Growth Responses and Regrowth to Low Temperature of Nine Native Moss Species

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ABSTRACT

Moss is used as an important material in indoor landscaping as well as outdoor landscaping. Moss is vivid green during growth and excellent in ornamental value. But when temperature drops, moss stops growth, turns brown or loses its ornamental value. In the present experiment, for the purpose of classifying native mosses according to the growth response to low temperature, the temperature of the plant growth chamber was set to 15°C/5°C (16h/8h, day/night) and 5°C (24h) for 8 weeks using nine native moss species. Thereafter, the temperature of the plant growth chamber was set to 20°C, and then the changes of moss block area and moss color were measured. The changes of moss block area and moss color were measured using a Photoshop program, after each moss block was photographed. As a result, *Atrichum undulatum* (Hedw.) Beauv., *Etodon luridus* (Griff.) A. Jaeger, *Bachythecium plumosum* (Hedw.) Schimp, *Plagiomnium cuspidatum* (Hedw.) T.J. Kop, and *Hypnum plumaeforme* Wilson showed a small decrease in moss block area at low temperature, and their recovery were the fastest at 20°C. These three species had higher green values at low temperature compared to other species, and the greenness increased rapidly at 20°C. On the other hand, *Atrichum undulatum* (Hedw.) Beauv., *Marchantia polymorpha* L., and *Thuidium cymbifolium* (Mitt.) A. Jaeger showed the smallest block area at low temperature and the lowest recovery even at 20°C. Their green values also decreased significantly at low temperature, and maintained low green value even at 20°C. These results showed that these three moss species are sensitive to low temperature. The remaining *Myuroclada maximowiczii*, *Plagiomnium cuspidatum*, and *H. erectiusculum* showed moderate responses to low temperature compared to other six species of mosses.

Keywords: Bryophyte, green interior, image analysis, leaf color, resistance to low temperature

Introduction

By botanical classification, moss is somewhere between chlorophyte and pteridophyte, divided into mosses (musci) and liverworts (hepaticae), with approximately 14,000-16,000 species (National Institute of Biological Resources, 2015). Moss is comprised of stems, leaves and rhizoids, and some types have the form of cormus differentiated from stem and leaf, and some in which the leaves and stems are not differentiated like *Marchantia* and Anthocerotophyta (Choi, 1980). They have roots but most are comprised of rhizoids,

without vascular bundle developed (Choi, 1980). Thus, most bryophytes absorb moisture through the entire plant body (During, 1992). The gametophyte generation is the plant body of moss we commonly see, living in the form of cormus or thallus. In the sporophyte generation, zygotes made by pollination of sperm nucleus and egg nucleus are germinated on gametophytes, thereby becoming sporophytes. Sporophytes create sporangia in which spores are created. New gametophytes are generated when spores germinate, and gametophytes live an autotrophic life as they have chloroplasts.

This study was supported by the 2018 research grant from Gyeongnam National University of Science and Technology.

Received: November 24, 2019, **Revised:** December 4, 2019, **Accepted:** December 11, 2019

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Most mosses grow on the ground, epiphytically on moist land, rocks, decayed trees and tree trunks, but some of them grow epiphytically on the leaves of broad-leaved trees. We can commonly see mosses around us, which mostly grows well in shaded and moist areas. Mosses also do not die out even in an extremely dry condition, and it undergoes photosynthesis as its resilience improves quickly with moisture supply (During, 1992; Oliver et al., 2005). Although there are slight differences among types of bryophytes, most mosses can contain moisture up to 50-200% of dry weight (Schofield, 2001). Mosses not only can hold much moisture but also has high utilization value as indoor plants as they grow well in shades (Kim et al., 2009). Moreover, bryophytes have the ability to purify heavy metal under-water (Choi, 1992), and are also known to have the ability to remove toluene from indoor air pollutants (Kim et al., 2010). With such excellent functions, the utilization value of bryophytes is recently increasing as materials for indoor landscaping, especially wall planting and miniature landscape, and in some cases moss gardens are formed for landscaping, using moss as the main material.

Despite the increasing use of mosses, there are only a few studies on the cultivation conditions of some native mosses (Cho and Lee, 2013a, 2013b), but barely any research on the selection of native bryophytes according to the environmental factors. When the temperature drops significantly in winter like in Korea, mosses brown easily or stop growing. Thus, it is necessary to select moss species with great adaptability to low temperature, which can be easily recovered at normal temperature even though damaged by low temperature.

Therefore, this study was conducted to provide some basic data for use of native mosses by determining their growth responses at low temperature as well as their resilience at room temperature by using nine native moss species.

Research Methods

Plant materials

Total 9 species of mosses were used in this study: *Atrichum undulatum* (Hedw.) P. Beauv., *Etodon luridus*

(Griff.) A. Jaeger, *Marchantia polymorpha* L., *Thuidium cymbifolium* (Mitt.), *Myuroclada maximowiczii* (G.G. Borshch.) Steere & W.B. Schofield, *Bachythecium plumosum* (Hedw.) Schimp, *Plagiomnium cuspidatum* (Hedw.) T.J. Kop, *Hypnum plumaeforme* Wilson, and *Hypnum erectiusculum* Sull. & Lesq. All mosses were collected from a mountain near Jinju in June with permission of the owner, which were then maintained and cultivated at a vinyl greenhouse in Gyeongnam National University of Science and Technology. Moss samples were sent to the National Institute of Biological Resources to verify the species, and the species were identified by a moss classification expert. Mosses were used by chopping them with a sharp steel frame (7cm²) and creating blocks in a fixed size. The mosses were placed on two layers of white non-woven fabric (10cm²) and put into a white plastic basket with many holes at the bottom. This basket is put into a plastic tray with 3cm edges on all four sides, and water was supplied through bottom watering.

Low temperature and recovery temperature treatment

To determine growth responses of mosses at low temperature, we set the plant growth temperature (JSPC-420C, JSR. INC., Korea) into two levels for the experiment: day/night temperature set at 15±2/5±1°C (16h/8h), and day/night temperature set at 5±1°C (24h). For 8 weeks after low temperature treatment, we regularly examined the changes in moss block size and moss color. To determine the resilience of mosses when they are placed at room temperature after low temperature treatment, we set the plant growth temperature at 20±2°C. We examined the changes in block area and color at one-week intervals. Light intensity for plant growth (PPFD) was set at 50±3 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (16h), and relative humidity at 70±3%.

Experimental design and photographing

The experiment began from the beginning of August, and we designed the experiment in a 10-times repetition using 10 blocks for each moss species. For 8 weeks after low temperature treatment, we examined the growth responses at low temperature. After that, the day/night tem-

Fig. 1. Image analysis by Adobe Photoshop program. After opening a moss photograph, only moss block was selected by the magic wand (A). The moss block area was obtained by the pixel value and moss color was obtained by the medium value of each color (red, green, and blue) on the histogram window (B).

perature was reset at 20°C, and we examined their recovery for 6 weeks. Mosses have extremely small plant body, and thus there are many difficulties in examining growth. We took photographs regularly to investigate the changes in moss block area and moss color. Photographs were taken right before low temperature treatment, at 4 and 8 weeks after low temperature, and second, 4 and 6 at room temperature. With the photographs, we conducted image analysis using the Photoshop program (Photoshop CS6, Adobe, USA) to obtain moss block area and color.

Image analysis and statistical analysis

The photos were opened on Photoshop program and the Magic Wand tool was used to select only moss, and the area of the selected part on Histogram was obtained in pixels (Fig. 1). At the same time, the moss color (red, green, and blue) value of the selected block was obtained. An analysis of variance was conducted on statistics using the SPSS 12.0 program (IBM, New York, USA), and significance among means was tested at the 5% significance level using Duncan's multiple range test.

Results and Discussion

Growth responses of native mosses at low temperature

At the low temperatures of 15/5°C or 5°C, all mosses

Fig. 2. Changes of moss block area during exposure to low temperature of 15/5°C (day/night, 16/8 h; gray circles) or 5°C (24 h; black circles) and during at 20°C (24 h). GLM repeated measures ANOVA was conducted before treatment (BT), 4, and 8 weeks during low temperature and 2, 4, and 6 weeks during recovery. Results are represented as the mean (n=10). (A) *A. undulatum*, (B) *E. luridus*, (C) *M. polymorpha*, (D) *T. cymbifolium*, (E) *M. maximowiczii*, (F) *B. plumosum*, (G) *P. cuspidatum*, (H) *H. plumaeforme*, (I) *H. erectiusculum*.

showed a significant decrease in block area regardless of temperature, which dropped down to 50-70% of the initial area at first one month (Fig. 2). After that, there was not much difference in moss block area for further one month.

This suggests that when the minimum temperature drop to 5°C, mosses are stunted in growth or discolored, showing a rapid decrease of area, but after that they showed almost no growth. Mosses respond sensitively to environmental changes, and thus their growth is easily discontinued, and they enter dormancy if the weather becomes too dry or cold. Stunted growth of mosses at low temperature varied among species. *Etodon luridus* and *Hypnum erectiusculum* showed the least area decrease (Fig. 2B, 2I), whereas *Marchantia polymorpha*, *Bachytheceium plumosum*, and *Hypnum plumaeforme* showed the most area decrease (Fig. 2C, 2F, 2H). The other five types were similar.

After low temperature treatment, the plant growth temperature was set to 20°C and the recovery degree of mosses was examined for 6 weeks. It was found that moss block area rather decreased in the first 2 weeks, showing approximately 20% decrease than the low temperature state (Fig. 2). After that, moss block area gradually increased over time. Moss block area rather decreased in the first 2 weeks even when growth temperature increased to 20°C, which is the optimum growth temperature of moss. It seems likely that any symptom of low temperature injury was not shown, because most mosses easily stop growth or begin dormancy at 5°C, but the symptoms of injury appeared on the surface after metabolic activities started at optimum growth temperature. The recovery of mosses at optimum

growth temperature varied among moss species. *Etodon luridus*, *Myuroclada maximowiczii*, and *Hypnum erectiusculum* showed the most recovery in moss block area compared to other mosses (Fig. 2B, 2E, 2I). They showed 70-80% recovery of the initial area at 6 weeks at room temperature. The mosses having the biggest injury at the low temperature and the least recovery at room temperature were *Atrichum undulatum*, *Marchantia polymorpha*, *Thuidium cymbifolium*, and *Hypnum plumaeforme*, which were merely 50% of the initial area in 6th week at 20°C (Fig. 2A, 2C, 2D, 2H).

Changes in moss color after low temperature and at room temperature

When mosses are used in indoor or outdoor landscaping, they look fresh in green and thus green mosses have high utilization value. However, mosses easily enter dormancy when the temperature drops in winter and the color turns brown, thus likely to lose ornamental value. Therefore, the ability of moss quickly to recover at room temperature is very important characteristics. Table 1 and Fig. 3 show the changes in moss color after low temperature treatment and at optimum growth temperature. When mosses are treated at low temperatures of 15/5°C (16/8h, day/night) or 5°C (24h), red, green and blue values all decreased greatly.

Table 1. Changes of moss color during exposure to low temperatures (15/5°C, day/night, 18/6 h or 5°C, 24 h) and during recovery (20°C, 24 h)

Temperature	Scientific name	Color	Low temperature			Recovery temperature		
			0 week	4 weeks	8 weeks	2 weeks	4 weeks	6 weeks
15°C/5°C (16h/8h)	<i>Atrichum undulatum</i>	Red	100	36.8d ^z	34.7d	34.9c	37.8d	32.0c
		Green	100	41.7d	45.1de	47.6d	47.9de	46.5ef
		Blue	100	36.3d	30.3d	29.4d	35.0d	27.7e
	<i>Etodon luridus</i>	Red	100	67.0b	60.5bc	58.4ab	83.2a	79.2a
		Green	100	88.1a	86.9a	79.9bc	91.3a	86.5ab
		Blue	100	60.1b	48.5bc	52.8bc	66.4b	79.7ab
	<i>Marchantia polymorpha</i>	Red	100	71.9ab	59.9bc	63.7ab	47.8cd	47.9cd
		Green	100	57.4bcd	43.5e	47.8d	36.7e	38.2f
		Blue	100	80.3a	73.9a	79.3a	77.8a	83.4a
	<i>Thuidium cymbifolium</i>	Red	100	80.3a	82.8a	73.2a	72.3ab	62.8ab
		Green	100	60.1bc	61.6c	52.7d	49.0de	40.1f
		Blue	100	61.9b	63.8b	65.2b	62.3bc	44.3cd

Table 1. (continued)

Temperature	Scientific name	Color	Low temperature			Recovery temperature		
			0 week	4 weeks	8 weeks	2 weeks	4 weeks	6 weeks
15°C/5°C (16h/8h)	<i>Myuroclada maximowiczii</i>	Red	100	54.0bc	46.4cd	49.8bc	60.2bc	60.3b
		Green	100	70.5b	67.2bc	67.8bcd	73.3bc	88.3ab
		Blue	100	49.2c	45.5cd	50.9bc	58.9bc	63.2bc
	<i>Brachythecium Plumosum</i>	Red	100	44.0cd	52.0bc	59.0ab	62.5abc	57.0b
		Green	100	63.6bc	69.7b	89.8a	89.0ab	82.7ab
		Blue	100	38.5d	36.0cd	41.7c	50.5c	51.1cd
	<i>Plagiomnium cuspidatum</i>	Red	100	53.3bc	66.3ab	59.3ab	70.3ab	64.3ab
		Green	100	49.2cd	55.7cd	62.0cd	68.8bcd	61.5de
		Blue	100	46.0cd	55.8d	51.1bc	61.4bc	50.2cd
	<i>Hypnum plumaeforme</i>	Red	100	40.6c	57.8bc	50.4bc	72.4ab	67.9a
		Green	100	82.5a	84.9ab	84.8ab	106.4a	98.7a
		Blue	100	46.2cd	61.2b	49.5bc	67.4b	52.1cd
	<i>Hypnum erectiusculum</i>	Red	100	50.0bc	61.0bc	61.8ab	64.3abc	65.4ab
		Green	100	54.3bcd	68.4bc	67.6bcd	65.4cd	64.4cd
		Blue	100	44.1cd	47.6c	55.6bc	45.4c	39.5de
	<i>Atrichum undulatum</i>	Red	100	36.7d	36.5d	37.3d	37.9d	28.0e
		Green	100	41.2e	43.4d	46.3e	45.7e	34.5e
		Blue	100	37.5d	37.7cd	40.9c	45.5cd	29.9e
	<i>Etodon luridus</i>	Red	100	60.0b	82.1a	74.3ab	88.9a	75.5a
		Green	100	78.3a	88.5a	86.4a	91.1a	92.0a
		Blue	100	68.4bc	64.9b	72.3a	88.2a	88.0a
	<i>Marchantia polymorpha</i>	Red	100	60.6b	61.2bc	61.0bc	56.2bc	46.4cd
		Green	100	50.9cde	49.2cd	49.1de	46.4e	40.3d
		Blue	100	78.9ab	78.0a	77.3bc	77.0b	73.0b
	<i>Thuidium cymbifolium</i>	Red	100	79.3a	70.1ab	84.0a	72.7b	62.b
		Green	100	67.9abc	57.8c	58.1d	57.0cde	46.3ab
		Blue	100	88.8a	81.5a	70.4b	81.1ab	85.2ab
5°C (24h)	<i>Myuroclada maximowiczii</i>	Red	100	56.5bc	45.1cd	43.6cd	48.9cd	57.4bc
		Green	100	66.3abc	65.3b	65.5c	66.1cde	70.9bc
		Blue	100	55.1bcd	48.5cd	54.7bc	73.9b	69.6bc
	<i>Brachythecium Plumosum</i>	Red	100	41.0cd	42.4d	47.0cd	51.3cd	38.2de
		Green	100	52.6bcd	59.2bc	67.1bc	74.6c	70.2bc
		Blue	100	36.9d	30.8d	35.5c	46.7c	51.2c
	<i>Plagiomnium cuspidatum</i>	Red	100	43.1cd	47.6cd	50.9cd	61.7bc	59.1bc
		Green	100	43.8de	49.2cd	54.2cd	52.9de	50.4cd
		Blue	100	42.5cd	48.6cd	56.3bc	66.0bc	78.9bc
	<i>Hypnum plumaeforme</i>	Red	100	48.9bcd	41.2d	47.6cd	52.0cd	59.8bc
		Green	100	68.3ab	68.0b	80.3b	87.8b	90.7a
		Blue	100	51.4bcd	44.0cd	54.2bc	42.0cd	49.7cd
	<i>Hypnum erectiusculum</i>	Red	100	49.3bcd	48.3cd	49.2cd	59.2bc	63.3ab
		Green	100	59.3bcd	60.4bc	60.8cd	69.3bcd	66.8c
		Blue	100	43.3cd	39.5cd	45.2c	36.7d	35.9d

Note. The data are % of starting value.

^aMean separation within columns for each low temperature treatment by Duncan's multiple range test $p \leq .05$.

Fig. 3. Changes of moss color during exposure to low temperature of 15°C/5°C (16/8 h, day/night) or 5°C (24 h) and during recovery at 20°C (24 h).

All color values that decreased at 4 weeks of low temperature treatment regardless of the temperature remained almost the same by 8 weeks as well. After the temperature was transferred to optimum temperature later, the recovery of green varied among moss species. Red and blue did not show any correlation with moss block area or growth state, and only green showed a correlation with moss block area or growth state, which is why we will explain changes in green values here. In fact, it is more important to maintain greenness in reality as well.

At low temperature of 15/5°C, *Etodon luridus* showed 87% of initial value in green color, and 90% at room temperature. *Hypnum plumaeforme* also had 85% of the initial greenness at low temperature, and increased to 98% at room temperature, showing that it is well maintaining greenness despite the reduced area by low temperature and revealing more greenness at room temperature. *Bachythecium plumosum* decreased in greenness to 70% of initial value at low temperature, but recovered up to 90% at room temperature. Therefore, these three mosses are strong against low temperature and quickly recover at room temperature after low temperature treatment.

On the other hand, the species that showed the most remarkable decrease in greenness at low temperature were *Atrichum undulatum* and *Marchantia polymorpha*, which had their greenness drop to below 50% of the initial greenness. This suggests that even when the temperature setting was changed to room temperature, the greenness was not recovered and thus completely withered, or showed barely any recovery at all. *Thuidium cymbifolium* damaged by low temperature, failed to recover even at room temperature, and the greenness gradually decreased from 60% to 40%. The other three species such as *Myuroclada maximowiczii*, *Plagiomnium cuspidatum*, and *Hypnum erectiusculum* showed a moderate decrease in greenness at low temperature and moderate recovery of greenness at room temperature.

Treatment at 5°C (24 h) also showed similar results with 15/5°C temperature treatment. *Etodon luridus* and *Hypnum plumaeforme* showed the least decrease in greenness at low temperature, and showed high greenness at room temperature. Only *Bachythecium plumosum* showed little change in greenness at 15/5°C but showed decrease of up to 60% of the initial value, while also rarely showing any recovery.

On the other hand, *Atrichum undulatum*, *Marchantia polymorpha*, and *Plagiomnium cuspidatum* showed decrease in greenness by 50% of the initial value, and there was barely any change in greenness even at room temperature. *Thuidium cymbifolium*, like at 15/5°C, showed continuous decrease in greenness even at room temperature.

This tendency was found more precisely in actual photographs (Fig. 3). *Etodon luridus* maintained greenness without any injury at the two low temperatures and retained vivid green at room temperature, showing greatest tolerance to cold. *Bachythecium plumosum* did not show severe injury at low temperature of 15/5°C, and also seemed healthy at room temperature. *Hypnum plumaeforme* showed decrease in greenness due to low temperature, but the green was vivid on living mosses, and showed more vivid greenness due to room temperature.

In summary of the moss block area, greenness and actual images, *Etodon luridus* that showed the least change in moss block area and also in green value (Table 1, Fig 3). *Bachythecium plumosum* showed a great decrease in moss block area at 15/5°C, but maintained the greenness without much decrease due to low temperature. These results indicated that these 2 species are relatively strong against low temperature. *Hypnum plumaeforme* showed a great decrease in moss area due to low temperature, but living mosses did not turn brown but showed vivid green, thereby maintaining ornamental value. *Hypnum erectiusculum* shown a little decrease in moss block area due to low temperature as well as high resilience, showed a significant decrease in greenness, indicating that it is not strong against low temperature.

On the other hand, *Atrichum undulatum*, *Marchantia polymorpha*, and *Thuidium cymbifolium* which showed a great decrease in moss area at low temperature and did not show a great increase in moss area at recovery temperature, had significantly lower greenness than other species, thereby proving to be sensitive to low temperature. The other species such as *Myuroclada maximowiczii*, *Plagiomnium cuspidatum* and *Hypnum erectiusculum* showed moderate responses low temperature.

Furness and Grime (1982) also examined growth responses to temperature by cultivating 40 species of bryophytes in various temperature ranges, and found out that

most mosses showed the best growth at 15-25°C, but only *Dicranella palustris* and *Racomitrium lanuginosum* grew well at the low temperature of 12-13°C. More than half of the species showed a decrease in growth rates to below 50% at 5°C, indicating that responses to low temperature varied among species.

Atrichum undulatum has leaves that curl up when dry and uncurled when there is water, thereby having a great ornamental value (Choi, 1980), but it is sensitive to low temperature. *Myuroclada maximowiczii* (G.G. Borshch.) Steere & W.B. Schofield has round leaves and smooth stems and branches, thus suitable for indoor landscaping, but they stop growing and turn brown when the temperature drops to below 5°C in winter. Indoor or outdoor landscaping of building using mosses can reduce heating and cooling costs and alleviate the phenomenon of urban heat island (Lee et al., 2005), and thus use of mosses is expected to increase for roof or wall planting. However, when using mosses, it is important to choose the ones with high resistance to low temperature as well as resilience.

Conclusion

As a result of reviewing resistance of nine species of Korean native mosses to low temperature as well as their resilience at room temperature, it was found that three species such as *Etodon luridus*, *Bachythecium plumosum*, and *Hypnum plumaeforme* did not show a significant decrease in moss area and greenness even at low temperature compared to others, and their recovery was also quick at room temperature. On the other hand, three species such as *Atrichum undulatum*, *Marchantia polymorpha*, and *Thuidium cymbifolium* showed a significant decrease in moss area and greenness at low temperature, and little recovery at room temperature. *Myuroclada maximowiczii*, *Plagiomnium cuspidatum*, and *Hypnum erectiusculum* showed moderate responses to low temperature compared to the other six species. Therefore, the results above must be considered in using native mosses for roof or wall planting, and outdoor and indoor landscaping.

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