アゾラの生存と再生に及ぼす温度と水分の影響
Effects of Temperature and Moisture on Azolla Survival and Regeneration

ルアングシリ マツラダ* 、 中嶋 佳貴*
RUNAGSIRI Mathurada*, NAKASHIMA Yoshitaka*

Introduction

Azolla spp. is a free-floating aquatic fern widely used as green manure due to its rapid growth and high nitrogen content (3-6% N by dry weight) (Watanabe et al., 1981). It serves as an excellent bio-fertilizer, capable of fixing atmospheric nitrogen through a symbiotic association with the cyanobacterium *Anabaena azollae* (Carrapico, 2002).

The Ministry of the Environment in Japan has designated foreign Azolla as a regulated species under the Invasive Alien Species Act, citing the risk of hybridization with native species and prohibiting its use. While Japan has native Azolla species, they are sensitive to high summer temperatures. (Watanabe, 2006). Azolla can also be harmful to the environment if it grows rapidly and covers the water surface, as it can block sunlight and reduce oxygen levels, potentially leading to the death of native aquatic species.

However, Azolla is an important nitrogen source for organic farming. To prevent unintended environmental impacts, we propose utilizing dried Azolla and ensuring it is free from contamination before application. Thus, this study aimed to examine 1) the effects of temperature on Azolla survival and regeneration and 2) The investigation of the effect of moisture loss on the survival potential and regeneration ability of Azolla.

Materials and Methods

1. The effects of temperature on Azolla survival and regeneration

Fresh Azolla was collected on Mar 6, 2025 and weighed, with 3 g allocated for each treatment. The samples were then incubated under four different temperature conditions: 30℃, 40℃, 50℃, and room temperature (24℃). Azolla samples were taken out daily from the incubator (30℃, 40℃, and 50℃) to monitor biomass from Day 1 to Day 14. After the incubation period, the Azolla from each treatment was transferred to water and floated on the surface. To assess regeneration ability, the samples were incubated at 25℃ under a light intensity of 30,000 lux for 7 days.

2. The influence of moisture loss on Azolla survival and regeneration.

Fresh Azolla (3 g) was collected on Apr 2, 2025 and placed in a petri dish. The samples were gradually exposed to room temperature to allow slow moisture loss. The weight of Azolla was measured every 3 hours to track the rate of moisture reduction for 48 hrs. After various durations of moisture loss, the Azolla samples were submerged in water and incubated under controlled conditions (25℃, 30,000 lux light intensity) for 21 days to assess their survival rates and to determine the critical moisture threshold below which Azolla cannot survive.

＊岡山大学大学院環境生命自然科学研究科 (Graduate School of Environmental, Life Science, and Technology, Okayama University) キーワード: 10. 環境保全：環境影響評価, 水環境

Results

1. The effects of temperature on Azolla survival and regeneration

The dry weight of Azolla decreased significantly on the first day of incubation, with weights of 0.13 g at 30℃, 0.14 g at 40℃, 0.15 g at 50℃, and 0.49 g at room temperature (Figure 1A). Higher temperatures caused greater biomass reduction. From Day 2 to 14, the weight stabilized at 3-6% of the initial weight. At room temperature, some Azolla collected on Day 1 remained green and regenerated after rehydration, while the brown parts from 30℃, 40℃ and 50℃ died (Figure 1B).

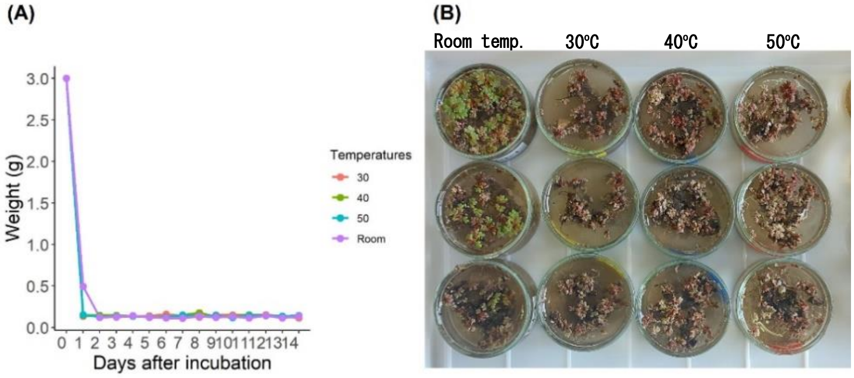

Figure 1. Azolla response after incubation. (A) Dry weight of Azolla incubated at different temperatures over a 14-day period. (B) Visual observation of Azolla floating on the water surface collecting on Day 1 after rehydration.

2. The influence of moisture on Azolla survival and regeneration.

The moisture content of Azolla decreased significantly over time (R^2 = 0.97), indicating a steady loss of moisture when exposed to air, reaching nearly 0% within 48 hours (Figure 2A). A positive correlation was observed between moisture content and survival rate (R^2 = 0.49) (Figure 2B), suggesting that samples with higher moisture at the time of rehydration had greater regeneration capacity. The critical moisture threshold for survival was identified at below 8%, beyond which Azolla could not survive (Figure 2B). However, even at very low moisture levels (8%), some Azolla remained viable, indicating a potential risk of unintentional environmental spread if not completely dried.

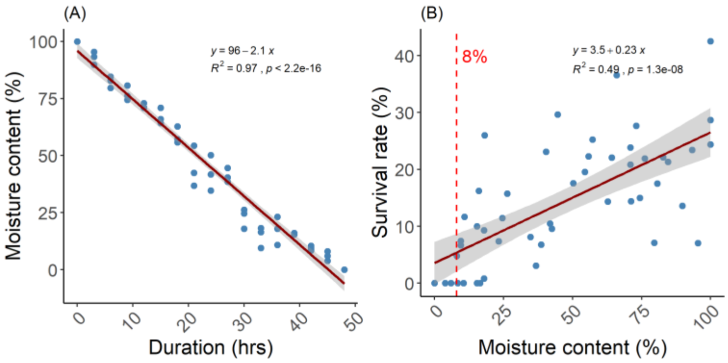

Figure 2. Regression analysis showing (A) the relationship between duration of moisture loss (hours) and moisture content (%), and (B) the relationship between moisture content (%) and survival rate (%) of Azolla after rehydration.

Conclusion

These results demonstrated that Azolla was unable to survive. Incubation at higher temperatures caused greater biomass loss and further reduced survival potential with 24 hrs. The Azolla samples taken on Day 1 remained alive and successfully regenerated after rehydration. In addition, Azolla rapidly loses moisture when exposed to air, reaching nearly 0% within 48 hours. The ability of Azolla to survive was positively correlated with its moisture content at the time of rehydration, with a critical threshold identified at 8%. Further research on spore drought tolerance is needed to clarify survival mechanisms under desiccation.