

Comparative Study of in Vitro and in Vivo Inflorescence of Feathered Amaranth

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Abstract

This study aimed to compare the inflorescence, florets and pollen formed by the plantlets of feathered amaranth [*Celosia argentea* var. *plumosa* (Burvenich) Voss] developed and grown under in vitro conditions with those formed by the plants grown under in vivo conditions. Plantlets were first derived from nodal explants of feathered amaranth cultured on Murashige and Skoog (MS) semi-solid medium under in vitro aseptic conditions, and then the plantlets produced in vitro inflorescences, florets and pollen. In parallel, inflorescences, florets and pollen of plants raised from feathered amaranth seeds under in vivo conditions were obtained for the comparative purpose. The results revealed that florets from both sources were largely similar except the size. The in vitro florets were mainly smaller than those of the in vivo ones. Also in vivo pollen exhibited a higher germination percentage and average pollen tube length than in vitro pollen when cultured on a pollen germination medium.

Keywords: Amaranthaceae, *Celosia argentea* var. *plumosa*, Floret, In vitro flowering, Pollen germination

1 Introduction

The flowers of many plant species are attractive commercially. Globally the floriculture market has been estimated to be worth about US \$60 billion [1]. Flower formation in whole plants grown in the glasshouse or under field conditions (herewith referred to as the in vivo conditions) are under the complex control of many factors [2]. In vitro flowering may be useful to aid gaining a better understanding of regulation of flowering as this phenomenon could occur in many different plants under highly controlled in vitro environmental conditions [3]–[7].

Studies on in vitro flowering are not only of academic interest but also of applied interest [2]. For

example, in vitro flowering could be used to aid plant breeding [8]. Moreover, test-tube plantlets that have formed flowers or inflorescences have been offered as products from the test-tube gift or bouquet industry [7], [9]. Therefore, it would be necessary to investigate if the in vitro flowers could be appropriate for basic and applied purposes compared to the in vivo flowers formed under natural conditions.

Feathered amaranth [*Celosia argentea* var. *plumosa* (Burvenich) Voss] is an annual plant of the Amaranthaceae family with eye-catching feather-like inflorescences which can be in a wide range of colors such as red, purple, orange, pink, salmon, yellow or creamy white [10]. In Thailand, it is available commercially as a popular pot plant, a bedding plant,

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or a cut flower. In vitro feathered amaranth plantlets that have formed flowers would be another addition to the floriculture industry. Therefore, in this research, we firstly initiated in vitro inflorescences of feathered amaranth and then evaluated its floret parts and pollen characteristics with in vivo inflorescences.

2 Materials and Methods

2.1 Plant materials

Feathered amaranth [*Celosia argentea* var. *plumosa* (Burvenich) Voss] seeds (number 5) were purchased from Thai Seed and Agriculture Co., Ltd. Bangkok, Thailand. To obtain the explants for in vitro culture, seeds were soaked in distilled water overnight, subsequently surface-sterilized by immersing for 10 min in 15% (v/v) Clorox (a commercial bleach solution containing 5.25%, w/v, sodium hypochlorite) and two drops of Tween-20 were also added and then rinsed by soaking three times with sterile distilled water (5 min for each rinsing). The surface-sterilized seeds were germinated in glass culture containers ten in each container with 10 ml basal MS medium [11] kept in a controlled growth room under 16 h illumination from white fluorescent lamps at a PPF of $21.78 \mu\text{mol m}^{-2}\text{s}^{-1}$ and 8 h darkness at $25 \pm 2^\circ\text{C}$ for two weeks. Then shoot explants (each one cm long) were excised from the aseptically grown seedlings and transferred to the basal MS medium, two shoots in a container, and stored in the same controlled growth room. Next, nodal explants (one cm long) were excised from the 2-week-old plantlets and placed on the same medium, one nodal explant per container, and maintained in the same controlled growth room for 12 weeks. For plants grown under in vivo conditions, 2-week-old seedlings were transferred from a sowing basket to a pot and in vivo inflorescences were observed after seven weeks.

2.2 Investigations of in vitro and in vivo inflorescences

Heights of in vitro plantlets and in vivo plants and their inflorescences were measured. Morphology of florets and their parts such as the bract, tepal, anther, filament, ovary and style from 5-week-old inflorescence (number of week was counted from the floral bud emerging at week 7 of in vitro plantlets derived from nodal explants and at week 4 after seed had been sown under in vivo

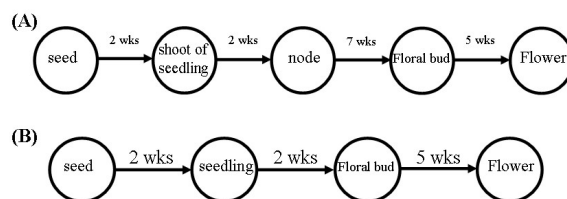


Figure 1: Time courses of the different growth stages leading to flowering in (A) in vitro and (B) in vivo feathered amaranth plants.

conditions (Figure 1) of in vitro plantlets and in vivo plants were investigated under both a stereomicroscope (EMZ-TR, Meiji Techno Co., Ltd.) and a compound light microscope (ML2000, Meiji Techno Co., Ltd.) and photos were taken by using a digital camera (Olympus C-760). For both in vitro and in vivo pollen germination test, the modified Mercado medium [12] comprising 0.1 mM boric acid, 1 mM calcium chloride, and 20% (w/v) sucrose was used. The pollen germinating medium was adjusted to pH 5.7, gelled with 0.9% (w/v) agar, and autoclaved at 121°C and 15 psi for 20 min before it was poured into Petri dishes (80 mm diameter). Anthers in the in vitro and in vivo florets at anthesis from five different inflorescences were collected and placed on the pollen germination medium. Pollen grains from the dehiscent anthers were then left over the surface of the medium and incubated at $25 \pm 2^\circ\text{C}$ in a dark room. Germination of pollen and pollen tube length from both in vitro and in vivo florets were examined under a light microscope (ML2000, Meiji Techno Co., Ltd.) over a period of 24 h. At least three fields in each Petri dish were examined to count pollen grains with or without pollen tubes and photos were taken using a digital camera (Olympus C-760).

2.3 Data analysis

Analysis of differences in the mean plant and inflorescence heights, length of floral parts, pollen morphology and germination, was carried out using independent samples t-test [13] at $P < 0.05$ level.

3 Results and Discussion

In the present research, it was found that the shoots developed from nodal explants of feathered amaranth during in vitro culture was able to initiate inflorescence

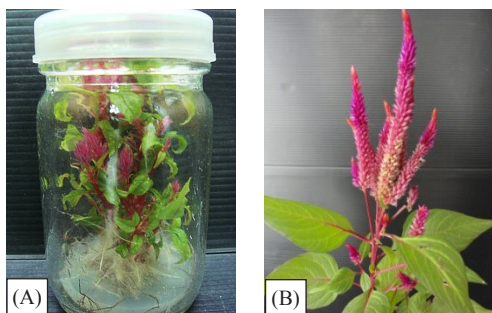


Figure 2: In vitro (A) and in vivo (B) inflorescence on plantlet and plant, respectively, of feathered amaranth.

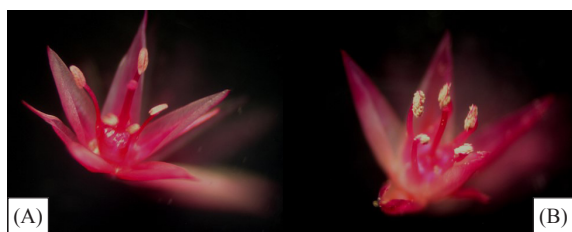


Figure 3: In vitro (A) and in vivo (B) florets of feathered amaranth under a stereomicroscope.

without any plant growth regulators (Figure 2). After seven weeks from the start of nodal explant culture, flower buds were formed in vitro which then developed into inflorescence in five weeks (Figure 3). In preliminary experiments it was observed that more shoots developed from nodal rather than shoot tip explants (data not shown). Moreover, nodal explant was competent for flowering during culture without any plant growth regulator requirement.

From start of nodal explant culture to the development of in vitro inflorescence it took about 12 weeks compared to about nine weeks from sowing seed to formation of in vivo inflorescence. Like under natural conditions, the in vitro flowering process requires the alteration of the vegetative shoot meristem into a floral or an inflorescence meristem which develops all the floral parts of a flower. So far, there have been just few reports comparing in vitro flower or inflorescence with the natural one [4], [14], [15], while the length of in vitro and in vivo vegetative and reproductive stage has never been evaluated before. Under in vitro conditions, once the feathered amaranth shoot grew out from the nodal explants, it took 5 weeks for the meristem of the in vitro shoot to develop into inflorescence in vitro. Seedlings grown in vivo also required five weeks to

transition from shoot meristem to an inflorescence (Figure 1). Thus, the reproductive phase of both in vivo and in vitro feathered amaranth is of similar duration (Figure 1).

It was found that the average height of the in vitro plantlets was about 5–6 times shorter than the in vivo plants. Also, in vivo inflorescence was about 12.7 times taller than the in vitro one (Table 1, Figure 2). When the florets and their parts from 5-week-old feathered amaranth inflorescences of both sources were assessed, no difference was found in their forms, color and numbers (Table 2, Figure 3). However, the bract and tepal lengths, the lengths of the anther, filament, ovary and style of the in vivo florets were greater than the in vitro florets (Table 2). In *Nicotiana tabacum* L. “Samsun,” the anther of the in vitro flowers was significantly smaller than the counterpart of the in vivo flowers. Furthermore, the numbers of anther and pistil were statistically different between in vitro and in vivo flowers [14]. Likewise, in *Gentiana triflora* Pall. var. *axillariflora*, the corolla length and anther number of in vitro flowers were dissimilar to the ex vitro flowers while the percentages of fully developed stigma from both sources were the same [4]. For *Bambusa arundinacea*, lemma, palea, anther and gynoecium length of the in vivo florets were greater than the in vitro florets [15]. The results from these previous reports and the present study indicated that the length and number of floret or flower parts in vitro might or might not be the same as the ex vitro floral parts depending on plant species. In in vitro inflorescence of feathered amaranth, the non-sexual parts of in vitro florets such as the bract and tepal were of a similar length and number compared to the in vivo florets. There was no difference in the number of the sexual parts such as the stamen and carpel between in vivo and in vitro florets but those of the in vivo florets were longer (Table 2).

Table 1: Plant and inflorescence heights of feathered amaranth grown in vitro and in vivo

Sources	Plant height (cm)	Inflorescence height (cm)
In vitro	7.27 ± 3.43a	1.15 ± 0.66a
In vivo	41.61 ± 2.60b	14.57 ± 1.36b

Values are means of 30 replications ± SD. Data marked by different letters in a column are significantly different ($P < 0.05$).

Table 2: Number and length of floral parts from in vitro and in vivo florets of feathered amaranth

Floral parts	Sources			
	In vitro		In vivo	
	number	length (mm)	number	length (mm)
Bract	2.00	4.61 ± 0.53a	2.00	4.67 ± 0.34a
Tepal	5.00	2.85 ± 0.23a	5.00	2.94 ± 0.53a
Anther	5.00	0.70 ± 0.08a	5.00	0.77 ± 0.07b
Filament	5.00	1.48 ± 0.24a	5.00	1.70 ± 0.29b
Ovary	1.00	1.69 ± 0.25a	1.00	1.90 ± 0.21b
Style	1.00	2.44 ± 0.42a	1.00	2.85 ± 0.21b

Values are means of 20 replications ± SD. Data marked by the same letter in a row are not significantly different ($P < 0.05$).

The morphology of in vitro and in vivo feathered amaranth pollen looked the same. Their pollen grains were apolar, spheroidal and pantoporate (Figure 4) which resembled the spherical shaped *Amaranthus* species pollen with a polypantoporate, or golf ball-like, aperture arrangement [16]. The diameter of pollen from both in vivo and in vitro feathered amaranth florets was also similar (Table 3). Furthermore, when pollen germination was evaluated, in vitro and in vivo pollen exhibited differences in pollen tube lengths and germination percentages (Table 3, Figure 4). This finding is similar to the previous report on *G. triflora* showing that the germination percentage of in vivo pollen was three times higher than the in vitro pollen [4].

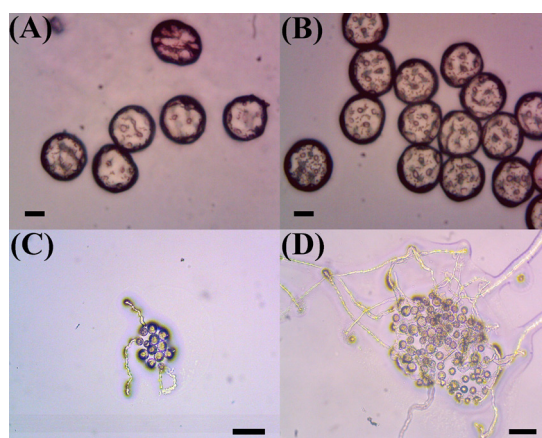
Table 3: Pollen morphology and germination from in vitro and in vivo florets of feathered amaranth, PD = pollen diameter; PL = pollen tube length, PG = pollen germination

Parameter	Sources	
	In vitro	In vivo
PD (mm) ¹	0.0231 ± 0.00165a	0.0232 ± 0.00171a
PL (mm) ¹	0.20 ± 0.08a	0.65 ± 0.20b
PG (%) ²	20.7 ± 12.9a	30.8 ± 14.2b

¹Values are means of 50 replications ± SD.

²Values are means of 25 replications ± SD. Data marked by the same letter in a row are not significantly different ($P < 0.05$).

In conclusion, there were some slight differences in the morphology of in vitro and in vivo florets or inflorescence of feathered amaranth as well as in germinative physiology of in vitro and in vivo pollen. However, the miniature and beautiful in vitro inflorescence of feathered amaranth could be considered to be included in the test tube bouquet industry for sale as a souvenir or gift.

**Figure 4:** Pollen morphology and germination from in vitro (A and C) and in vivo (B and D) florets of feathered amaranth. (A and C scale bar = 0.01 mm; B and D scale bar = 0.1 mm)

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