100
Average (± standard error)	0.4^a±0.12	3.1^b±0.06	5.9^c±0.03	6.1^c±0.02
Maximum	19.5	14.6	11.4	10.1
Minimum	−14.0	−0.4	3.4	3.3

Values not followed by the same superscripted letters are significantly different at $\alpha<0.05$, based on Tukey's studentized range (HSD) tests.

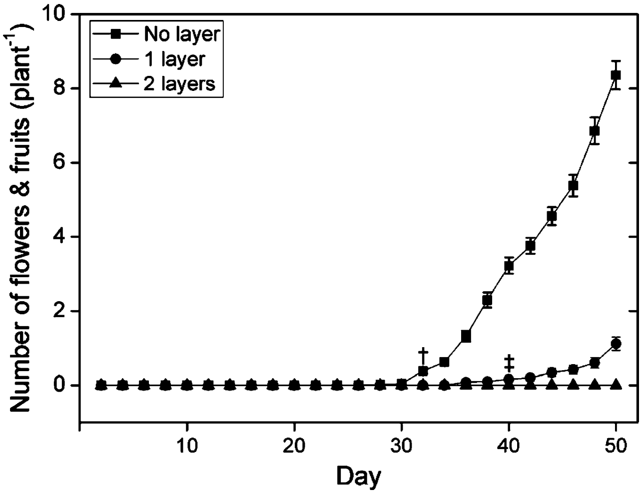

Fig. 1 Number of flowers and fruits from *Ranunculus kadzusensis* after 50 days of growth under different shading conditions. *Bars* represent mean±standard error [*dagger* indicates point at which values become significantly different among three treatments ($p<0.01$); *double dagger* point at which values differ significantly for one-layer and two-layer netting treatments ($p<0.05$), ANOVA]

were recorded. MSD was determined by measuring the major diameter of internodes along the main stem.

Data Analyses

Data were evaluated by analysis of variance (ANOVA), using SAS Enterprise Guide 4.1 software (SAS Institute Inc. 2006). Differences between groups were compared with Tukey's studentized range (HSD) tests. Significance levels were set at $\alpha<0.05$.

Results

Effect of Shading on Plant Growth

Numbers of *Ranunculus* flowers and fruits differed significantly across treatments. The non-shaded group flowered earliest, on day 28 of this experiment, followed on day 36 by plants covered with a single layer of netting. Plants with two layers did not blossom during this trial. The total

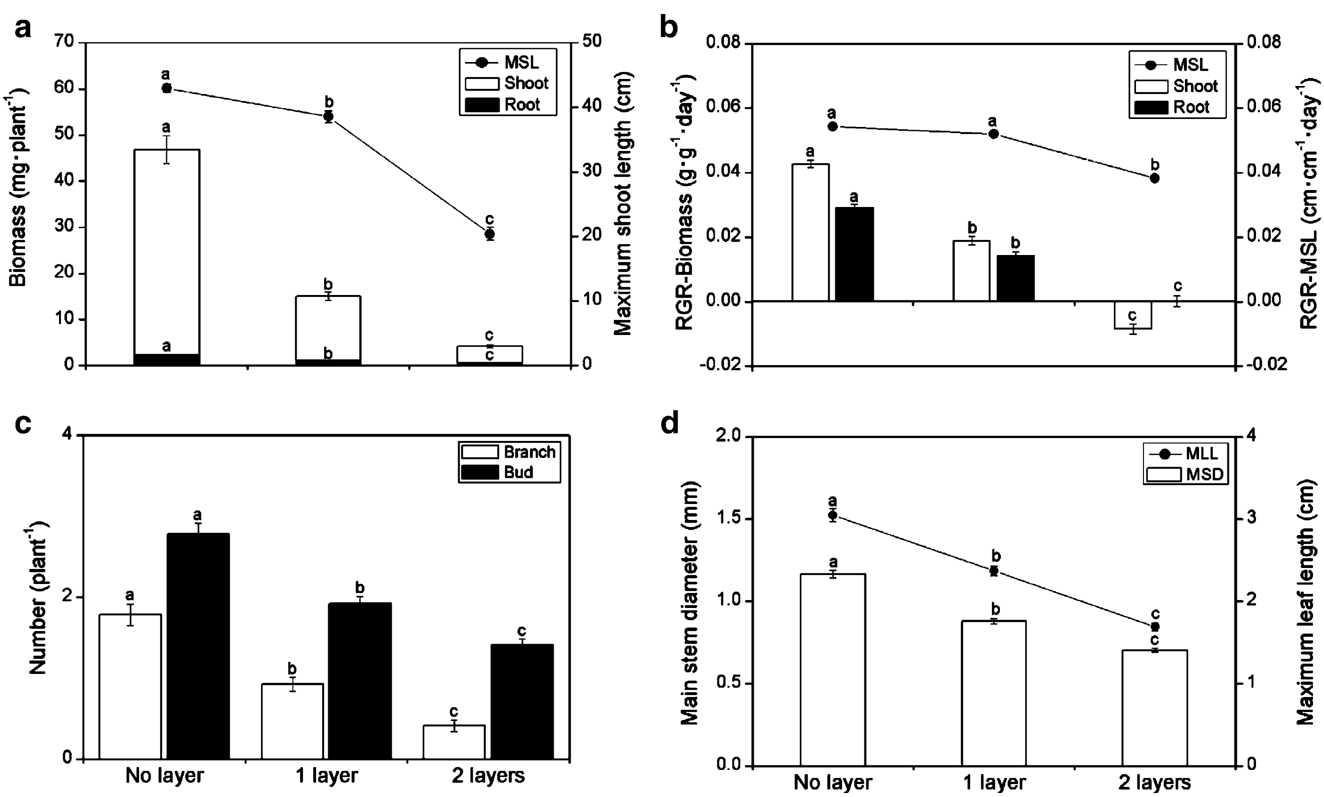

Fig. 2 **a** Biomass and maximum shoot length (MSL), **b** relative growth rates (RGRs) of shoot and root biomasses and MSL, **c** number of branches and buds, and **d** main stem diameter (MSD) and maximum leaf length (MLL) for *Ranunculus kadzusensis* after 50 days under different shading conditions. *Bars labeled with same letter* do not differ significantly according to Tukey's studentized range (HSD) test ($\alpha<0.05$). *Bars* represent mean±standard error

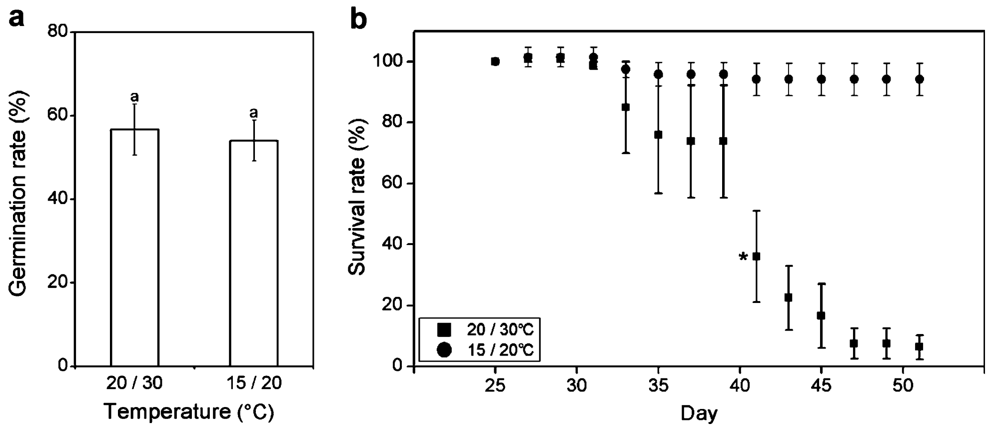

Fig. 3 **a** Percent germination and **b** survival rate of *Ranunculus kadzusensis* at different temperatures and under 12-h photoperiod. *Bars labeled with same letter* do not differ significantly; *asterisk* is point at which two treatments differ significantly according to Tukey's studentized range (HSD) test (α<0.05). *Bars* represent mean±standard error

number of flowers and fruits decreased as the amount of shading increased (Fig. 1).

At day 50, the biomass of shaded plants was lower than that of non-shaded plants. The lowest values for shoots and roots were recorded from plants with two layers of netting, and shaded plants were shorter than non-shaded shoots (Fig. 2a). The RGR-biomass of shoots and roots decreased significantly in response to increased shading; however, no significant difference in RGR-MSL was found between non-shaded and single-layer-shaded groups (Fig. 2b). The number of branches and buds, MSD, and MLL also were significantly lower with greater intensities of shading (Fig. 2c, d).

Effect of Temperature on Plant Growth

Germination rates did not differ between treatments at 30/20°C and 20/15°C (Fig. 3a). Plants maintained under that first regimen gradually lost their green color and slowly died, whereas those grown at 20/15°C survived. From day

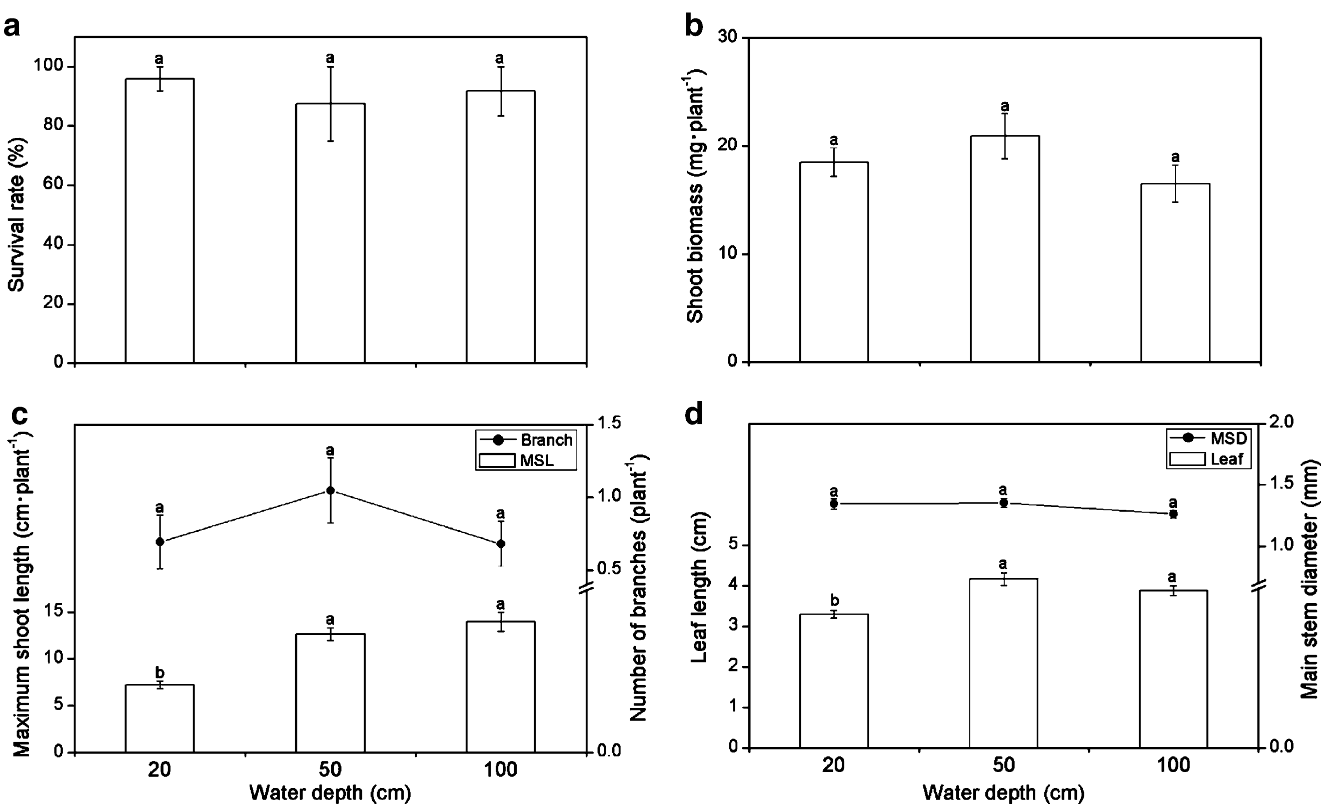

Fig. 4 **a** Survival rate, **b** shoot biomass, and **c**, **d**, several morphological characteristics of *Ranunculus kadzusensis* at different water depths during Winter. *Bars labeled with same letter* do not differ significantly according to Tukey's studentized range (HSD) test (α<0.05). *Bars* represent mean±standard error

41, survival rates differed significantly between plants at 30/20°C and those at 20/15°C (Fig. 3b).

Effect of Water Depth on Plant Growth

None of our plants placed at the water surface survived. Although no significant differences in mortality rates were found among plant groups at various depths (Fig. 4a), those set at 50 or 100 cm was taller and had longer leaves than those at 20 cm (Fig. 4c, d). No significant differences in shoot biomass, branch number, or MSD were found among water depths (Fig. 4b, c, d).

Discussion

Effect of Shading

Shade inhibited the growth of *R. kadzusensis*. At 10% light transmission, RGR-shoot was negative. Values for MSL, MSD, MLL, and the numbers of branches, flowers, and fruits all decreased as shade levels increased. These results are similar to those of Garbey et al. (2006), who reported that the biomass and stem length of *R. peltatus* (in the same subgenus *Batrachium* as *R. kadzusensis*) also are reduced when light transmission is inhibited. In contrast, some aquatic plants tend to be taller when under heavier shading, and are usually distributed at a wide range of water depths (Barko and Smart 1981; Tobiessen and Snow 1984).

Rice paddies, the main habitat for *R. kadzusensis* in Korea and Japan, are open areas with no shade occurring during its life cycle. Although that setting provides several conditions that are favorable to such plant growth, e.g., abundant water and eutrophic soil, light appears to be the most important factor for this species. This may explain why it has a higher survival rate in paddies than in ditches and ponds, where many other species compete for light.

Effect of Temperature

Although species vary in their preferences, the optimal temperature for many submerged macrophytes is relatively high (Barko and Smart 1981; Tobiessen and Snow 1984; Barko et al. 1986). This wide range enables species with different temperature requirements to share the same environment without competition (Barko et al. 1986). Rice paddies are the most typical habitat for *R. kadzusensis*. Because those plants usually bloom in winter and spring, they are compatible with rice farming practices, and can avoid being eliminated when fields are cultivated (Ku et al. 2007).

Although germination occurred at both 30/20°C and 20/15°C, most plants died when exposed to the former regimen, especially after 41 days under those conditions. Because this species requires about 2 months to flower and fruit, it is important to maintain a cool environment for its propagation. Previous pond experiments conducted during winter have shown that *R. kadzusensis* is relatively well-adapted to cold water temperatures, and growth is limited when the habitat is warmer. Because most other plants are dormant during that season, this adaptation to cold water is beneficial to *Ranunculus* reproduction. In particular, it is advantageous that this species grows in paddies during winter and early spring, when rice is not being cultivated.

Effect of Water Depth

Water level is closely related to light transmission, with deeper water hindering the processes of scattering and absorption (Wetzel 1988). Submerged macrophytes usually extend into the depths in order to maximize their absorption of the light and CO_2 needed for photosynthesis; for example, *Hydrilla verticillata* is very effective in elongating its shoots (Barko and Smart 1981; Maberly and Madsen 2002).

In our wintertime pond experiment, none of the plants survived when set at ground level. Maximum shoot lengths were achieved at 50 and 100 cm deep, but plants also elongated, to a lesser extent, at 20 cm. In winter, the range in daily air temperature fluctuated more than did daily water temperature. The pond froze to a depth of more than 5 cm during this experiment, perhaps killing those surface plants and inhibiting plant development at 20 cm. Therefore, moderately deep water may be beneficial for the survival and growth of *R. kadzusensis* during winter. However, no significant differences were observed in shoot biomass, branch numbers, or MSD values among water depths. Because this experiment was conducted with fully mature plant fragments and because the usual growth rate of this species is slow in winter, water depth may have had little effect on its morphology. More significant variations might have resulted if such a trial were performed instead with seedlings.

In conclusion, *R. kadzusensis* is most typically found in the rice paddies of Korea and Japan. As yet, there is no definitive answer to why that habitat is preferred. Our study showed that this species can resist low temperatures but not shading, which might explain why this endangered plant species is commonly distributed in paddies that are not shaded during winter and early spring when low temperatures persist. Moreover, we suggest that one not underestimate the influence of anthropogenic activities, such as paddy management practices, on the survival of *Ranunculus* in such an environment.

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