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Break Dormancy by Trimming Immature *Globba* spp.

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ABSTRACT

Young *Globba winitii* seeds at 20 days after pollination were collected and trimmed at different parts of their seed coat, then cultured on MS medium supplemented with 10 mg/l BA, 1.0 mg/l NAA, 10 mg/l GA_3 and 30 g/l sucrose. The results showed that the trimming method could break dormancy, and young embryos germinated in the first week. Seeds trimmed down to a naked embryo had the highest germination rate, germination index and speed of emergence, which were 98.03%, 22% and 100%, respectively.

1. Introduction

The genus Globba (hundred species) is one of the largest genera in the primarily tropical Zingiberaceae. Globba along with the small genera Gagnepainia, Hemiorchis, and Mantisia comprise the Globbeae, one of the two tribes of subfamily Zingiberoideae (William *et al*, 2004). Globba species are distributed throughout tropical (and parts of subtropical) Asia, ranging from India to southern China, south and east to the Philippines and New Guinea, 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) enter dormancy from approximately November through

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April, while most species south of that point remain evergreen throughout the year. 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, Laos, Vietnam, and Cambodia. (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*., 2002). 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 (Williams et al,1999). 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). In some species (e.g., *G. marantina* L. and *G. bulbifera* Roxb.) seeds are rarely produced and plants produce bulbils as their primary means of reproduction. After the flower is pollinated and fertilized, a hard-shelled seed develops, which remains dormant until the next rainy season. Because, Suberin and pectin compounds give the seed shell its toughness. Water and air cannot pass through to the inside, so the seed does not sprout readily (Seliger and Mc Elroy, 1995). Multiplication New Shoots from Embryo Culture on Globba spp. has been reported by Jala *et al*. (2013).

Mature *globba* seeds remain dormant for a long time and have a low germination rate, which is an obstacle to commercial production. Presently, some new hybrid varieties have been created by crossing between different genera. However, these hybrid varieties are even more difficult to propagate due to problems of low germination, sterility or seed abortion. Plant tissue culture is a promising approach to overcome these difficulties. For instance, an embryo rescue technique, in which young embryos are cultured on synthetic media, is one method to increase the number of plantlets. Also, trimming the seed coat to break dormancy followed by micropropagation can yield a large number of plantlets within a short period.

The objectives of this research were to find suitable methods for trimming young *Globba winitii* seeds. During the embryos of seed embryos were often cut and destruction from equipment. Our goal is to culture them to increase the percentage of germination and obtain rapid shoot emergence in a short period.

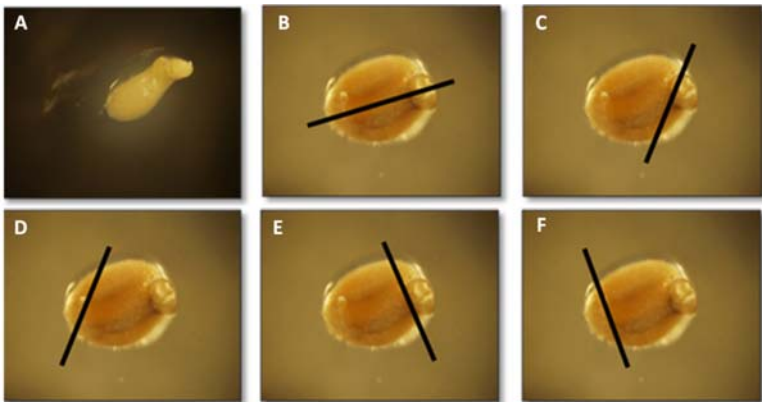

Figure 1: The position of *Globba winitii* (commercial white) seed, treated by trimming at different parts
(A) Naked embryo (B) Trimmed at the middle seed
(C) Trimmed one side of the micropyle (D) Trimmed one side at the base across micropyle
(E) Trimmed at the micropyle side (F) Trimmed at the end across micropyle

2. Materials and Methods

Young *Globba winitii* seeds at 20 days after pollination were collected and cleaned with liquid detergent, washed under running tap water for 15 min, soaked in 70% alcohol for 1 min and sterilized in 20% Clorox for 20 min, followed by 10% Clorox for 10 min and finally soaked with sterilized distilled water 3 times, 1 min each time. The seeds were randomly divided into 6 treatment groups that were trimmed at different parts of the seed: trimmed down to a naked embryo, trimmed at the end of the micropyle, trimmed on one side of the micropyle, trimmed at the base across the micropyle, trimmed on one side at the base and across the micropyle and not trimmed (control) (6 treatments as shown in Figure 1). All seeds were cultured on MS medium supplemented with 10 mg/l BA, 1 mg/l NAA, 10 mg/l GA_3 and 30 g/l sucrose. The medium was solidified with 0.8% agar after adjusting the pH to 5.6 and sterilized by autoclaving at 121° C (1.06 Kg^{-1} m^{-2}) for 20 min. The cultures were maintained at 25 ± 2° C under a 16-h photoperiod with illumination provided by cool fluorescent lamps at an intensity of 60 $\mu molm^{-2}$ sec^{-1} (TLD 36 w/853350 lm Phillips Thailand). Cultured seeds were subcultured into the same medium every 2 weeks to induce growth. After all seeds germinated, the embryos were transferred to MS medium supplemented with 2 mg/l BA for growing.

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3. Statistical Analysis

Experiment was set up in Completely Randomized Design (CRD) with 6 treatments; each treatment consisted of 20 replicates for the 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 trimming young *Globba winitii* seeds at various parts of the seed and culturing them on MS medium supplemented with 10 mg/l BA, 1 mg/l NAA, 10 mg/l GA_3 and 30 g/l sucrose, (They affect cell elongation by altering cell wall plasticity. They stimulate cambium, a subtype of meristem cells and affecting enzyme production that mobilizes food production used for growth of new cells in aleurone layer) the percentage of germination observed in trimmed seeds was significantly different from the control in the first and second week, as shown in Table 1.

Table 1: Germination Percentage of young *Globba winitii* seed trimmed at different part of seeds after culturing for two and three weeks. (Mean ±SD)

Method for trimmed	Percentage of germination *			GI*	SE (%)*
	1st week	2nd week	3rd week		
Naked embryo (control)	98.03±3.39 c	98.03 ± 3.39 d	98.03 ± 3.39 d	22.06 c	100 c
No trimmed (intact seed coat)	00.00 ±0.00 a	0.00±3.39 a	00.00 ±0.00 a	0 a	0 a
Trimmed at the micropyle side	78.33±3.37 c	96.66 ± 5.77 d	96.66 ± 5.77d	18.094c	83.56c
Trimmed at the base across the micropyle	72.03±1.38c	82.61 ±8.20 d	84.62 ±8.20 d	16.191c	74.518b
Trimmed one side of the micropyle	34.90 ±1.87b	55.08 ±1.84 c	56.10 ±1.84 c	8.818b	62.073b
Trimmed one side across the micropyle	68.69 ± 1.09c	85.00 ± 7.98 d	86.00 ± 7.98 d	15.887c	80.773c
Trimmed at the middle seed	1.33 ± 2.33 a	31.50 ± 1.04 b	31.50 ± 1.04 b	2.44 a	4.222 a

* significant difference ($p \leq 0.05$)
abc Average compared mean within column by Duncan's multiple range test at ($p \leq 0.05$)
GI = germination index was calculated as described by Association of Official Seed Analyst (AOSA,1991)
SE = Speed of emergence was calculated according to ISTA (1996)

This result (Table 1) confirms the theory that the dormancy period is affected by the hardness of the seed coat (Chien and Lin, 1994). When the seed coat was trimmed, it let water and oxygen pass through the seed coat to reach the micropyle and the embryo directly (Figure 2). With other species, scarification or other treatments to break down the impermeability of the seed coat have been shown to shorten dormancy and result in germination in a relatively short time. For instance, *Lupinus hispanicus* seed has been reported to have long dormancy due to the seed coat (Centenera et al., 1999).

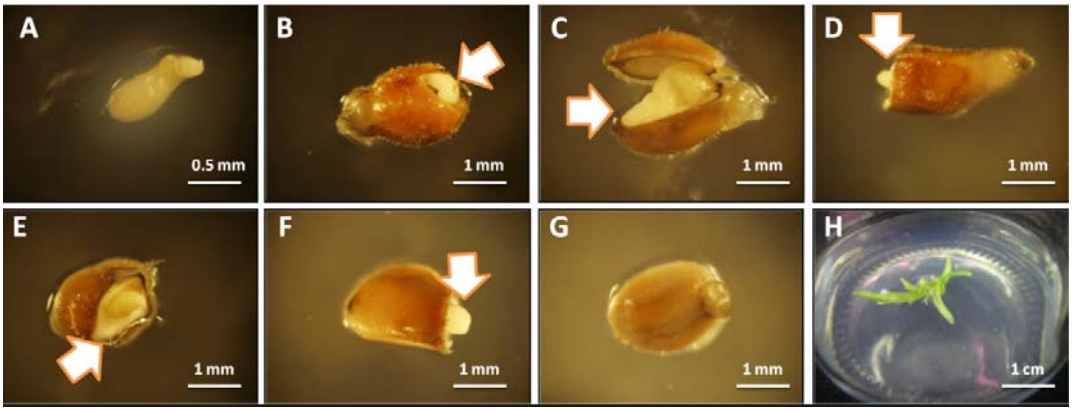

Figure 2: Young *Globba winitii* (commercial white) seed which treat by trimming at different parts of seed after culturing 3 weeks.
(A) Naked embryo, (B) Trimmed one side at the base across micropyle,
(C) Trimmed at the middle seed, (D) Trimmed at the end across micropyle,
(E) Trimmed one side of the micropyle (F) Trimmed at the micropyle side,
(G) Trimmless (Whole seed - no trimmed), and (H) Young plantlets from embryo.

In the present study, scarification (by way of trimming) resulted in the greatest imbibition, germination percentage, seedling establishment and also the highest values of seedling growth characteristics compared with untreated (control) seeds. However, partially trimming the seed did not improve the seed coat permeability much, and resulted in a low germination percentage (Table 1). The methods of trimming at the middle of the seed or trimming at one part of the micropyle gave the lowest percentages of germination at 1.33% and 34.9%, respectively. One possible explanation for this is that trimming at the middle of the seed may destroy or injure the embryo. From research by Eeckhaut et al. (2007), had done on *Rhododendon* which harvested 10 weeks after pollination and initiated in vitro, showed that seeds from inter-generic crosses had larger endosperm and the number of rescued embryos that germinated into new plantlets was greater. This research was similar to a report by Lili et al. (2008) on hybrid seedless grape (Emerald Seedless x Beichun), in which hybrid fruits were harvested 3 days after pollination and young embryos were cultured on WPM (woody plant medium, Lloyd and McCown, 1980). They obtained a high survival rate and new hybrid plants.

In the second week of this study, the germination index was again higher for the treatment groups of trimmed globba seeds than for the untrimmed control. This is compatible with results from the research of Jala (2011), Ellis and Robert (1981), Hangarter (1996), Warpeha and Kaufman (1989) and Winslow (1999), who reported that many plant species respond to the environment with optimal growth and development according to the availability of light, water and oxygen. In our experiment, the final germination percentage

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was higher for the bare embryo seed group than for either trimmed or intact seeds, with a statistically significantly difference among treatments (naked >trimmed >intact).

Partially trimming the seed coat or completely removing the seed coat apparently relieved any mechanical restraint and/or barriers to gas exchange, as these treatments greatly improved germination, just as they did of Genesis in a report by Duval and Ne Smith (2000). Comparing the mean germination rates of each seed coat trimming treatment, we found statistically significant differences. The group of seeds that was partially trimmed at the micropyle part and the group that was trimmed on one side across the micropyle both gave similar results, as shown in Table1. This result indicates that the seed coat was the major obstacle to seed germination. This is probably because the thick seed coat prevents water and oxygen from entering into the seeds. When the seed coat was removed, there was nothing to shield the inside. Water and oxygen could enter, stimulating the metabolism of the embryo to germinate into a seedling. When the germination index of each treatment was calculated, significant differences were found. Naked embryos had the highest germination index at 22.06 and the control group with intact seed coats had the lowest. In addition, the highest speed of emergence was recorded for the naked seed group, which was 100%. Data for speed of emergence followed the same trend as for germination index. This research agrees with Jala (2012) research on *Nepenthes mirabilis*. The speeds of emergence in young seeds which were trimmed at the micropyle part and those that were trimmed on one side at the base across the micropyle were the next fastest after the naked seed group.

5. Conclusion

Young embryos could be induced to germinate by trimming the young seeds at 20 days after pollination to break seed coat dormancy. Immature embryos could germinate within the first week and naked embryos with the seed coat entirely removed showed the highest germination percentage, germination index and seed emergence rate at 98.03%, 22% and 100%, respectively. But, Trimming at the micropyle side of the *Globba* seeds is the best method for enhance germination and got shot time which the same as the naked embryos. Explants from *globba* varieties cultured on MS medium supplemented with difference BA gave non significance difference in this experiment. But, Khao Burma cultured on MS medium supplemented with 2 mg/l BA gave the highest average number 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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