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Effect of Cytokinin in Tissue Culture in the Ornamental Aquatic Plant – Pearl Grass

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Summary

In recent years, interest in aquariums has increased in Japan, and the demand for aquatic plants used to decorate aquariums is on the rise. Many of them are grown by tissue culture and sold commercially as sterilized, pesticide-free products. The production of these tissue culture transplants is exclusively carried out by venture companies. As the results, there is little research information published about the growth of aquatic plants by tissue culture. We believe that there is still a lot of room for optimization, including the composition of the culture medium, and have presented the results of tissue culture experiments of three aquatic plants. In previous experiments plant growth regulators were not added to the medium due to the risk of tissue culture mutations, but this time, cytokinin was added to promote growth and improve propagation efficiency. Without the addition of cytokinin, shoots developed and elongated from the axillary buds of both species of pearl grass and rooting occurred, but the addition of cytokinin suppressed leaf formation and rooting, especially in thidiazuron. In the cytokinin-added medium where leaf formation was inhibited, green globular masses that are thought to be derived from shoot axillary buds were formed.

INTRODUCTION

In recent years, interest in aquariums has increased in Japan, and the demand for aquatic plants used to decorate aquariums is on the rise. The aquatic plants used are native to Southeast Asia, Central and South America, Australia, etc., and many of them are grown by tissue culture and sold commercially as sterilized, pesticide-free products. The production of these tissue culture transplants is exclusively carried out by venture companies. As the results, there is little research information published about the growth of aquatic plants by tissue culture.

We believe that there is still a lot of room for optimization, including the composition of the culture medium, and have presented the results of tissue culture experiments of three aquatic plants of the Scrophulariaceae family at the 20th IPPS-J Gifu annual meeting (Niki and Amaki, 2014) and the 24th IPPS-J Okinawa annual meeting (Minamiyama et al., 2017). In previous experiments plant growth regulators (PGRs) were not added to the medium due to the risk of tissue culture mutations, but this time, cytokinin was added to promote growth and improve propagation efficiency.

Here, we report the formation of green globular masses that can be used as intermediate propagules in the process of tissue culture of pearl grass, which is a member of the Linderniaceae family, in a medium supplemented with cytokinin.

MATERIALS AND METHODS

New large pearl grass (*Micranthemum umbrosum* (J.F. Gmel.) S.F. Blake) and Cuban pearl grass (*Micranthemum callitricoides* (Griseb.) C. Wright) of the Linderniaceae family, which are commercially available and produced by Aqua Design Amano Co., Ltd. (Niigata, Japan), were purchased and used in this experiment. The culture vessels were flat-bottomed glass test tubes measuring φ40 × 130 mm, and 30 mL of medium was dispensed into each tube. The medium was a 1/2 concentration MS (Murashige and Skoog, 1962) composition with 20 g/L sucrose and 2 g/L gellan gum (Wako Pure Chemical Industries Ltd., Oosaka, Japan) added, the pH was adjusted to 5.8, and the medium was sterilized in an autoclave at 121°C for 15 minutes. The apical part of each shoot (cutting) was prepared to about 1 cm long, and 5 cuttings were cut (inserted) into the one test tube respectively. Those test tubes were plugged with double layer aluminum foil for multiplication of shoots, and the in vitro shoots after subcultures for multiplication were used in subsequent experiment.

The cytokinin used were benzyladenine (BA; Wako Pure Chemical Industries Ltd., Oosaka, Japan) and thidiazuron (TDZ; Wako Pure Chemical Industries Ltd., Oosaka, Japan), and two levels of addition concentration were 0.5 and 1.0 mg/L. All culture conditions, including multiplication by subculture, were 23 ± 1°C, 16 hours of illumination (40 μmol・m^{-2}・s^{-1} PPFD) under cool white-fluorescent lamps (FLR40S・EX-N/M-H, Toshiba Lighting and Technology Co., Ltd., Yokosuka, Japan) / 8 hours of darkness. Two months after the start of culture, the fresh weight of 5 explants per test tube was measured and morphological observation was carried out.

RESULTS AND DISCUSSION

Without the addition of cytokinin, shoots developed and elongated from the axillary buds of both species of pearl grass and rooting occurred, but the addition of cytokinin suppressed leaf formation and rooting, especially in TDZ (**Table 1**, **Figs. 1 and 2**).

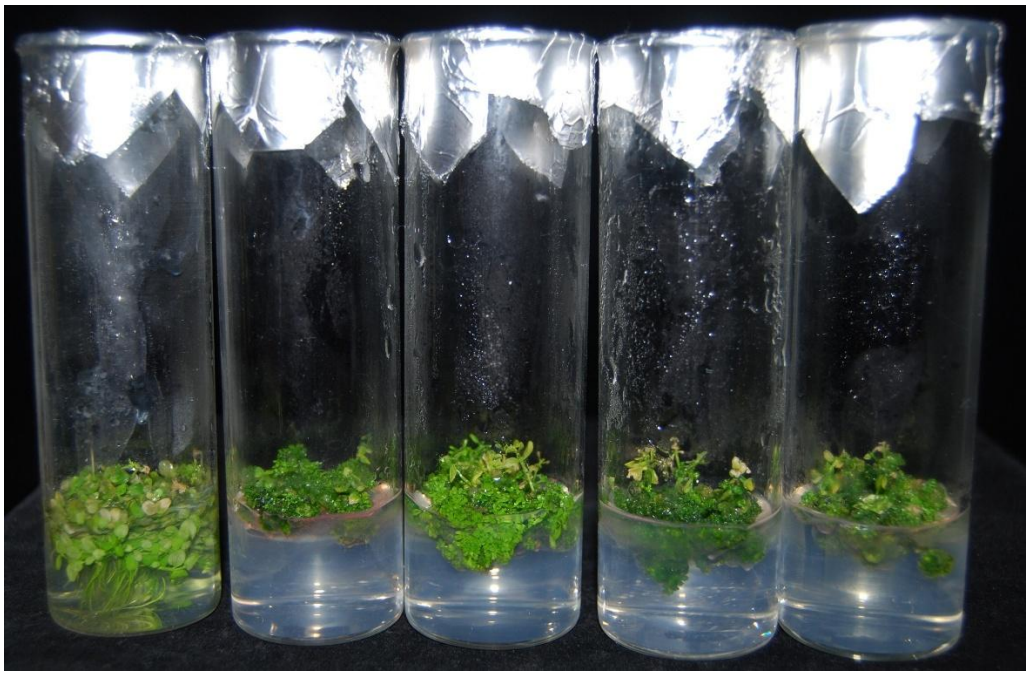

Figure 1. Effect of cytokinin on the growth of new large pearl grass. From left: no addition (0 mg/L), 0.5 mg/L BA, 1.0 mg/L BA, 0.5 mg/L TDZ, 1.0 mg/L TDZ.

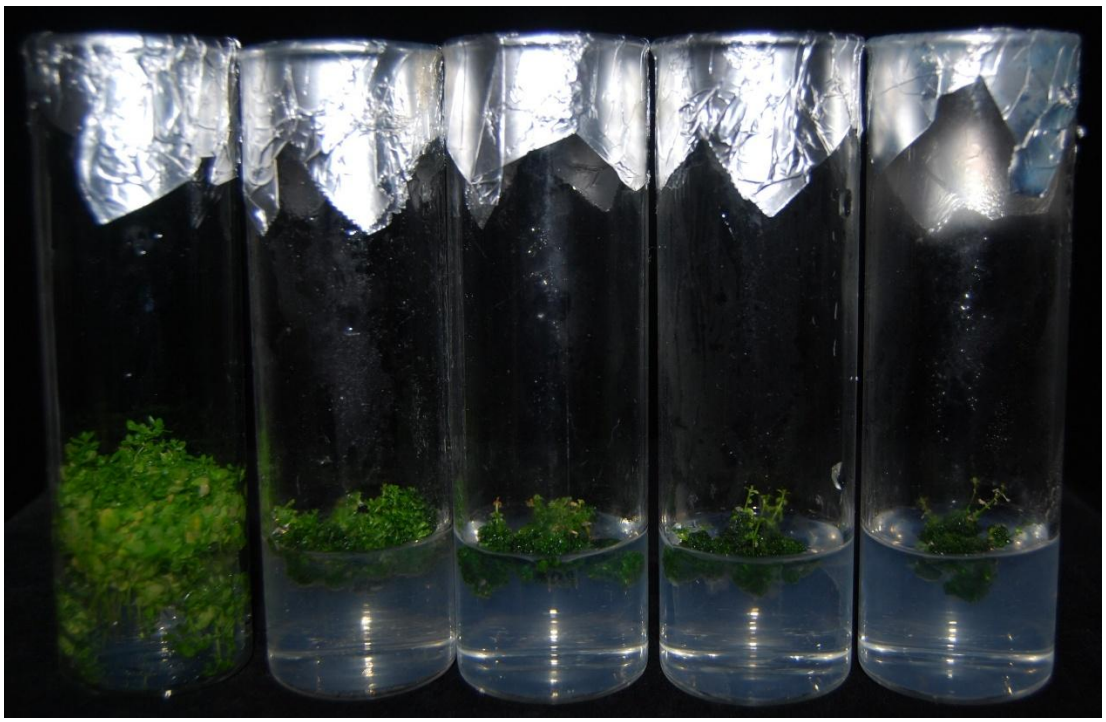

Figure 2. Effect of cytokinin on the growth of Cuban pearl grass. From left: no addition (0 mg/L), 0.5 mg/L BA, 1.0 mg/L BA, 0.5 mg/L TDZ, 1.0 mg/L TDZ.

Table 1. Effect of cytokinin on the growth of pearl grass (n = 5).

Plant name	Cytokinin name	Concentration (mg/L)	Total fresh weight (mg/explant)
New large pearl grass	No addition	0	708 ± 72
	Benzyladenine	0.5	432 ± 56
		1.0	694 ± 46
	Thidiazuron	0.5	384 ± 42
		1.0	382 ± 58
Cuban pearl grass	No addition	0	964 ± 0.0
	Benzyladenine	0.5	424 ± 36
		1.0	288 ± 32
	Thidiazuron	0.5	248 ± 26
		1.0	190 ± 16

In the cytokinin-added medium where leaf formation was inhibited, green globular masses that are thought to be derived from shoot axillary buds were formed (**Fig. 3**). In many general plants, cytokinin promotes shoot development from axillary buds, but it was inhibited in pearl grass. A similar effect was confirmed in ferns (Higuchi et al. 1987; Amaki, 1997), and it has been shown that the tissues formed can be used as intermediate propagules for tissue culture propagation as GGBs (green globular bodies) (Suneetha and Hegde, 2022). In tissue culture transplants of aquatic plants, endophytic contaminating microorganisms that are not detected during the micropropagation process often become apparent after sale. It may be possible to use green globular masses induced by cytokinin as a new micropropagation method, because it is easy to observe the presence of endophytic contaminating microorganisms.

Figure 3. Green globular masses formed from the axillary buds of explants. Cuban pearl grass, medium with 1.0 mg/L TDZ added.

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