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Multiplication New Shoots from Embryo Culture on *Globba* spp.

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ABSTRACT

An in vitro propagation system was developed for comparison of six varieties of young *globba* embryos cultured on MS medium supplemented with various concentrations of BA. The result showed non significance Duncan's multiple range tests. The G-75, G-52, G-08 and commercial white varieties, cultured on MS medium supplemented with 5 mg/l BA, gave the highest average number of new shoots.

1. Introduction

Globba species belongs to the family Zingiberaceae and distributed throughout tropical (and parts of subtropical) Asia, ranging from India to southern China, south and east to the Philippines and New Guinea (Smith, 1988 and Boyce, 2006), with the center of distribution in monsoonal Southeast Asia, especially Thailand and Myanmar. Virtually all species distributed north of the Isthmus of Kra (most species of *Globba* and all species of the remaining genera). The other three genera of Globbeae are more restricted in distribution and fall completely within the range of *Globba* itself. Gagnepainia is found primarily in Thailand,

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Laos, Vietnam, and Cambodia, while Hemiorchis and Mantisia are distributed in northeastern India, Myanmar, and Bangladesh. (Seliger and Mc Elroy, 1995).

Flowers in the Globbeae, like all Zingiberaceae, are among the most highly derived in angiosperms (Endress, 1994; Kress et al., 2012). This species produced fantastic pendulous bracts with delicate white, yellow and orange inflorescences on the top of the leafy shoot. Calyces in the Globbeae are highly reduced, with petals replacing most of their protective function. Standard petal function (i.e., pollinator attraction and mechanical assistance to pollination) has been co-opted by elaborate staminodes that have replaced four of the six stamens that were fertile in ancestral species of Zingiberales (the fifth stamen is aborted in the Zingiberaceae and the sixth remains fertile; Kirchoff, 1988). *Globba* flowers are distinctive in having a relatively small staminodal labellum and a greatly elongated, arched stamen that is as long or longer than the floral tube and staminodes. However, the hallmark of most (90%) *Globba* species are the small linear to triangular appendages along the sides of the anther. The colorful bracts and flowers seen in many species are useful taxonomically and have attracted horticultural interest, especially for *G. winitii* C. H. Wright. Most, if not all, species of *Globba* can reproduce through the production of asexual vegetative bulbils in the inflorescence, a rare occurrence in the rest of the family (Larsen *et al*., 1998).

Also, trimming the seed coat to break dormancy followed by micropropagation can yield a large number of plantlets within a short period (Chanchula *et al*., 2013).

The objectives of this research were to find suitable methods for micropropagation young *Globba winitii*. During the embryos of seed, embryos were often cut and destruction from equipment. Culturing them to increase the percentage of germination and obtain rapid shoot emergence in a short period, and to find the most suitable concentration of BA for increasing the number of new shoots in 6 varieties of globba by using embryo culture.

2. Materials and Methods

For this experiment, selecting the best trimmed of seed for embryo cultures 6 varieties of *globb*a (Khao Burma was *G. magnifica,* G-75 was *G. winitii* “Rubby Queen”, G-52 was *G. schumberkii* “Burmese Dancing Girl”, G-03 was *G. winitii* “Purest Angel”, G-08 was *G. winitii* “Pristina pink” and Commercial white was *G. winitii* “White Dragon”) cultured on

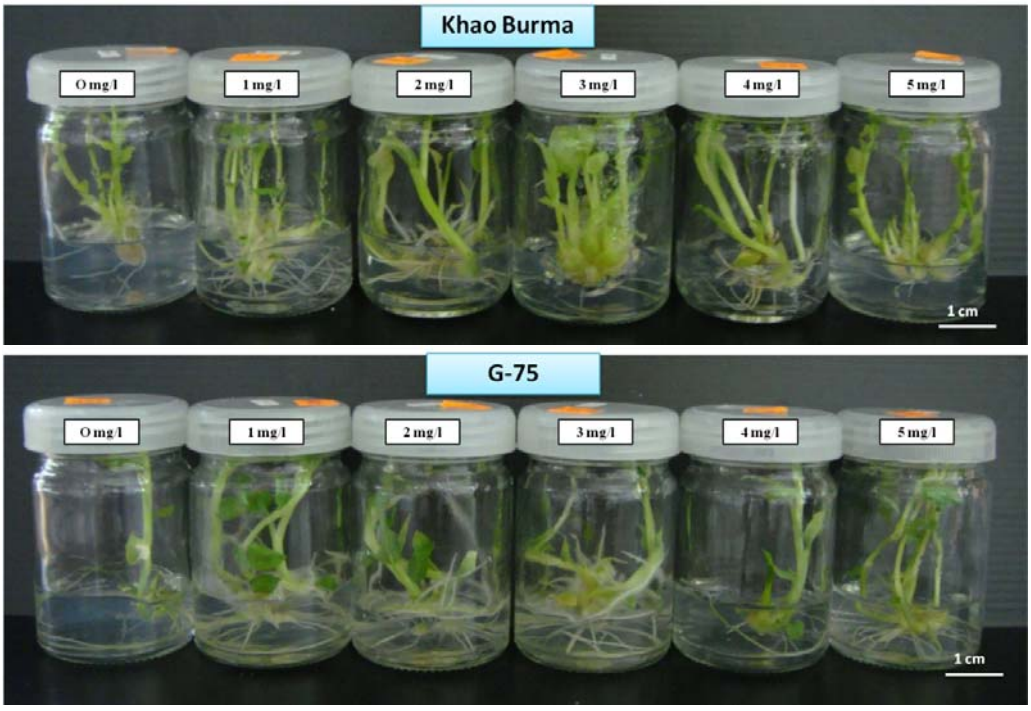
Murashige and Skoog (MS,1962) medium. After all seeds germinated, the embryos were transferred to MS medium supplemented with various concentrations of BA (0, 1, 2, 3, 4 and 5 mg/l) to stimulate new shoot formation and and subcultured at 2 weeks intervals for further growth to the same medium for total 4 times. Data were collected on the number of plantlets.

3. Statistical analysis

Experiments were set up in Completely Randomized Design (CRD) with 6 treatments; each treatment consisted of 25 replicates for this experiment. The test of statistical significance was done by applying Duncan's Multiple Range Test (DMRT) at 5% confidence level using SAS statistical software, Release 6.03 (SAS Institute Inc., Cary, NC).

4. Results and Discussion

After cultured embryos of 6 varieties of Globba spp. on MS medium supplemented with various concentration of BA (0, 1, 2, 3, 4 and 5 mg/l) for 6 weeks. The result is discussed below.



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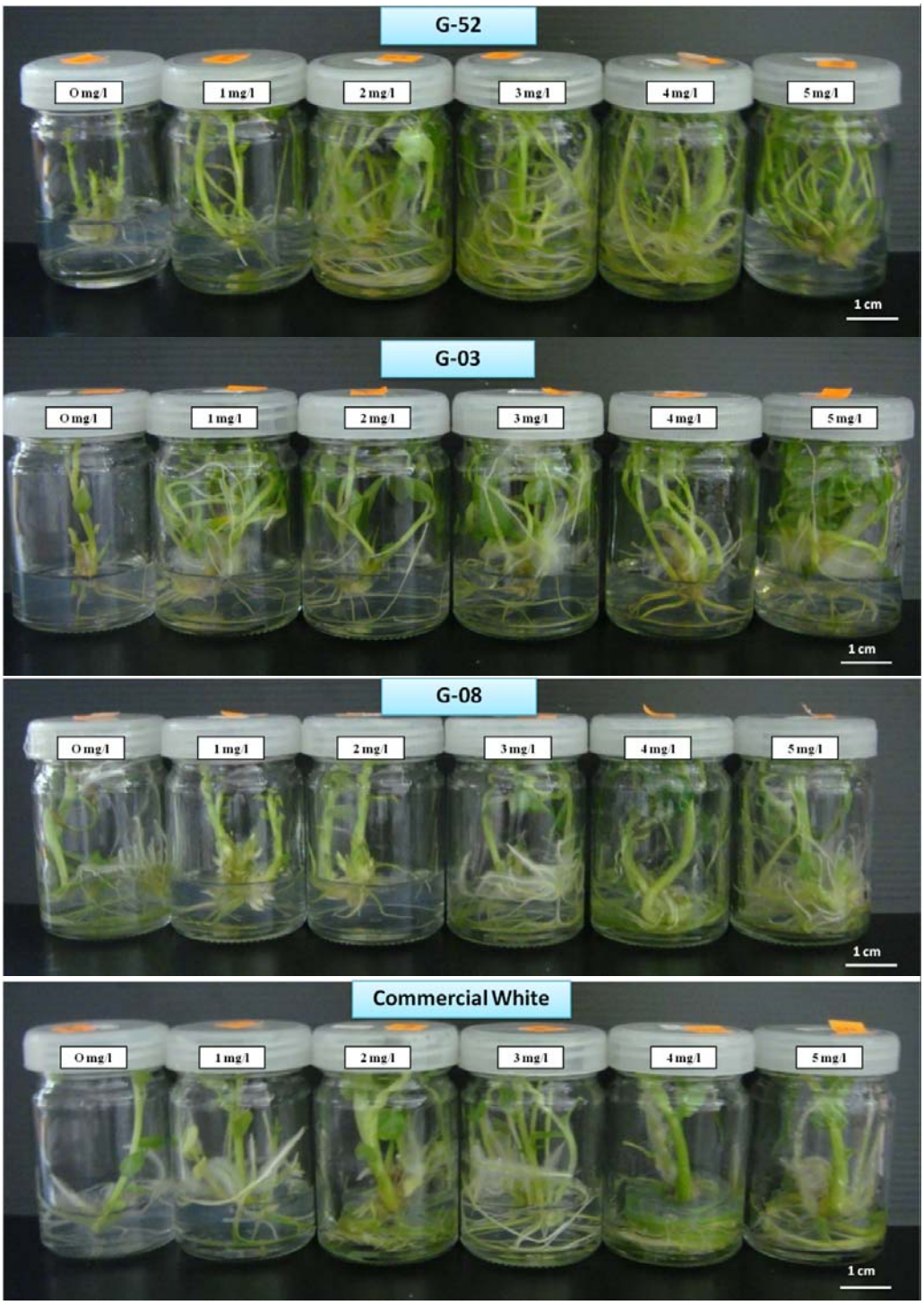

Figure 1: Number of new shoots from each varieties of *Globba* sp. cultured on MS medium containing different concentrations (0, 1, 2, 3, 4, 5 mg/l) of BA after 6 weeks of culture.

4.1 Shoot Induction

The number of new shoots tips emerging from embryos of 6 varieties of globba after transfer to MS medium supplemented with different concentrations of BA (0–5 mg/l) for 60 days are shown in Table 1. The number of new shoots formed from direct somatic embryo in

each concentration of BA were not significantly different. However, in this experiment, different varieties of *globba* responded differently to different concentrations of BA. For instance, Khao Burma cultured on MS medium supplemented with 2 mg/l BA gave the highest number of new shoots (Figure 1A). This result was different from the other varieties. *Globba* (G) varieties number 08, 52, 75 and commercial white which gave a higher number of new shoots when cultured on MS medium supplemented with 5 mg/l BA (Figure 1B, 1C, 1D). In contrast, young shoots of variety G-03 responded best to 1 mg/l BA and gave the highest number of new shoots (4.00 shoots) on that medium (Figure 1E). This result is compatible with the findings of Jala (2011), who reported that *Globba winitii* (white bract) gave the highest number of new shoots when cultured on MS medium supplemented with 2 mg/l BA, and another report by Jala (2012), in which the highest number of new shoots of *Nepenthes mirabilis* was obtained when the plants were cultured on MS medium supplemented with 2 mg/l BA.

Table 1: Number of new shoots from young embryos which were cultured on MS medium containing different concentrations of BA after 8 weeks of culture.

Globba varieties	Number of new shoots at different concentration of BA (mg/l) ^{NS}					
	0	1.00	2.00	3.00	4.00	5.00
Khao Burma	1.00±00	3.00±1.00	4.33±3.21	3.66±1.15	3.66±2.08	4.00±1.00
G-75	2.33±0.57	1.66±0.57	2.33±0.57	2.33±0.57	3.33±0.57	8.66±2.08
G-52	1.33±0.57	3.00±1.00	1.66±0.57	2.33±1.00	2.00±1.00	5.33±1.15
G-08	1.66±0.57	1.33±0.57	4.33±1.5	2.33±0.57	1.33±0.57	5.33±0.57
G-03	1.66±0.57	4.00±1.11	2.00±0.00	1.00±0.00	1.33±0.57	1.66±0.57
Commercial white	1.66±0.57	3.00±0.00	2.00±1.00	3.00±1.00	3.00±0.00	5.33±0.57

(Average mean ± SD)
NS – non significant different among treatments.
Descriptive Statistic Significance level 0.05

5. Discussion

For induction of multiple shoots via direct organogenesis, induction of multiple shoots through shoot-tip culture was initiated on MS medium supplemented with different concentration BA. Thus, growth of shoot tips and subsequent multiplication could not be achieved in medium without PGR as reported earlier (Rout et al., 2000). All treatments which incorporated with BA were able to induce multiple shoots and spontaneous root. As Kho *et al*. (2010) reported in vitro propagation of *Globba brachyanthera* by culturing on Gamborg B5 medium supplemented with 3.0 mg/L BAP and get the highest multiplication rate of 6.6

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shoots per explants. Jala (2011) had done with Curcuma longa and used 2mg/l BA gave 2.6 shoots per explants. As Shukla et al.(2007) had done with *Curcuma angustifolia* Rozbi which used 3 mg/l BAP could produced 6.9 shoots per explants within 6 weeks. In *Zingiber officinale* Rosc. which related family to turmeric, was reported by Balachandran *et al* (1990). All treatments which incorporated with PGR were able to induce multiple shoots and spontaneous root has been reported earlier for a few species of Zingiberaceae (Balachandran *et al*., 1990; Borthakur *et al*., 1992; Kuruvinashetty *et al*., 1982).

6. Conclusion

Young embryos could be induced to germinate by trimming the young seeds. However, there is no significant difference on mean number of shoots induced from *globba* varieties cultured on MS medium supplemented with various BA concentrations. Nevertheless, Khao Burma cultured on MS medium incorporated with 2 mg/l BA gave the highest average multiplication rate of new shoots at 4.33 shoots, while varieties G-75, G-52, G-08 and commercial white cultured on MS medium supplemented with 5 mg/l BA gave the highest average number of new shoots at 8.66, 5.33, 5.33, and 5.33 shoots, respectively.

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