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Short communication

Response of six *Azolla* species to transient high-temperature stress

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Abstract

Response of eight *Azolla* strains from six species to transient exposure to high-temperature stress (above 40°C) was examined. Each *Azolla* strain showed differential tolerances to the stress. Based on growth and survival after the treatment, we concluded that the order of tolerance of six species to transient high-temperature stress was as follows: *A. pinnata*>*A. microphylla*, *A. mexicana*>*A. caroliniana*, *A. filiculoides*>*A. rubra*. Treatment with high-temperature stress also caused the rapid abscission of root and branches of *Azolla*. The temperature and time that caused rapid abscission depended on the species. In *A. filiculoides* and *A. microphylla*, the abscised branches were alive and proliferative when the temperature stress was short-term. © 1999 Elsevier Science B.V. All rights reserved.

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Azolla, a genus of floating aquatic ferns with seven extant species, is distributed throughout tropical and temperate regions of the world (Watanabe et al., 1992). The sporophyte of *Azolla* is 10–40 mm in diameter and multibranched. Each branch includes a stem with bilobed leaves and adventitious roots. The abscission of branches or roots allows the fragmentation of these plants and facilitates vegetative propagation (Addicott, 1982; Peters and Calvert, 1983). *Azolla* possesses the ability to utilize atmospheric N_2 due to a symbiosis with the blue-green alga *Anabaena Azollae*, which grows in the cavities of *Azolla* leaflets. *Azolla* has been used extensively and effectively for green manure in rice fields instead of chemical fertilizer in Asia. Interest in the use of this plant as a biological filter for the renovation of waste water has increased (Kitoh and Shiomi, 1984; Scharpenseel and Knuth, 1987; Debusk and Reddy, 1987; Kitoh et al., 1993). The success of macrophyte-based biomass production or waste water treatment systems is contingent upon maintaining an adequate year-round plant growth, and therefore high- and low-

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temperature tolerance (especially high-temperature tolerance in tropical regions) is an important factor in the management of macrophytes in various aquaculture applications. Several groups have studied the temperature tolerance of *Azolla* and have concluded that mean air temperatures more than 30–35°C (varying according to species and growth conditions) often have a direct negative impact on the growth and survival of *Azolla* (Peters et al., 1980; Tung and Watanabe, 1983; Watanabe and Berja, 1983; Debusk and Reddy, 1987). The temperature in some regions can fluctuate widely over the course of one day. In addition, it is estimated that in Japan, the temperature of water in ponds or pools in which *Azolla* lives temporarily exceeds 40°C in the noon heat in summer, particularly in ponds or pools that are small and shallow. Further information about the effects of high temperature within a short term, as well as mean temperature, on the growth and survival of *Azolla* is thus needed for the year-round management of this plant. In this paper we present results of experiments on the effects of transient high-temperature stress (above 40°C) on eight *Azolla* strains from six species.

A. microphylla 4018, *A. microphylla* 4021, *A. mexicana* 2503, *A. caroliniana* 3017 and *A. pinnata* var. *pinnata* 7002 were transplanted from IRRI (International Rice Research Institute) (Watanabe et al., 1992). *A. filiculoides* 1090, *A. rubra* Obaru and *A. pinnata* var. *imbricata* were collected from Japan. All *Azolla* strains were maintained in a growth cabinet. The details of the composition of the culture medium (Uheda, 1986) and plant growth condition (Uheda et al., 1995a) have been described previously. For the determination of growth after treatment with high-temperature stress, plantlets of *Azolla* (0.5 g) were rinsed with distilled water and transferred to an Erlenmeyer flask (volume, 550 ml) that contained 140 ml of the culture medium for *Azolla* (Uheda, 1986). The culture medium was maintained at various temperatures by immersing the flasks in water at desired temperatures. Flasks were irradiated using fluorescent lamps (60 μmol m^{-2} s^{-1} at plant level) and incubated with gentle shaking at 80 rpm. After 30, 60 and 120 min, plant materials were transferred to a tray that contained fresh culture medium and cultured for 10 days under a 16 h light/8 h dark cycle at 24°C. The plants were illuminated by fluorescent lamps and tungsten lamps (90 μmol m^{-2} s^{-1} at plant level). The culture medium was changed every four days. The fresh weight of harvested plants was measured after they had been blotted dry. For the determination of survival, a plantlet of *Azolla* was transferred to an Erlenmeyer flask (volume, 140 ml) that contained 40 ml of the culture medium and treated with temperature stress as described above. A plantlet was then transferred to a tray that contained fresh medium. When a plantlet abscised its branches after treatment with high temperature, a plantlet and abscised branches were cultured together in the same tray. Plantlets were cultured for 10–20 days as described for the growth experiment. When plantlets were dead, they eventually became brownish. By contrast, when plantlets were alive, green tips appeared and grew. For examinations of abscission, a plantlet of *Azolla* was transferred to an Erlenmeyer flask and treated with temperature stress as described for the survival experiment. After treatment, the number of abscised branches and detached roots more than 10 mm in length were determined separately. The number of remaining attached roots more than 10 mm in length was also determined. Abscised branches were then transferred individually to separate trays that contained fresh medium and cultured as described for the growth experiment above. After culture for 10–20 days, the number of alive and dead branches were determined.

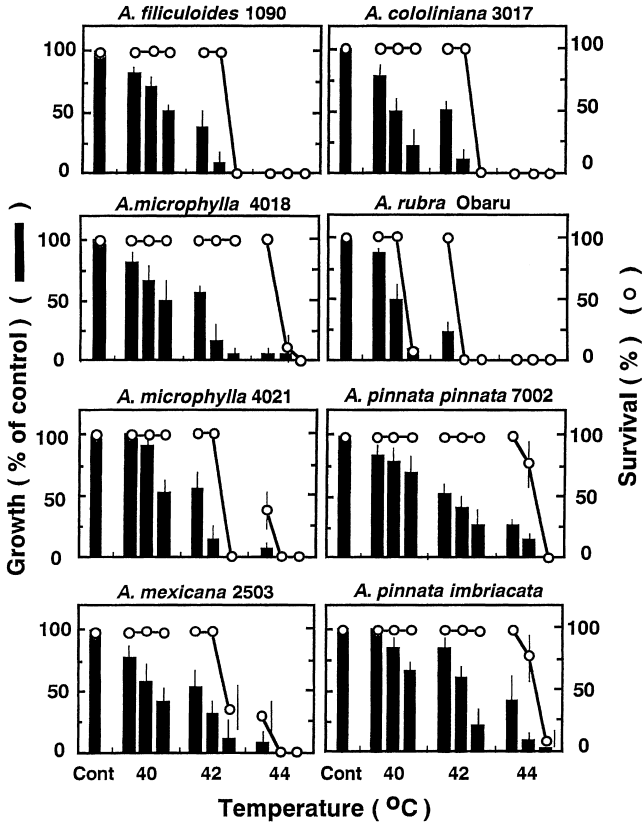

Fig. 1. Growth (■) and survival (○) of various *Azolla* strains after treatment with high-temperature stress. Plantlets were treated for 30 (left column), 60 (middle column) and 120 min (right column) at various temperatures. Controls were treated at 25°C for 2 h. Growth rate values are expressed as percentage of the control. The fresh weights (g/pod) in the control experiments were 6.8 (*A. filiculoides* 1090), 7.7 (*A. microphylla* 4018), 5.0 (*A. microphylla* 4021), 3.7 (*A. mexicana* 2503), 5.4 (*A. caroliniana* 3017), 1.7 (*A. rubra* Obaru), 2.9 (*A. pinnata* var. *pinnata* 7002) and 3.8 (*A. pinnata* var. *imbricata*). Determinations were performed twice using separate preparations. Results were reproducible. Data show one set of results. Values are presented as means±standard deviations (n=3 and 15 for the growth and survival data, respectively).

The treatment with water temperature above 40°C caused a marked decrease in the growth rate of the plants and an increase in the number of dead plants (Fig. 1). However, each *Azolla* strain showed different tolerance to the stress. Among the strains tested, *A. pinnata* var. *imbricata* and *A. pinnata* var. *pinnata* 7002 had the highest tolerance to the temperature stress. Most fronds of these strains survived after the treatment at 42°C for 2 h or at 44°C for 1 h, though the growth rate decreased markedly. However, even these strains did not survive after treatment at 46°C for 30 min (data not shown). By contrast, *A. rubra* Obaru had the lowest tolerance to the high-temperature stress. This strain did not survive after treatment at 40°C for 2 h or at 42°C for 1 h. Based on the data of growth and survival after the treatment, we concluded that the degree of tolerance of

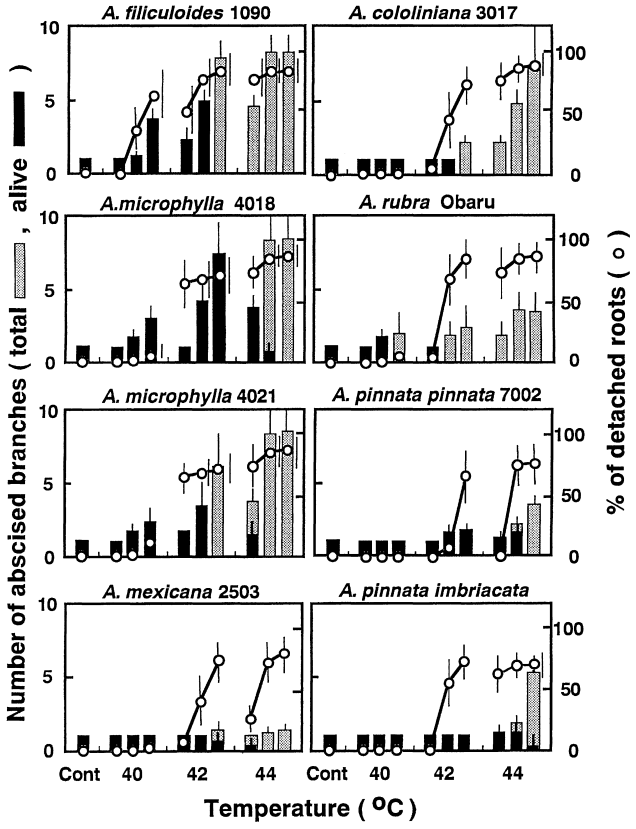

Fig. 2. Abscission of branches and roots after treatment with temperature stress. Plantlets were treated for 30 (left column), 60 (middle column) and 120 min (right column) at various temperatures. Controls were treated at 25°C for 2 h. Number of total (□) and proliferative (■) branches abscised. (○) Number of abscised roots longer than 10 mm as a percentage of total roots longer than 10 mm after treatment with temperature stress. Determinations were performed twice using separate preparations. Results were reproducible. Data show one set of results. Values are means±standard deviations (n=5).

the various strains to transient high-temperature stress was in the following order: *A. pinnata* var. *imbricata*, *A. pinnata* var. *pinnata* 7002>*A. microphylla* 4018>*A. mexicana* 2503, *A. microphylla* 4021>*A. caroliniana* 3017, *A. filiculoides* 1090>*A. rubra* Obaru. The order of tolerance of individual species was consistent with the reports by the other workers (Peters et al., 1980; Tung and Watanabe, 1983). They determined the tolerance of various species to mean air temperature below 40°C.

Unlike many other plants, in *Azolla*, a rapid abscission of roots and branches took place under high-temperature stress (Fig. 2). The rapid abscission took place in all of the *Azolla* strains tested, though the temperature and time that caused a rapid abscission and the magnitude of rapid abscission varied among these strains. Among the strains tested, particularly in *A. filiculoides* 1090, *A. microphylla* 4018 and *A. microphylla* 4021, the abscised branches were alive and proliferative when the temperature stress was short-

term. The maximum number of proliferative branches was increased by five times, seven times and four times, respectively, by the treatment. These strains may utilize rapid abscission to counteract the death induced by high-temperature stress. Under environmental and experimental conditions, *Azolla* plants often grow in clumps. In such a case, the stems and roots of individual sporophytes are entangled with each other above and in the water, respectively. The sporophytes then cannot be easily moved or dispersed over a wide area by air currents and/or movements of the water surface. The rapid abscission of branches and roots not only leads to an increase in its numbers but may also simultaneously allow easy movement and/or dispersion to better conditions. Rapid abscission may therefore increase the chances of *Azolla* for survival against high-temperature stress. When these species would be used, the rapid abscission and the resultant dispersion and propagation should be concerned. With respect to the strains other than *A. microphylla* and *A. filiculoides*, rapid abscission seemed not to contribute to the propagation. However, since the times and temperatures that caused rapid abscission and the death of the plants were similar, further precise examinations are necessary for clarification of this point.

Extant *Azolla* species are divided into two sections, *Euazolla* and *Rhizosperma. Anabaena azollae* of the sections *Euazolla* and *Rhizosperma* are genetically different (Peters and Meeks, 1989). Thus, as well as the differences in the host plants, the differences in the symbiotic *Anabaena* strains may have anything to do with the differences in response to individual *Azolla–Anabaena* associations being observed. However, the detailed mechanism by which individual *Azolla–Anabaena* associations showed differential response to high-temperature stress is unknown. In order to clarify the details, further investigations are needed. With regard to a unique rapid abscission system in *Azolla*, we reported previously that sodium azide, 2,4-dinitrophenol and carbonyl cyanide *m*-chlorophenylhydrazone, all well-known inhibitors of respiration, caused the abscission of roots and branches (Uheda and Kitoh, 1994; Uheda et al., 1994; Uheda et al., 1995b). The process of the rapid abscission is very similar to that of normal abscission with respect to hydrolyzation of the middle lamella and the separation of intact cells. Abscission induced by high temperature may take place in a manner similar to the rapid abscission induced by inhibitors of respiration. However, the details of this abscission are not known; the mechanism of rapid abscission is currently being studied.

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References

Addicott, F.T., 1982. Abscission. University of California Press, Berkeley.

Debusk, W.F., Reddy, K.R., 1987. Growth and nutrient uptake potential of *Azolla caroliniana* Willd. and *Salbinia potundifolia* Willd. as a function of temperature. Environ. Exp. Bot. 27, 215–221.

Kitoh, S., Shiomi, N., 1984. Nutrient removal by *Azolla* from the mineral nutrient solution and waste water. Water Purif. Liq. Wastes Treat. 25, 561–567in Japanese.

Kitoh, S., Shiomi, N., Uheda, E., 1993. The growth and nitrogen fixation of *Azolla filiculoides* Lam. in polluted water. Aquat. Bot. 46, 129–139.

Peters, G.A., Toia Jr., R.E., Evans, W.R., Crist, D.K., Mayne, B.C., Poole, R.E., 1980. Characterization and comparisons of five N_2-fixing *Azolla–Anabaena* associations. I. Optimization of growth conditions for biomass increase and N content in a controlled environment. Plant Cell Environ. 3, 261–269.

Peters, G.A., Calvert, H.E., 1983. *Azolla–Anabaena Azollae* symbiosis. In: Goff, L.J. (Ed.), Algal Symbiosis. Cambridge University Press, Cambridge, pp. 109–145.

Peters, G.A., Meeks, J.C., 1989. The *Azolla–Anabaena* symbiosis: basic biology. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40, 193–210.

Scharpenseel, H.W., Knuth, K., 1987. Use and importance of *Azolla–Anabaena* in industrial countries. In: *Azolla* Utilization. International Rice Research Institute, Los Banõs, Laguna, Philippines, pp. 154–167.

Tung, H.F., Watanabe, I., 1983. Differential response of *Azolla–Anabaena* associations to high temperature and minus phosphorus treatments. New Phytol. 93, 423–431.

Uheda, E., 1986. Isolation of empty packets from *Anabaena*-free *Azolla*. Plant Cell Physiol. 27, 1187–1190.

Uheda, E., Kitoh, S., 1994. Rapid shedding of roots from *Azolla filiculoides* plants in response to inhibitors of respiration. Plant Cell Physiol. 35, 37–43.

Uheda, E., Nakamura, S., Kitoh, S., 1994. Events associated with the rapid separation of cells from detached roots of *Azolla filiculoides* depend on pH. J. Exp. Bot. 45, 1451–1457.

Uheda, E., Kitoh, S., Dohmaru, T., Shiomi, N., 1995a. Isolation and analysis of gas bubbles in the cavities of *Azolla* leaves. Physiol. Plant. 93, 1–4.

Uheda, E., Nakamura, S., Kitoh, S., 1995b. Aspects of the very rapid abscission of *Azolla* branches: anatomy and possible mechanism. Int. J. Plant Sci. 156, 756–763.

Watanabe, I., Berja, N.S., 1983. The growth of four species of *Azolla* as affected by temperature. Aquat. Bot. 15, 175–185.

Watanabe, I., Roger, P.A., Ladha, J.K., Van Hove, C., 1992. Biofertilizer germplasm collections at IRRI. International Rice Research Institute, Los Banõs, Laguna, Philippines.