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Investigation of the Propagation of Pink-flowered *Haemanthus* by Inflorescence Culture

Ayaka Ohuchi and Hiroaki Ohashi

Graduate School of Agriculture, Ehime University, 3-5-7, Tarumi, Matsuyama, Ehime, 790-8566, Japan

ohashi@agr.ehime-u.ac.jp

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Summary

Adventitious bud formation in H*aemanthus* tissue culture was successful using floret peduncles as the original explant. It was also possible to get organogenesis from callus cultures originally taken from ovary tissue after exposing cultures to low temperatures.

INTRODUCTION

Haemanthus species is bulbous plant of the Amaryllidaceae family and about 60 species have been identified, mainly in South Africa. In Japan, *H. albiflos* Jacq. is the most popular species of the genus, which is widely cultured and distributed. On the other hand, red to pink-flowered *Haemanthus* are expensive and distribution amount is few, because it takes a long time to propagate through division. The more efficient propagation method for this species is needed to meet demand. *Haemanthus* is

bulbous plant, so it has dwarf stem inside of the bulb. The inflorescence of *Haemanthus* plants is enclosed in bract leaves. These are specialized leaves with axillary buds in the leaf axils that become flowers. For example, *Haemanthus* has about sixty florets present inside the bract leaves, blooms with the bract leaves slightly open. *Haemanthus albiflos* propagates by division and seeds.

Flower stalk and inflorescence culture are a beneficial method, in the plants that have dwarf stem which is underground or at the surface of the earth, and where the flower stalk is the only elongating stems. The reasons are as follows: low rate of contamination, high rate of organogenesis and callus formation, cause little damage to mother plant (Ohashi *et*. *al*., 2009, Matsumoto and Ohashi, 2015).

In this study, we aim to create the propagation method of pink-flowered *Haemanthus*. We used the inflorescence because could have the availability of adventitious bud differentiation.

Allium fistulosum L. var. *viviparum* Makino and *A. macrostemon* Bunge, which is Amaryllidaceae family same with *Haemanthus*, set bulbils at inflorescence. Especially, *A. macrostemon* sets florets and bulbils at inflorescence in the same timing. Based on *Allium* plants, inflorescence of *Haemanthus* could make bulbils by inflorescence culture.

MATERIAL AND METHODS

Pink-flowered *Haemanthus*, presumed to be derived from interspecific hybrids, were used in the experiment. Inflorescences were collected in late September 2023, when the inflorescences elongated about 4 cm from the scale (**Fig. 1**).

Figure 1. Inflorescence of pink flowered *Haemanthus* before cutting.

The inflorescences were collected with the flower stalks attached about 2 cm from the bract leaf base (BLB) by using box-cutter, and the cut ends were immediately sealed with melted wax to prevent the entry of bacteria. The bract leaves were cut at a position that would not damage the BLBs, and the florets were cut off perianths etc., leaving the ovaries (**Fig. 2**). Then, the BLBs were sterilized with sodium hypochlorite solution supplemented with agrochemical spreader for 8 minutes for surface sterilization. After that, BLBs are rinsed in sterile distilled water. After that, flower stalks and florets detached from the BLBs, leaving about 2 mm above and below the BLBs.

BLBs which were divided into three pieces, and ovaries of florets were placed on the culture medium, which were MS medium (Murashige and Skoog, 1962) supplemented with 30g・L^{-1} sucrose, 2.5 g・L^{-1} Gellan Gum. Plant growth regulators (PGRs) combination was shown Table 1, which is consisted with 0, 1, 2 mg・L^{-1} 6-Benzylaminopurine (BA), 0, 1, 2 mg・L^{-1} 1-

Naphthaleneacetic acid (NAA). The medium pH adjusted to 5.8 prior to autoclaving at 121℃ for 15 minutes. The culture condition kept under a 16/8h light/dark regimen at 21±2℃.

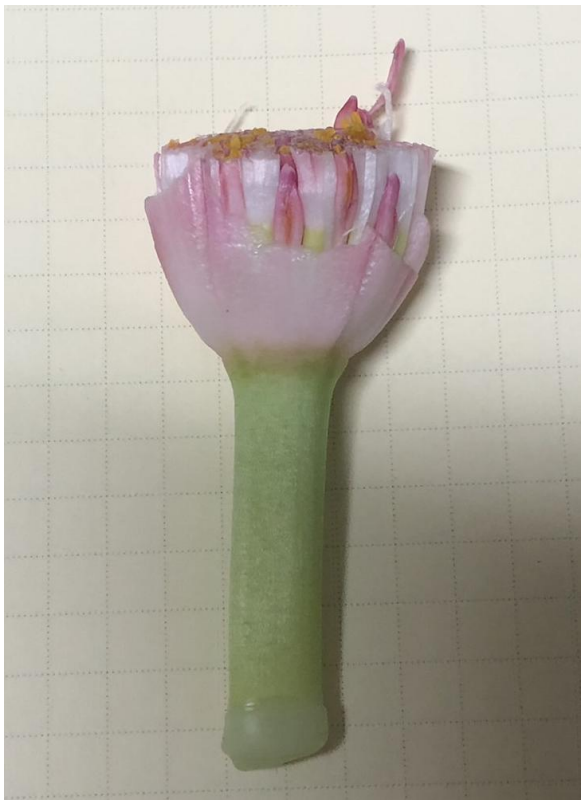

Figure 2. The inflorescence before surface sterilization. It was collected with the flower stalks attached about 2 cm from the bract leaf base, and the cut ends were immediately sealed with melted wax. The bract leaves were cut at a position that would not damage the BLB, and the florets were cut off perianths etc.

BLBs culture was conducted with 6 repetitions in 5 treatments; 3 repetitions in control (**Table 1**), and ovaries culture conducted with 9 repetitions in 5 treatments (**Table 2**).

RESULTS AND DISCUSSION

After placement, survival, contamination, callus formation, and organogenesis recorded periodically. After 56 days, organogenesis observed in all PGRs combination BLBs culture, including the control section (**Table 1**). Organogenesis observed from the base of the cut end of floret peduncles (**Fig. 3**), and although number of organogenesis tended to be higher in the PGRs combination, there was also a higher tendency for the organogenesis to be malformation.

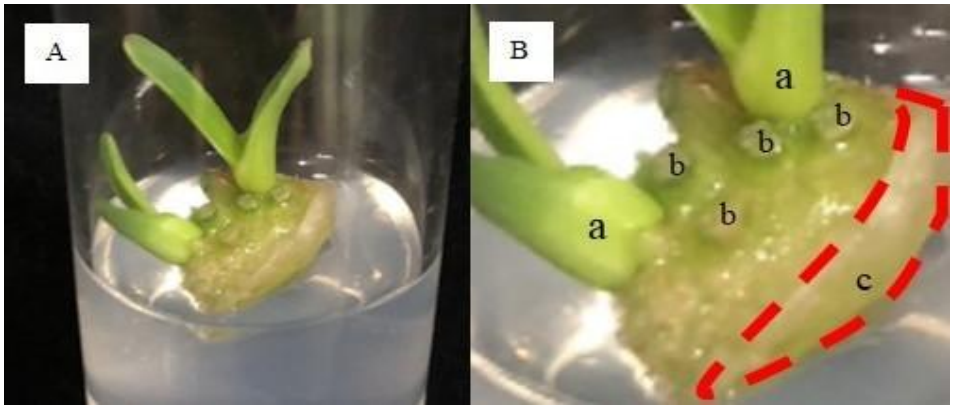

Figure 3. Organogenesis on control on bract leaf base culture. A) Whole explant with adventitious bud formation. B) Enlargement of A. (a) Adventitious buds. (b) The base of the floret peduncles. (c) Cutting surface of bract leaf.

Table 1. Effects of plant growth regulators for organogenesis on bract leaf base explants of pink flowered *Haemanthus* (after 56 days incubation at 21±2℃).

PGRs (mg·L^{-1})		Explants for testing (No.)	Survived explants (No.)	Explants with observed organogenesis (No.)	Rate of organogenesis (%)
NAA	BA				
0	0	3	2	2	100
1	1	6	4	3	75.0
1	2	6	5	3	60.0
2	1	6	4	3	75.0
2	2	6	4	4	100

PGRs: Plant growth regulators (PGRs). NAA: 1-Naphthaleneacetic acid, BA: 6-benzylamino-purine.

In addition, the contamination rate was about 30% in total. It might be because the bract leaves were slightly open when the inflorescences were collected, which increased the contamination rate of the inflorescence interior by some insects and fungi, as well as the fact that the surface fungicide did not sufficiently penetrate to the culture area (BLB) due to the morphological characteristics of the inflorescences.

After 56 days, no organogenesis observed in all PGRs combination ovaries culture, but callus formation observed around cut end of peduncle side (**Table 2**).

Table 2. Effects of plant growth regulators for organogenesis and callus formation on ovary explants of pink flowered *Haemanthus* (after 56 days incubation at 21±2℃).

PGRs (mg·L^{-1})		Explants for testing (No.)	Survived explants (No.)	Explants with observed organogenesis (No.)	Explants with observed callus (No.)	Callus formation (%)
NAA	BA					
0	0	9	5	0	1	20.0
1	1	9	9	0	5	55.6
1	2	9	9	0	4	44.4
2	1	9	9	0	5	55.6
2	2	9	8	0	2	25.0

PGRs: Plant growth regulators (PGRs). NAA: 1-Naphthaleneacetic acid, BA: 6-Benzylamino-purine. Rate of callus formation = (No. of explants observed callus / No. of survived explants) x 100.

Flowering timing of *Haemanthus* sp. is from late September to early October, after that, vegetative growth stage has come, incubation at low temperature would occur organogenesis. After 5 months incubation at 21±2℃, we moved ovaries to growth chamber, which was set 15℃, 20℃ and 25℃. After 230 days incubation, we got organogenesis from ovaries via callus at PGRs combination of 15℃ and 20℃; no organogenesis was observed at 25℃ (**Table 3**).

Table 3. Effects of plant growth regulators and temperature for organogenesis on ovary explants of pink flowered *Haemanthus* (after 230 days incubation).

Incubation temperature	PGRs (mg·L^{-1})		Explants for testing (No.)	Explants observed organogenesis (No.)	Rate of organogenesis (%)
	NAA	BA			
15	0	0	1	0	0
	1	1	2	0	0
	1	2	4	2	50.0
	2	1	3	0	0
	2	2	2	0	0
20	0	0	-	-	-
	1	1	3	2	66.7
	1	2	3	1	33.3
	2	1	3	1	33.3
	2	2	3	0	0
25	0	0	1	0	0
	1	1	3	0	0
	1	2	2	0	0
	2	1	3	0	0
	2	2	2	0	0

PGRs: Plant growth regulators (PGRs). NAA: 1-Naphthaleneacetic acid, BA: 6-Benzylaminopurine. After 5 months incubation at 21±2℃, we moved ovaries to 15℃, 20℃ and 25℃.

In conclusion, we got organogenesis which include adventitious buds from inflorescences of *Haemanthus* sp. BLBs culture at 21±2℃, we got adventitious buds directly from the base of the floret peduncles. The number of organogenesis was

higher at PGRs combination, there was also a higher tendency for the organogenesis to be malformation.

In ovaries culture, it was difficult to get organogenesis at 21±2℃, but by changing to low-temperature culture at 15℃ and 20℃, we got organogenesis via callus from ovaries.

We will study the effect of low temperature on BLBs culture to establish more efficient propagation method of *Haemanthus*. In addition, we will conduct multiplication by using adventitious buds which were obtained from this study.

LITERATURE CITED

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