SHORT REPORT

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Comparison of in vitro and in vivo inflorescence of common cockscomb (*Celosia argentea* var. *cristata*)

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ABSTRACT: Inflorescence of common cockscomb (*Celosia argentea* var. *cristata*) was induced in vitro from plantlets regenerated from nodal explants cultured for 5 weeks on semi-solid basal MS medium supplemented with or without 0.5 mg/l benzyladenine under aseptic and light conditions. In vitro inflorescence of 12-week-old plantlets was compared with that formed under natural conditions. Florets from both sources had the same number of floral parts and exhibited similar characteristics (floret shape and percentage of pollen germination). However, the florets formed in vitro were obviously smaller than those formed under natural conditions.

KEYWORDS: in vitro flowering, pollen germination, pollen morphology, sexual reproduction

INTRODUCTION

Common cockscomb (*Celosia argentea* var. *cristata*) is a herbaceous plant and has been classified as a member of the family Amaranthaceae. This annual plant has a distinctive characteristic inflorescence which looks like the crest of a rooster or convoluted brain after it has developed fully[1]. Owing to the dazzling and attractive inflorescence colour, it is a popular ornamental plant in all regions of Thailand. Additionally, this plant could produce some useful chemicals such as antiviral proteins, betalains, and anthocyanin[2–4] that can be applied in many beneficial ways.

In vitro flowering has been reported for different kinds of flower morphology: solitary in *Capsicum annuum*[5] and rose[6], or inflorescence in *Amaranthus*[7] and bamboo[8], or even complex inflorescence as in Chicory[9] and *Pentanema indicum*[10]. To explore the potential practical applications of in vitro flowering, for example, for breeding valuable plants under controlled, high-health conditions, an important prerequisite is to investigate if the flowers formed under in vitro conditions are basically free of gross aberrations when compared to those formed on plants grown in soil. Henceforth the former will be referred to as in vitro flowers and the latter as in vivo flowers. In vitro and in vivo flowers have previously been studied and compared to only a limited extent[11–13]. Furthermore, inflorescence obtained from both sources of plant in the family Amaranthaceae have never been evaluated. Therefore, in this study, in vitro and in vivo inflorescence of common cockscomb were investigated and compared for the first time to ascertain if the in vitro inflorescence of common cockscomb would retain the same dazzling features and potential for pollination as the in vivo ones.

MATERIALS AND METHODS

Seeds of common cockscomb, *Celosia argentea* var. *cristata*, were purchased from TSA Ltd., Bangkok. Surface-sterilization of seeds began with immersing the seeds in distilled water for 3 h and then soaking for 10 min in 15% (v/v) Clorox (a commercial bleach solution containing 5.25%, w/w, sodium hypochlorite as available chlorine) to which 2–3 drops of Tween-20 were added. After this, they were rinsed 3 times with sterile distilled water (5 min for each rinse) before they were placed on basal MS medium[14] and kept in a growth room for 3–4 weeks under 16 h illumination from white fluorescent lamps (44.57 μmol m^{-2} s^{-1}) and 8 h darkness at 25 ± 2 °C. To increase the number of nodes, shoot cuttings (1 cm long) were excised from the axenically grown seedlings and placed on plant growth regulator-free MS medium. After the shoots had grown for 3–4 weeks, 3 to 4 nodal explants (each 0.8 cm long) were excised and transferred to MS medium supplemented with 0 or 0.5 mg/l benzylade-

nine (BA).

After inflorescence had developed on the 12-week-old in vitro plantlets, they were investigated and compared with those from pot plants obtained from a local market in Bangkok at the same age (lower part of ex vitro inflorescence had started producing seeds). Floret morphology was examined under both a stereomicroscope (EMZ-TR, Meiji Techno Co., Ltd.) and a compound microscope (ML2000, Meiji Techno Co., Ltd.) and photographs were taken using a digital camera (Olympus C-760).

The modified medium of Mercado et al[15] consisting of 0.1 mM boric acid, 1 mM calcium chloride, and 20% (w/v) sucrose was used for both in vitro and in vivo pollen germination tests. This medium was adjusted to pH 5.7, gelled with 0.8% (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 inflorescence 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 ± 2 °C in a dark room. Germination of pollen from both in vitro and in vivo florets was examined under a light microscope (ML2000, Meiji Techno Co., Ltd.) over a period of 24 h. At least 3 fields in each Petri dish were examined to count pollen grains with or without pollen tubes and pictures were taken using a digital camera (Olympus C-760).

RESULTS AND DISCUSSION

In vitro flowering in common cockscomb (*Celosia argentea* var. *cristata*) was first reported by Yamada et al[16]. This was shown in various cultures including seedlings (on MS basal medium), shoot apices (on 1/2 MS basal medium), nodal segments (on 1/2 MS basal medium), and leaf segments (on MS medium supplemented with 3 mg/l kinetin and 0.3 mg/l IAA). In the present experiments, it was found that induction of common cockscomb's inflorescence in vitro from plantlets regenerating from nodal explants occurred on MS medium with or without 0.5 mg/l BA. This result confirmed that external cytokinin may not be necessary for in vitro inflorescence initiation in common cockscomb. The in vitro and in vivo inflorescence were of the same shape which was spicate with a crested terminal. Furthermore, the present study revealed that inflorescence of common cockscomb formed under in vitro and in vivo conditions were largely indistinguishable except for the floret size (Table 1 and Fig. 1). Adding BA to the medium had no effect on the formation and the characteristics of inflorescence (Table 1 and Fig. 2). However, callus was formed on the cut end of the nodal explants placed on MS medium supplemented with 0.5 mg/l BA and the number of roots formed on this medium was also obviously less than that formed on plant growth regulator free-medium (Fig. 3).

Both in vitro and in vivo florets have the same number of bracts, tepals, stamens, ovaries, and styles (2, 5, 5, 1, and 1, respectively). Various parts of the in vitro florets were generally found to be smaller than those of in vivo florets, except the ovary length (Table 2). This suggests that development of in vitro florets proceeded in the same way as under natural

Table 1 Comparison of size between in vivo and in vitro inflorescence of common cockscomb (*Celosia argentea* var. *cristata*).

Sources	Inflorescence size (cm)	
	width	length
In vivo	1.51 ± 0.14^{a}	3.87 ± 0.34^{a}
In vitro MS	1.00 ± 0.11^{b}	1.22 ± 0.25^{b}
In vitro MS + BA	0.96 ± 0.11^{b}	1.19 ± 0.20^{b}

Values are means of 10 replications $\pm$ SD. Data marked by same letter in a column are not significantly different (ANOVA, $P < 0.05$).

Fig. 1 Appearance of in vivo (left), in vitro MS (middle) and in vitro MS + BA (right) inflorescence on plant and plantlets of common cockscomb.

Fig. 2 Close-up view of in vivo (left), in vitro MS (middle) and in vitro MS + BA (right) inflorescence on plant and plantlets of common cockscomb.

Fig. 3 Number of roots formed on in vitro MS plantlet (left) was higher than in vitro MS + BA plantlet (right) because of the formation of callus (arrow) on the cut end of nodal segment.

Table 2 Comparison of the lengths (in mm) of floral parts for in vivo and in vitro florets.

Floral part	In vivo	In vitro MS	In vitro MS + BA
Bract	3.14 ± 0.45^{a}	2.14 ± 0.34^{b}	2.21 ± 0.48^{b}
Tepal	4.63 ± 0.45^{a}	3.72 ± 0.32^{b}	3.65 ± 0.27^{b}
Anther	1.05 ± 0.16^{a}	0.92 ± 0.11^{b}	0.89 ± 0.19^{b}
Filament	1.91 ± 0.42^{a}	1.61 ± 0.20^{b}	1.59 ± 0.35^{b}
Ovary	1.07 ± 0.23^{a}	0.99 ± 0.16^{a}	0.97 ± 0.07^{a}
Style	3.22 ± 0.38^{a}	2.33 ± 0.42^{b}	2.10 ± 0.31^{b}

Values are means of 20 replications $\pm$ SD. Data marked by same letter in a column are not significantly different (ANOVA, $P < 0.05$).

conditions. This is consistent with the previous in vitro flowering studies of Nadgauda et al [12] on bamboo and Zhang and Leung [13] on gentian.

It is important to investigate in greater details the pollen produced by flowers formed under in vitro conditions [17, 18]. In the present investigation, we initially germinated in vitro and in vivo pollen of common cockscomb on the medium of Mercado et al [15] as we did in *Capsicum annuum* [5]. However, very little of the common cockscomb pollen germinated in this medium (data not shown). The germination medium was then modified by increasing sucrose concentration from 5% (w/v) to 20% (w/v). As a consequence, a higher percentage of pollen germination resulted. This reveals that pollen germination of common cockscomb requires a higher sucrose concentration than *Capsicum annuum*.

When both in vitro and in vivo pollen were compared under a light microscope, they exhibited similar shape, size and germination percentage (Table 3 and Fig. 4). Pollen grains from both sources are spherical

Table 3 Comparison of the pollen morphology and germination from in vivo and in vitro florets from 12-week-old plants and plantlets of common cockscomb. PL = pollen length in equatorial view; PG = pollen germination.

Sources	PL (mm)	PG (%)
In vivo	0.0226 ± 0.0017^{a}	28.0 ± 7.3^{b}
In vitro MS	0.0219 ± 0.0015^{a}	26.9 ± 8.5^{b}
In vitro MS + BA	0.0217 ± 0.0019^{a}	24.8 ± 6.2^{b}

Values are means of 15 replications $\pm$ SD. Data marked by same letter in a column are not significantly different (ANOVA, $P < 0.05$).

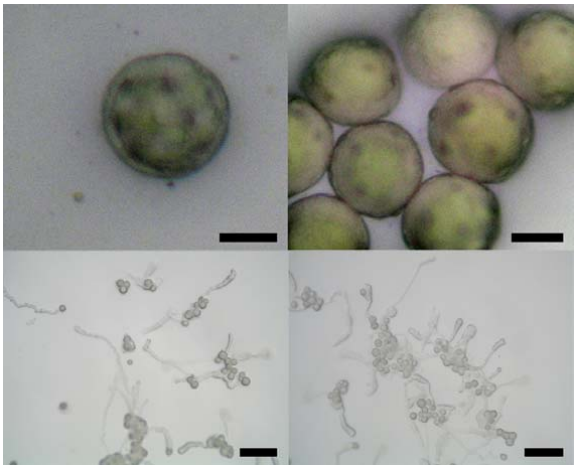

Fig. 4 Light microscope observations of morphology and germination of pollen from (*left*) in vivo and (*right*) in vitro florets of common cockscomb. Scale = 0.01 mm and 0.1 mm for upper and lower figures, respectively.

(diameter about 0.02 mm) and periporate (i.e., have numerous pores). In both cases, the pollen started to germinate 2 h after the start of the germination test and the germination rate was about 25%. However, seed formation has not been observed under the present culture conditions. Since common cockscomb is a wind and insect pollinated plant, in a standstill aseptic culture in vitro pollination was unlikely to occur without any experimental manipulation, as in the present study. Thus, it would be of interest to investigate further if artificial pollination could assist in vitro seed set of common cockscomb.

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