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A PRELIMINARY STUDY ON ALLELOPATHIC ACTIVITY OF BRYOPHYTES UNDER LABORATORY CONDITIONS USING THE SANDWICH METHOD

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ABSTRACT. Allelopathy is a common phenomenon in plants, with environmental impacts in ecosystems through interference of allelochemicals that inhibit or promote growth and activity of other life. In this study, allelopathic activities of bryophytes, represented by *Sphagnum palustre*, *Racomitrium japonicum*, *Dicranum japonicum*, and *Hypnum plumaeforme*, were detected using the sandwich method for bioassay of allelopathic effect for lettuce under laboratory conditions. Most of the representatives more or less showed inhibition or promotion of radicle elongations of lettuce seedlings. These results suggested that bryophytes also have allelopathic activities, and the influence would provide the ecosystems one of the factors for decelerating progressive succession in an early stage of succession of vegetation.

INTRODUCTION

The concept of allelopathy has been observed for over 2,000 years, and the term allelopathy was coined by Molisch (1937). Originally he termed the word as a means of beneficial and detrimental chemical interaction among plants, including microorganisms, with direct or indirect, inhibitive or promotive, offensive or defensive influence. This allelopathic influence is a form of chemical competition through interference chemicals and results from combinations of some allelochemicals to inhibit or promote growth and activity of other life (Rice 1984; Kobayashi 2004; as reviews). In most situations, the influence appears as inhibition that additively or synergistically acts to inhibit growth of neighboring plants. These phenomena have been widely observed from not only vascular plants but also some lichens because of their static habitat. Many allelopathic plants, particularly such vascular plants as *Pinus*, *Asparagus*, *Juglans*, *Brassica*, *Solidago*, *Eucalyptus*, and *Pteridium*, have been reported (e.g., del Moral & Cates 1971; Bell & Muller 1973; Newman & Rovira 1975; Gliessman & Muller 1978; Lodhi & Killingbeck 1980, 1982; Kil & Yim 1983; de Scisciolo et al. 1990; May & Ash 1990; Alonso-Amelot et al. 1992; Fujii 1994; Dolling et al. 1994; Konar & Kushari 1995; Tolliver et al. 1995; Dolling 1996; Nakamura & Nemoto 1996; Sivagurunathan et al. 1997; Shiraishi et al. 2002; Fujii et al. 2003). In agriculture and

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agroforestry, phytotoxic plants, including some allelopathic cover crops, with the capability to form allelochemicals were used to control weeds using their allelopathic inhibition (e.g., Tukey 1969; Putnam & DeFrank 1979, 1983; Rice 1984; Einhellig 1996; Rizvi et al. 1999).

Living organisms form ecosystems, interacting with each other, and the role of bryophytes in ecosystems is very important (e.g., Glime 2001, as a review). Allelopathy is a phenomenon observed in diverse plant groups of vascular plants and lichens and that has considerable environmental impact in ecosystems. For example, some bryophytes, such as *Sphagnum palustre* L., *Racomitrium japonicum* (Dozy & Molk.) Dozy & Molk. and *Hypnum plumaeforme* Wilson, are known to be dominant in plant communities and sometimes to form large, pure colonies in Japan. Many biologically active compounds, such as some terpenoids and phenolic compounds, have also been isolated from bryophytes (e.g., Asakawa 1981, 1995, 1998, 2004; Basile et al. 1993, 1998a, b; So & Chan 2001; Frahm 2004). Thus, it is possible that these occurrences of dominance are due to allelopathic influence from such species of bryophytes. However, there are few reports on bryophytes as donors with allelopathic effects on vascular plants. The objectives of this study are (1) to confirm the presence of the allelopathic properties of bryophytes affecting other organisms, and (2) to demonstrate some examples of allelopathic influence from bryophytes.

Materials and Methods

The allelopathic activity of bryophytes as donor plants was assessed under laboratory conditions in using the sandwich method proposed by Fujii (1994) and Fujii et al. (2004). As a test (receptor) plant material, lettuce (*Lactuca sativa* L. cv. Melbourne, Tohoku Seeds Co. Ltd, Utsunomiya, Japan) was used, since it is universally used and is easy to handle, and shows rapid and uniform growth response (Reynolds 1975a, b; Negm & Smith 1978; Cameron & Julian 1980; Duke et al. 1983; Fujii 1994; Konar & Kushari 1995; Fujii et al. 2004). The condition of the bioassay was optimized by Fujii et al. (2004) who used a gelling material as a growth medium for the lettuce seedling growth and determined the effects of the concentration of the medium on the growth of the radicle and hypocotyl of the lettuce.

In this study, four species of mosses, *Sphagnum palustre*, *Racomitrium japonicum*, *Dicranum japonicum* Mitt., and *Hypnum plumaeforme*, which form pure colonies, were used for allelopathic donor plants. These mosses were collected from May to July, 2005, on the campus of Hiroshima University, Higashi-hiroshima-shi, Hiroshima, Japan. The voucher specimens of the bryophytes were deposited in HIRO (HT-5797–5804). Clean parts of shoots were used for the tests; if needed, shoots from a single clump were washed several times with deionized water (DW) before drying. Whole plants including rhizoids, except for a part of bases of stems and dead shoots, were used for the samples. Different drying conditions (heated in 80°C for 8 hrs and non-heated with silica gel at room temperate) were compared for the same samples to detect thermal tolerance of allelochemicals in effects on the allelopathic activity. Little fungal and bacterial growth was observed without use of antibiotics within the three-day incubation (in this case, some growths of them were detected within one or two weeks after the incubation). Effects of contamination of algae were not tested, and a few algae, such as green algae and diatoms, were observed under a light mi-

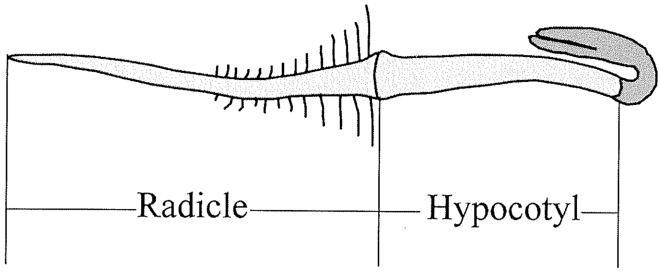

Fig. 1. Radicle and hypocotyl of a lettuce seedling.

croscope.

For the controls of the seeding test, deionized water was used for the negative control; 2-[4-(2-Hydroxyethyl)-1-piperadinyl]ethansulfonic acid (HEPES) buffer, which has a low level of cell toxicity, for pH adjustment for *Sphagnum* and its control; and a Japanese native basil *Glechoma hederacea* L. var. *grandis* (A.Gray) Kudo [Lamiaceae] for the positive control.

Samples of the bryophytes and basil for control were ground to a rough powder using a crushing machine (Toyobo, Kurashiki, Japan). The powder (100 mg dry weight in each plate, that is ca. 4.2 mg in each well) was placed into every well of a 24-well multidish plastic plate (the diameter and depth of well are 15.7 and 17.2 mm, respectively). The plates were segmented into two or four experimental provisions. Preparation of the growth medium with 0.5% (w/v) agar (Nacalai Tesque, Kyoto, Japan) was added to each well of the multidish plate and gelatinized for ca. 30 min. After gelatinizing the agar, the upper layer of the agar was added, and gelatinized completely for ca. 60 min to prevent direct contact of seeds with the powdered donor plants. Final volume of agar in each well was ca. 1 ml. After gelatinizing the agar, three seeds of lettuce were placed on the surface of each well of the plate. After seeding, the plate was incubated for three days at room temperature (ca. 24°C) in a black box to maintain completely dark conditions. Treatments for each experimental provision were performed using a total of 36 seeds.

After incubation for three days, the lengths of radicles and hypocotyls of lettuce seedlings were measured using a digital caliper (Fig. 1). A Student *t*-test ($p<0.05$, n=33–36) was used to test the difference between the means of an independent provision and the untreated control with DW. The germination percentage of the 36 seeds was also recorded.

RESULTS

The evaluation of allelopathic activity of the four species of bryophytes and controls with an application of the sandwich method is shown in Fig. 2. Most of the donor plants, except for *Racomitrium japonicum*, significantly inhibited radicle elongation of the recep-

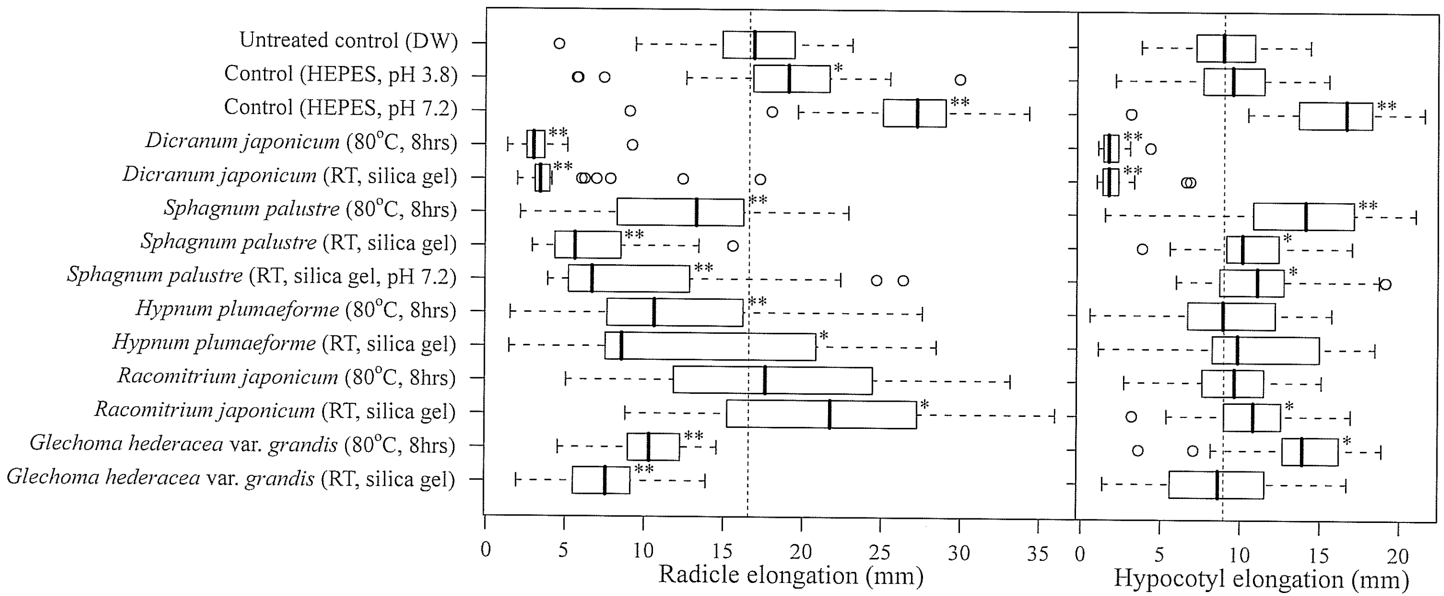

Fig. 2. Radicle and hypocotyl elongations of lettuce growing on 0.5% agar gel containing powdered bryophytes species tested by the sandwich method. The difference of the means compared to untreated control (DW) was tested by the Student *t*-test (n=33–36), and the significances are indicated as asterisks (*,**=$p<0.05$, 0.01, respectively) at the fore near the box in the graph. The boxplots (box and whisker diagrams) indicate the median and range of the data, containing a measure of central location (the median meant by midline in the graph), two measures of dispersion (the range as the two extremes by two whisker ends, and inter-quartile range as versions of the first and third quartiles by box), the skewness (from the orientation of the median relative to the quartiles by bar), and potential outliers (marked individually by circles).

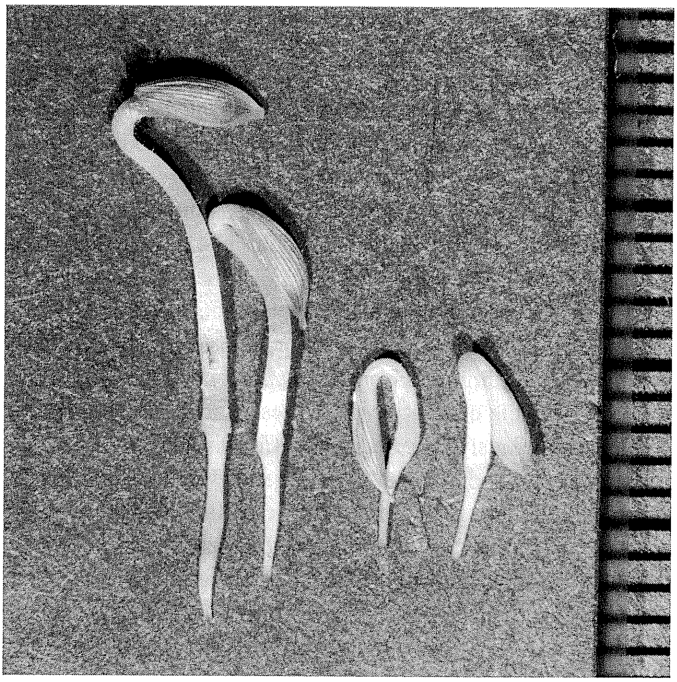

Fig. 3. Mean representatives of the seedlings treated with the donor plants *Sphagnum palustre* (RT, silica gel; two on left) and *Dicranum japonicum* (80°C, 8 hrs; two on right). Scales = 1 mm.

Table 1. Difference in radicle and hypocotyl elongations of lettuce growing on 0.5% agar gel between the heated drying and non-heated drying conditions (80°C, 8 hrs, and RT, silica gel) tested by the sandwich method.

Species	Radicle	Hypocotyl
Dicranum japonicum	*	—
Sphagnum palustre	**	**
Hypnum plumaeforme	—	—
Racomitrium japonicum	—	*
Glechoma hederacea var. *grandis*	**	**

The difference of the means between the drying conditions was tested by the Student *t*-test. *,** = $p <$ 0.05, 0.01, respectively. n=33–36.

tor lettuce seedlings, although allelopathic activities on seedlings varied with the donor plants as seen in the growth of the lettuce (Fig. 3). Little inhibitory effect from the donors, except for *Dicranum japonicum*, on lettuce germinations was observed (91.2–100% germination percentage). The difference between the heated dried and non-heated drying conditions for each species is shown in Table 1.

Dicranum japonicum caused very strong inhibitory effects for the test plants. Radicle elongations showed significant difference between the heated dried and non-heated drying conditions for *D. japonicum*, and hypocotyl elongations showed no significant difference between the conditions for *D. japonicum*. *Sphagnum palustre* showed the same level of inhibitory effects for radicle elongation of the test plants as in the positive control plant, *Glechoma hederacea* var. *grandis*, and the other donor plants previously reported by Fujii (1994), Shiraishi et al. (2002), and Fujii et al. (2003). When the pH condition of *S. palustre* was controlled at pH 7.2, it also showed inhibitory effects (57% in the means) for the test plants. Both radicle and hypocotyl elongations for *S. palustre* exhibited significant differences between the drying conditions. *Hypnum plumaeforme* showed the same or low level of inhibitory effects for radicle elongation of the test plants as in the positive control plant and other donor plants previously reported. Only *H. plumaeforme* exhibited no significant differences in both radicle and hypocotyl elongations between the drying conditions. *Racomitrium japonicum* showed no or promotory effects in radicle and hypocotyl elongations on the test plants. *R. japonicum* exhibited a significant difference in hypocotyl elongation between the drying conditions. The HEPES controls (pH 7.2 and 3.8) showed some significantly promotory effects for the test plants.

These results suggest that *Dicranum japonicum* shows a strong allelopathic inhibitory effect for the seedling radicle and hypocotyl elongations, and *Sphagnum palustre* and *Hypnum plumaeforme* also show some inhibitory effects for radicle elongations. Also, radicle elongations of lettuce seedlings were significantly affected by the powdered bryophytes, except for *Racomitrium japonicum* (dried in 80°C, 8 hrs). Only one species of the applied plants, *D. japonicum*, significantly inhibited hypocotyl elongation of lettuce. These results show that the elongation was more strongly inhibited in the radicle than in the hypocotyl for applied plants. Also, effects of the difference in the drying conditions were different among all the donors.

Discussion

Most of the representatives more or less showed inhibition or promotion of radicle elongations of lettuce seedlings. The results obtained in this study suggested that most of these bryophytes show some allelopathic effects, and allelopathic influence is maybe a common and widely observed phenomenon in bryophytes, although only four species of the donor plants were applied in this study. For bryophytes with a lower rate of growth than some vascular plants, allelopathy would be one of the strategies to survive in ecosystems, especially at the beginning of succession or a static habitat, appearing as inhibition to other plants. Allelopathy would provide the ecosystems with one of the factors for decelerating progressive succession in an early stage of succession of vegetation, and some bryophytes, such as *Sphagnum* spp. and *Hypnum plumaeforme*, can be dominant in plant communities and sometimes form pure colonies with their rapid covering rate.

The comparison between the different dried conditions (heated and non-heated with silica gel) showed the compounds have some thermal tolerance (not heat-labile) in *Di-*

cranum japonicum and *Hypnum plumaeforme*, suggesting that the allelopathic phenomenon is caused by low-molecular substances with their activity as allelochemicals having action similar to plant hormones, such as auxins, gibberellins (GA) and brassinosteroids (BR), fatty acids, or steroids, not by high-molecular substances, such as proteins and polypeptides that lack thermal tolerance with the activity as allelochemicals. *Sphagnum palustre* exhibited differences between the drying conditions, suggesting that some parts of the allelopathic effects in *S. palustre* are caused by low-molecular substances and other parts of the effects are caused by high-molecular substances.

The effect of acidic pH is also believed linked to inhibitory effects on seedlings. For *Sphagnum*, the pH condition in its habitat is distinctly acidic (pH 2–6, tending to less than 5.7) in many cases, as shown in many studies (e.g., Skene 1915; Clymo 1963; Clymo & Hayward 1982; Hingley 1993) or our previous observations (data not shown), wherein the acid conditions would be produced by the *Sphagnum* itself (Clymo 1963; Dainty & Richter 1993). In this study, the pH condition of the culture medium was buffered to 7.2 by HEPES, although the optimum pH-range of HEPES buffer is 7–8. To obtain more perspicuous results, use of the other buffering materials with wide range would be preferable. Some additional experiments would be needed to check the effects of various donors of bryophytes, powdering of plant materials, and buffering with HEPES. Effects of contamination of some algae or fungi also should be tested using axenic bryophyte strains.

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