

A Study on Seed Germination and Seedling Development of *Spathoglottis* Bl. Orchids

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ABSTRACT

The experiments were conducted to study characteristics of seed from self-pollination, intraspecific and interspecific crosses, seed germination and seedling growth of *S. plicata* and *S. kimbaliiana* *in vitro*.

Seeds of *S. plicata* from capsule at 15, 20, 25 and 30 days and of *S. kimbaliiana* at 30, 35 and 40 days of age after pollination were germinated in modified VW solid and liquid media *in vitro*. The results showed that immature seeds in 15-day-old capsules of *S. plicata* had the lowest percentage of fertile seeds, germination rate and germination index. Self-pollinated seeds of *S. kimbaliiana* had lower percentage of fertile seeds in 30-day-old capsule while there was no difference in either intraspecific and interspecific crosses. Seeds in liquid medium showed faster and better germination percentage than that on solid medium.

After *in vitro* germination at 33 ± 2 °C and 3,000 lux of light intensity, seeds were swollen and showed green embryos in liquid medium in 17-22 days which was 6 days faster than in solid medium. The germination rate and index were observed one month after sowing the seeds. There was no germination in 15-day-old capsule of all crosses of *S. plicata*. The 25- and 30-day-old capsules of *S. plicata* and 30-, 35- and 40-day-old capsules of *S. kimbaliiana* showed high germination rate and germination index. Seeds in liquid medium developed better than those in solid medium. After one month from germination, seeds in 15 day capsule of *S. plicata* germinated and developed to large-sized seedlings within 4 months.

The 0, 0.01, 0.1 and 1 mg/l paclobutrazol had no significant effects on seedling growth of interspecific cross (*S. kimbaliiana* x *S. plicata*) while 100 g/l blended banana in modified VW medium promoted seedling growth in fresh weight, plant height, leaf number, leaf length and root number.

Key words: *in vitro* germination, *Spathoglottis* Bl., paclobutrazol, banana, seedling growth

INTRODUCTION

Spathoglottis is one of the most popular terrestrial orchids and the most important horticultural cultivated species because it is attractive, fast growing and easy to cultivate and had ever-blooming in the garden. Many hybrids of

this genus have beautiful flowers. Seeds germinate mostly on certain media *in vitro*. The problem is that the seeds of some hybrids are difficult to germinate and the seedling growth are not uniform.

Orchids are one of the most difficult plants to propagate by seed in nature. The seeds have tiny elongated shape, ranging from about one-quarter

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millimeter to two millimeters long depending on variety.

The flower is pollinated when the pollinia are placed on the stigma of an orchid flower. The ovary beneath it also begins to grow, and soon attains its full size. In the case of *Spathoglottis*, it is cylindrical, green, and hanging downwards. When it is ripe, the sides gape open with three slits, and the seeds are free to fall out, which they do at any puff of wind. Each seed consists of a tiny round embryo surrounded by a loose translucent coat, and so has low density and consequently a slow rate of falling. Orchid seeds vary much in the details of their size, the shape of the seed coat, and their color, but all are very small (Holttum, 1957).

Both commercial and amateur growers are in need of appropriate media, which insure a high percentage of germination and rapid seedling growth (Arditti and Lawrence, 1964). Over the years orchidologists have devised a number of nutrient formulae for the aseptical germination of a large variety of orchid genera. Many of these formulae contain one or more ingredients of relatively undefined composition, e.g. coconut water (Withner, 1959). On the other hand, Arditti and Lawrence (1964), reported that when all additive (banana, tomato juice, coconut milk, vitamins, etc.) were combined, it was highly effective on a variety of genera (Anderson, 1967).

Banana powder was first used in a medium for orchid seed germination in Brazil (Graeflinger, 1950). The use of banana in culture media for orchid seedlings became popular thereafter. Some growers homogenized banana fruit pulp with their media, whereas others stirred puree into their solutions. Opinion varied at the time as to whether green bananas (Hey and Hey, 1966) enhanced growth better than the ripe ones, and preferences still exist among growers (Ernst, 1967; 1975).

Paclobutrazol (proposed common name for ICI-PP-333) is a pyrimidine derivative has shown growth-controlling activity in a wide range of agronomic and ornamental crops. It is an inhibitor

of gibberellin biosynthesis that has exhibited little or no phloem mobility (McDaniel, 1983). Paclobutrazol has shown growth-retarding activity on a wide range of ornamental species (Shanks, 1980; Witfret, 1981; Barrett and Bartuska, 1982, Freeborg and McDaniel, 1981; Sterrett, 1985), and it has also been shown to reduce inter-node elongation (Barrett and Bartuska, 1982; McDaniel, 1983), to reduce leaf surface area, and to cause leaves to appear darker green (Wample and Culver, 1983).

Paclobutrazol may also promote rooting indirectly by retarding shoot growth, thereby increasing the partitioning of assimilates and/or hormones to the base of the cutting. Whatever the case, paclobutrazol may be useful in promoting rooting and controlling shoot growth simultaneously. It is of interest that relatively low concentration of paclobutrazol is effective in promoting root formation compared with some other synthetic root-promoting agents (Davis *et al.*, 1985).

Thus, the objectives of experiment were to study the characteristics of *Spathoglottis* Bl. orchid seeds from self-pollination, intraspecific and interspecific cross capsules, the effects of liquid and solid media on seed germination, and the effects of low concentration of paclobutrazol and banana on growth of seedlings.

MATERIALS AND METHODS

Two species of terrestrial orchids *S. plicata* Bl. (purple flower), and *S. kimballiana* (Hort.) Sander (yellow flower), were used in this study. Three experiments were designed and conducted as follows:

Experiment 1. Characteristics of seeds at different ages

After the two orchid species were self-pollinated, intraspecific and interspecific crossed, the capsules were harvested after pollination at the

ages of 15, 20, 25 and 30 days for *S. plicata*, and 30, 35 and 40 days for *S. kimbaliiana*. Seed size, percentage of fertile seeds and seed weight per capsule were observed.

Experiment 2. Effects of solid and liquid media on seed germination

Seeds from the first experiment were *in vitro* germinated in solid and in liquid medium. Capsule ages were set up as treatments. There were four treatments for *S. plicata* (15-, 20-, 25-, and 30-day old capsules) and three treatments for *S. kimbaliiana* (30-, 35-, and 40-day old capsules). The experiment was designed in CRD (Completely Randomized Design) with four replications (1 capsules/replication) in which four bottles were used for one replication.

Seeds, obtained from pollinated capsules, were *in vitro* germinated in solid and liquid media. Capsules were washed with detergent and water. After that, the capsules were surface-sterilized by dipping in 95% ethyl alcohol and flame immediately in the laminar flow hood. Then, the sterilized capsules were cut in cross section into two parts by an aseptic blade. Seeds were taken out of each capsule using a blade or forceps, and sown in four bottles of solid and four bottles of liquid media. The culture was incubated on shelf at 33 ± 2 °C under 3,000 lux for one month.

In this experiment, the liquid and solid media of modified VW (Vacin and Went, 1949) were used for seed germination. Liquid medium was supplemented with 150 ml coconut water and 20g/l table sugar. Solid medium was supplemented with 150 ml coconut water, 20 g/l table sugar, 100 g/l blended banana, 100 g/l potato extract, 4.5 g/l agar, and 2 g/l charcoal dust. Nitric acid was used to adjust pH value to 5.0.

One month after germination, one bottle from each replication was checked for germination percentage and germination index.

The remaining three bottles in each replication were subcultured into modified VW

solid medium. Then, the culture was incubated at 32 ± 2 °C and 3000 lux of light intensity.

The germination percentage was calculated with the following formula:

$$\text{Percentage of seed germination} = \frac{100 (b + c + d)}{a + b + c + d}$$

The germination index was calculated with the following formula:

$$\text{Germination index} = \frac{(1b + 2c + 3d) 10}{a + b + c + d}$$

The parameter used to measure the germination were germination percentage and germination index. When germination does not occur at all, the germination index is 0; when all seeds are germinated they will reach the class value of 3, in which the germination rate is 100% and the germination index is 30 (Pierik *et al.*, 1988).

The parameters used to measure the germination are given below:

| Class value | Seed number | Characteristics |
|-------------|-------------|---|
| 0 | a | No germination |
| 1 | b | Seed containing a green swollen embryo |
| 2 | c | Green embryos rupturing the seed coat |
| 3 | d | Embryos completely out of the seed coat |

Experiment 3. Effects of paclobutrazol and banana on seedling growth of *S. kimbaliiana* (Hort.) Sander interspecies cross

The four months old seedlings from germination, which had leaves, roots and 5-6 cm in height, were subcultured into eight media in the 150-ml bottle (five plants/bottle). The cultures were kept under eight hours daily light at 22 ± 2 °C and 3,000 lux of light intensity. Four months after subculture, seedlings were taken out from each bottle to record the seedling weight, height, number of roots and leaves, and leaf size.

The solid modified VW medium as in

Experiment 2 was used as the basal medium. There were four levels of paclobutrazol and two levels of blended banana as additive in this experiment. The experiment was designed in a 4 × 2 factorial analysis in CRD with 10 replications (five seedlings per replication) were used in this experiment.

| Treatments | Media |
|------------|--|
| 1 | V W + 0 mg/l paclobutrazol + 50 g/l blended banana |
| 2 | V W + 0 mg/l paclobutrazol + 100 g/l blended banana |
| 3 | V W + 0.01 mg/l paclobutrazol + 50 g/l blended banana |
| 4 | V W + 0.01 mg/l paclobutrazol + 100 g/l blended banana |
| 5 | V W + 0.1 mg/l paclobutrazol + 50 g/l blended banana |
| 6 | V W + 0.1 mg/l paclobutrazol + 100 g/l blended banana |
| 7 | V W + 1 mg/l paclobutrazol + 50 g/l blended banana |
| 8 | V W + 1 mg/l paclobutrazol + 100 g/l blended banana |

RESULTS

Experiment 1. Characteristics of seeds at different ages

It was found that seed colors were not different among the types of pollination (self-pollination, intraspecific and interspecific crosses), but colors were affected by the ages of the capsule. It was noted that the same age of capsule produced the same seed color: the seed color of the 15-day-old capsule was light green, those of the 20- and 25-day-old capsule were cream, and that of the 30-day-old capsule was black.

Seed sizes of *S. plicata* were significantly different among capsule ages in three pollination types. The self-pollinated seeds of the 20-, 25-, and 30-day-old capsule were significantly longer

than that of the 15-day-old capsule, while the 30-day-old capsule was the largest and the 15-day-old capsule had the lowest seed width. For the intraspecific cross, seeds of the 25- and 30-day-old capsule were longer than those of the 15- and 20-day-old capsule, whereas seed width of the 15-day-old capsule was significantly narrower than those of the other three. For the interspecific cross, the 25- and 30-day-old capsules yielded larger seeds than the 15- and 20-day-old capsules. From the above observation, it could be concluded that the older the capsule age, the bigger the seed size (Table 1).

However, in the 15-day-old capsule, intraspecific cross and interspecific cross yielded longer and wider seed sizes than self-pollination. The 20-day-old capsule seed was longer in self-pollination than other two crosses, while the seed was wider in intraspecific cross than self-pollination and interspecific cross. On the contrary, interspecific cross of 25- and 30-day-old capsules yielded longer and wider seeds than other two crosses (Table 1).

The percentages of fertile seed per capsule and seed weight per capsule were significantly different among capsule age depending on type of pollination. The percentage of fertile seed was highest at the 30-day-old capsule, medium at the 25-day-old capsule and lowest at the 15- and 20-day-old capsule for self-pollination. For the intraspecific and interspecific cross, percentages of fertile seed at the 25- and 30-day-old capsules were higher than those of the 15- and 20-day-old capsules (Table 2).

Moreover, the seed weight per capsule was also different depending on capsule age. The seed weight of capsule aged 20 days was higher than those of 15, 25 and 30 days in self-pollination, and intraspecific cross, while there were no significant differences among capsule ages in interspecific cross.

However, the percentages of fertile seed of all capsule ages (15, 20, 25 and 30 days) were not

significantly different among three crosses. Furthermore, at 25- and 30-day-old capsule, seed weight was higher in interspecies cross than in self-pollination and intraspecific cross, while there were no significant differences among three crosses in 15- and 20-day-old capsules (Table 2).

Similar to the results of *S. plicata*, seed colors varied according to the ages of the capsule, but were not different among types of pollination. The observation showed that the seed color of 30- and 35-day-old capsules was cream. It was distinctly different from the black seed color of the 40-day-old capsule.

Seed sizes of *S. kimballiana* at different

capsule ages in different pollination types are shown in Table 3. It was observed that seed sizes were not different among capsule ages if the cross was made between species. For self-pollination, the differences among capsule ages were found only in seeds from 35-day-old capsule whose seed width was larger than those from the 30- and 40-day-old capsules. It was found that in intraspecific cross, seed sizes in the 35- and 40-day-old capsules were larger than that of the 30 day-old capsule.

However, self-pollination and interspecific cross at 30-day-old capsule yielded longer and wider seeds than intraspecific cross. For the 35-day-old capsule, the difference was found only in

Table 1 Seed size of *S. plicata*.

| Capsule age (days) | Length (mm) | | | F-test | Width (mm) | | | F-test |
|-----------------------|-------------|---------------------------------|-------|--------|------------|---------------------------------|-------|--------|
| | P⊗ | P ₁ x P ₂ | P x Y | | P⊗ | P ₁ x P ₂ | P x Y | |
| 15 | 0.47b | 0.65b | 0.64b | ** | 0.10c | 0.13b | 0.12b | * |
| 20 | 0.75a | 0.64b | 0.62b | ** | 0.15b | 0.17a | 0.09c | ** |
| 25 | 0.77a | 0.75a | 0.99a | ** | 0.14b | 0.16a | 0.19a | ** |
| 30 | 0.81a | 0.76a | 0.96a | ** | 0.17a | 0.17a | 0.19a | * |
| F-test | ** | ** | ** | | ** | ** | ** | |
| C.V. (%) | 11.84 | 11.64 | 9.48 | | 11.73 | 11.58 | 16.55 | |

ns = not significantly different, **significantly different at $P \leq 0.01$.

Means in each column followed by the same letters are not significantly different at $P \leq 0.05$ as determined by DMRT.

Table 2 Percentage of fertile seed, seed weight per capsule of *S. plicata*.

| Capsule age (days) | % Fertile seed/capsule | | | F-test | Seed weight (g)/capsule | | | F-test |
|-----------------------|------------------------|---------------------------------|--------|--------|-------------------------|---------------------------------|-------|--------|
| | P⊗ | P ₁ x P ₂ | P x Y | | P⊗ | P ₁ x P ₂ | P x Y | |
| 15 | 52.25c | 55.08b | 55.13b | ns | 0.246b | 0.230b | 0.246 | ns |
| 20 | 56.26c | 58.62b | 58.69b | ns | 0.313a | 0.328a | 0.341 | ns |
| 25 | 80.35b | 74.71a | 77.71a | ns | 0.256b | 0.267b | 0.394 | ** |
| 30 | 87.34a | 79.62a | 83.72a | ns | 0.234b | 0.219b | 0.354 | ** |
| F-test | ** | ** | ** | | ** | ** | ns | |
| C.V. (%) | 4.70 | 5.98 | 5.91 | | 8.16 | 10.94 | 18.63 | |

ns = not significantly different, **Significantly different at $P \leq 0.01$.

Means in each column followed by the same letters are not significantly different at $P \leq 0.05$ as determined by DMRT.

seed width, where self-pollination yielded wider seeds than intraspecific and interspecific crosses. It was noticed that there were no significant differences among the three types of pollination in the 40-day-old capsule (Table 3).

For self-pollination, the 40-day-old capsule produced 88.06 % fertile seeds, which was higher than the 30-day-old capsule. For the intraspecific and interspecific crosses, the percentages of fertile seeds were not different among the 30-, 35- and 40-day-old capsules.

The 30-day-old capsule yielded the highest seed weight (0.804 g), and the 35-day-old capsule produced the lowest seed weight (0.434 g) for self-pollination. For the intraspecific cross, the 30- and 40-day-old capsules yielded heavier seed weight

than the 35-day-old capsule. For the interspecific cross, the 30-day-old capsule yielded the heaviest seed weight, followed by the seed weight of the 35-day-old capsule. The seed weight of the 40-day-old capsule was found to be the lowest (Table 4).

However, in all 30-, 35- and 40-day-old capsules, self-pollination and interspecific cross yielded higher percentages of fertile seed than intraspecific cross, though seed weight was heavier in self-pollination than the other two crosses in all three capsule ages (Table 4).

Experiment 2. Effects of solid and liquid media on seed germination

The seeds of *Spathoglottis*, with the capsule

Table 3 Seed size of *S. kimballiana*.

| Capsule age (days) | Length (mm) | | | F-test | Width (mm) | | | F-test |
|-----------------------|-------------|---------------------------------|-------|--------|------------|---------------------------------|-------|--------|
| | Y⊗ | Y ₁ x Y ₂ | Y x P | | Y⊗ | Y ₁ x Y ₂ | Y x P | |
| 30 | 0.63 | 0.45b | 0.65 | ** | 0.17b | 0.11b | 0.18 | ** |
| 35 | 0.61 | 0.59a | 0.57 | ns | 0.20a | 0.18a | 0.17 | ** |
| 40 | 0.64 | 0.61a | 0.64 | ns | 0.18b | 0.18a | 0.18 | ns |
| F-test | ns | ** | ns | | ** | ** | ns | |
| C.V. (%) | 8.41 | 12.37 | 11.88 | | 11.67 | 8.74 | 9.76 | |

ns = not significantly different, **significantly different at $P \leq 0.01$.

Means in each column followed by the same letters are not significantly different at $P \leq 0.05$ as determined by DMRT.

Table 4 Percentage of fertile seed, seed weight per capsule of *S. kimballiana*.

| Capsule age (days) | % Fertile seed/capsule | | | F-test | Seed weight (g)/capsule | | | F-test |
|-----------------------|------------------------|---------------------------------|-------|--------|-------------------------|---------------------------------|--------|--------|
| | Y⊗ | Y ₁ x Y ₂ | Y x P | | Y⊗ | Y ₁ x Y ₂ | Y x P | |
| 30 | 73.89b | 52.86 | 80.90 | * | 0.804a | 0.374a | 0.441a | ** |
| 35 | 81.52ab | 54.88 | 89.89 | ** | 0.434c | 0.296b | 0.307b | ** |
| 40 | 88.06a | 60.33 | 91.57 | ** | 0.531b | 0.388a | 0.285c | ** |
| F-test | * | ns | ns | | ** | ** | ** | |
| C.V. (%) | 6.58 | 19.20 | 6.48 | | 2.11 | 2.03 | 3.02 | |

ns = not significantly different, *significantly different at $P \leq 0.05$, **significantly different at $P \leq 0.01$.

Means in each column followed by the same letters are not significantly different at $P \leq 0.05$ as determined by DMRT.

ages of 15, 20, 25 and 30 days for *S. plicata* and 30, 35 and 40 days for *S. kimballiana*, were cultured *in vitro* for one month. The seeds germinated rapidly in aseptic culture. The embryos swelled and emerged from the testa within 3-4 weeks, and protocorms covered with numerous hairs (rhizoids) were formed.

2.1 *S. plicata*

The germination percentage and

germination index of *S. plicata* on solid and liquid media are shown in Table 5.

Seeds from all crosses of *S. plicata*, the 25- and 30-day-old capsule, started to germinate in 23-28 days after pollination on solid media. Viable seeds were swollen and turned green. Embryos became so swollen that the seed coat began to rupture. However, seeds from the 15- and 20 day-old capsules did not show any germination signs at

Table 5 Germination percentage and germination index of *S. plicata* in one month on solid and liquid modified VW media.

| Capsule age (days) | Germination percentage | | T-test | Germination index | | T-test |
|--|------------------------|------------------|--------|-------------------|------------------|--------|
| | Solid medium | Liquid medium | | Solid medium | Liquid medium | |
| Self-pollination (P f) | | | | | | |
| 15 | 0.0b | 0.0b | ns | 0.0b | 0.0b | ns |
| 20 | 6.35b | 8.99b | ns | 0.63b | 0.90b | ns |
| 25 | 84.62a | 94.64a | * | 12.88a | 18.19a | ** |
| 30 | 67.72a | 72.29a | ns | 12.20a | 14.07a | ns |
| F-test | ** | ** | | ** | ** | |
| C.V. (%) | 50.73 | 48.35 | | 57.33 | 50.35 | |
| Intraspecific cross (P ₁ x P ₂) | | | | | | |
| 15 | 0.0b | 0.0b | ns | 0.0b | 0.0b | ns |
| 20 | 6.72b | 9.72b | ns | 0.67b | 1.03b | ns |
| 25 | 78.83a | 83.30a | ns | 12.45a | 15.12a | ns |
| 30 | 90.61a | 94.51a | ns | 15.53a | 17.75a | ** |
| F-test | ** | ** | | ** | ** | |
| C.V. (%) | 28.28 | 22.33 | | 28.37 | 28.13 | |
| Interspecific cross (P x Y) | | | | | | |
| 15 | 0.0c | 0.0c | ns | 0.0c | 0.0b | ns |
| 20 | 5.97b | 8.25b | ns | 0.60b | 0.91b | ns |
| 25 | 87.74a | 92.73a | ns | 13.90a | 17.35a | ** |
| 30 | 91.13a | 92.77a | ns | 16.43a | 17.36a | * |
| F-test | ** | ** | | ** | ** | |
| C.V. (%) | 9.44 | 5.06 | | 9.09 | 10.27 | |

*significantly different at $P \leq 0.05$, **significantly different at $P \leq 0.01$.

Means in each column followed by the same letters are not significantly different at $P \leq 0.05$ as determined by DMRT.

that time. One month after germination, seeds from the 25- and 30-day-old capsules developed into protocorms. However, seeds from the 20-day-old capsule were only at 5.97-6.72% germination rate which were lower than those of the 25 and 30 days, while those of 15 days did not show any germination signs in one month yet.

In liquid media, the embryos from the seeds of the 25- and 30-day-old capsules became swollen and started to turn green within 14-21 days after germination. In contrast, the seeds of the 15- and 20-day-old capsules did not show any germination signs at that time. In one month, many embryos from seeds of the 25- and 30-day-old capsules became swollen and green, and they even produced protocorms with or without shoot tips. However, embryos from the seeds of the 15-day-old capsule remained unchanged, while seeds of the 20-day-old capsule germinated at 8.25-9.72% started to swell and turned green.

One month after germination, the swollen, green seeds or protocorms were transferred to a modified VW solid medium and cultured for three months. The first primordial leaf appeared on the top of the protocorms in approximately 40-47 days after germination, and roots were formed around 63-70 days after germination. After roots

were formed, the complete plantlets developed, but not all plants grew simultaneously.

Four months after germination (three months after transflasking), the germination percentage and germination index of *S. plicata* were observed. It was found that, all crosses of 15-day-old capsules resulted in lower germination percentage and germination index than that of the 20-day-old capsule (Table 6).

The 15-day-old capsule of *S. plicata* self-pollination, intraspecific and interspecific crosses had germination rates at 32.41, 34.14 and 27.78% on solid medium as in liquid medium of 37.34, 39.44 and 31.62 %, respectively. The 20-day-old capsule showed greater rate of seed germination up to 65.10, 67.40 and 73.89 % on solid medium and 75.53, 82.60 and 80.68 % in liquid medium, respectively.

2.2 *S. kimballiana*

The germination percentage and germination index of *S. kimballiana* on solid and liquid media are shown in Table 7.

Seed germination of *S. kimballiana* on solid medium showed that the developmental stages were not different, though the differences were more or less 2 or 3 days, among the three types of

Table 6 Germination percentage and germination index of *S. plicata* in three months after subculture on solid modified VW medium (4 months from germination).

| Capsule age (days) | Germination percentage | | Germination index | |
|--|------------------------|---------------|-------------------|---------------|
| | Solid medium | Liquid medium | Solid medium | Liquid medium |
| Self-pollination (P ⊗) | | | | |
| 15 | 32.41 | 37.34 | 5.53 | 4.52 |
| 20 | 65.10 | 75.53 | 11.67 | 13.40 |
| Intraspecific cross (P ₁ x P ₂) | | | | |
| 15 | 34.14 | 39.44b | 4.90 | 4.90 |
| 20 | 67.40 | 82.60a | 11.76 | 17.54 |
| Interspecific cross (P x Y) | | | | |
| 15 | 27.78 | 31.62 | 4.53 | 3.89 |
| 20 | 73.89 | 80.68 | 15.12 | 14.13 |

pollination and among the three capsule ages. The germination started in approximately 14 days after isolation with swelling of the embryos. In 21 days, the embryos became so swollen that the seed coats began to rupture. In one month, the swollen embryos developed into protocorms with or without shoot tips.

All developmental stages were the same between the solid and liquid media in *S. kimbaliiana*. But in the liquid medium, the embryos swelled faster and greener than they did in the solid media. The germination rate was 81.94-97.98 % and the germination index was 15.57-19.82 %.

Experiment 3. Effects of paclobutrazol and banana on growth of *S. kimbaliiana* (Hort.) Sander interspecies cross

Seedlings were subcultured in modified VW media added with 0, 0.01, 0.1, 1 mg/l paclobutrazol mixing 50, 100 g/l blended banana in each level of paclobutrazol concentration for 4 months.

Table 8 showed that paclobutrazol concentrations did not significantly affect fresh weight, plant height, leaf number, leaf size, number and root length of seedlings, whereas seedlings in the treatment with 100 g/l blended banana were

Table 7 Germination percentage and germination index of *S. kimbaliiana* in one month on solid and liquid modified VW media.

| Capsule age (days) | Germination rate (%) | | T-test | Germination index | | T-test |
|--|----------------------|------------------|--------|-------------------|------------------|--------|
| | Solid medium | Liquid medium | | Solid medium | Liquid medium | |
| Self-pollination (Y \otimes) | | | | | | |
| 30 | 93.55 | 95.61 | ns | 17.16 | 17.80 | * |
| 35 | 93.19 | 96.81 | * | 17.20 | 18.11 | ns |
| 40 | 95.29 | 94.63 | ns | 18.21 | 18.79 | ns |
| F-test | ns | ns | | ns | ns | |
| C.V. (%) | 3.63 | 3.16 | | 4.37 | 6.36 | |
| Intraspecific cross (Y ₁ x Y ₂) | | | | | | |
| 30 | 93.70 | 97.98 | ** | 17.40 | 19.19 | ns |
| 35 | 94.03 | 94.38 | ns | 17.99 | 16.60 | ns |
| 40 | 92.19 | 96.24 | ** | 16.56 | 17.72 | ns |
| F-test | ns | ns | | ns | ns | |
| C.V. (%) | 2.37 | 2.73 | | 5.93 | 8.21 | |
| Interspecific cross (Y x P) | | | | | | |
| 30 | 86.75 | 86.75 | ns | 15.91 | 15.86 | ns |
| 35 | 81.94 | 90.25 | ns | 15.57 | 17.17 | ns |
| 40 | 97.87 | 97.55 | ns | 19.82 | 18.87 | ns |
| F-test | ns | ns | | ns | ns | |
| C.V. (%) | 14.52 | 13.32 | | 19.11 | 20.84 | |

ns = not significantly different. * = significantly different at $P \leq 0.05$, ** = significantly different at $P \leq 0.01$.

significantly higher in fresh weight, plant height, leaf number, leaf length, and root number than 50 g/l blended banana.

However, media with 0.1 and 50 g/l blended banana yielded the lowest fresh weight, leaf width and root number. Furthermore, leaf width was

Table 8 Factorial analysis of paclobutrazol and banana on fresh weight, plant height, leaf number/plant, leaf size, root number/plant and length of *S. plicata* x *S. kimballiana*.

| | Fresh weight (g) | Plant height (cm) | Leaf | | | Root | |
|-----------------|------------------|-------------------|--------|-------------|------------|--------|-------------|
| | | | Number | Length (cm) | Width (cm) | Number | Length (cm) |
| Paclo 0 mg/l | 2.24 | 17.72 | 3.09 | 12.46 | 0.65 | 8.27 | 7.47 |
| Paclo 0.01 mg/l | 2.24 | 18.28 | 3.26 | 12.59 | 0.63 | 8.30 | 7.60 |
| Paclo 0.1 mg/l | 2.06 | 18.12 | 3.23 | 12.56 | 0.61 | 7.41 | 7.61 |
| Paclo 1 mg/l | 2.29 | 17.76 | 3.40 | 12.46 | 0.68 | 7.64 | 7.68 |
| F-test | ns | ns | ns | ns | ns | ns | ns |
| Banana 50 g/l | 1.96b | 17.48b | 2.97b | 12.10b | 0.63 | 7.08b | 7.72 |
| Banana 100 g/l | 2.45a | 18.46a | 3.52a | 12.93a | 0.66 | 8.73a | 7.47 |
| T-test | ** | * | * | * | ns | ** | ns |
| Pa x Ba | ns | ns | ns | ns | ** | ns | ns |
| C.V. (%) | 26.44 | 10.12 | 33.36 | 12.17 | 17.26 | 19.71 | 15.56 |

ns = not significantly different, *significantly different at $P \leq 0.05$, **significantly different at $P \leq 0.01$.

Means in each column followed by the same letters are not significantly different at $P \leq 0.05$ as determined by DMRT.

Pa x Ba = paclobutrazol x Banana interaction.

Table 9 Effects of paclobutrazol and banana on fresh weight, plant height, leaf number/plant, leaf size, root number/plant and length of *S. plicata* x *S. kimballiana*.

| Paclo (mg/l) | Banana (g/l) | Fresh weight (g) | Plant height (cm) | Leaf | | | Root | |
|--------------|--------------|------------------|-------------------|--------|-------------|------------|---------|-------------|
| | | | | Number | Length (cm) | Width (cm) | Number | Length (cm) |
| 0.0 | 50 | 1.95ab | 17.23 | 2.81 | 12.43 | 0.71ab | 7.39abc | 7.56 |
| 0.0 | 100 | 2.52a | 18.21 | 3.38 | 12.49 | 0.59b | 9.15a | 7.39 |
| 0.01 | 50 | 2.00ab | 18.34 | 3.03 | 12.45 | 0.65ab | 7.76abc | 7.98 |
| 0.01 | 100 | 2.49a | 18.22 | 3.50 | 12.73 | 0.62b | 8.84a | 7.23 |
| 0.1 | 50 | 1.81b | 17.35 | 3.02 | 12.02 | 0.58b | 6.41c | 7.48 |
| 0.1 | 100 | 2.30ab | 18.89 | 3.45 | 13.10 | 0.64ab | 8.41abc | 7.74 |
| 1 | 50 | 2.08ab | 17.01 | 3.04 | 11.52 | 0.59b | 6.77bc | 7.86 |
| 1 | 100 | 2.49a | 18.50 | 3.76 | 13.41 | 0.77a | 8.51ab | 7.51 |
| F-test | | * | ns | ns | ns | ** | ** | ns |
| C.V. (%) | | 26.44 | 10.12 | 33.36 | 12.17 | 17.26 | 19.71 | 15.56 |

ns = not significantly different, *significantly different at $P \leq 0.05$, **significantly different at $P \leq 0.01$.

Means in each column followed by the same letters are not significantly different at $P \leq 0.05$ as determined by DMRT.

wider in media with 1 mg/l paclobutrazol and 100 g/l banana than the media without and with 0.01 mg/l paclobutrazol and 100 g/l banana, and with 0.1 and 1 mg/l paclobutrazol and 50 g/l banana treatments (Table 9).

DISCUSSION

Seed sizes and percentages of fertile seed of *S. plicata* were significantly different among capsule ages in three pollination types. It was noticed that the older capsules possessed larger and more fertile seeds than the relatively younger ones (Table 1 and Table 2). Therefore, older capsule ages of 25 and 30 days resulted in larger seed sizes and higher numbers of fertile seed, probably because the seeds were mature. But the younger capsules of 15 and 20 days still had more than 50% of fertile seeds.

In contrast to *S. plicata*, the older capsule age of *S. kimballiana* did not yield greater seed size, except for intraspecific cross. The high percentage of fertile seed in older capsule age was found only in self-pollination, however, in interspecific and intraspecific crosses, too, there was a potential for older capsules to produce higher percentage of fertile seed in comparison to relatively younger ones.

However, in both species, it was found that seed weights of young capsule were heavier than those of relatively older capsules. This was due to the lower seed moisture content in seeds from older capsules. The cell of seed coat died at maturity and developed transparent walls. Though both species could gain their potential capsule size and weights at the beginning stage of maturity, the capsule should be kept longer in the mother plant in order to nurture young seeds to maturity. So, the older capsules could produce more fertile seeds than did younger capsules.

One month after germination of *S. plicata*, the 25- and 30-day-old capsules, in all three pollination types, yielded higher germination

percentages and higher germination index than did the 15- and 20-day-old capsules in both solid and liquid media. Four months after germination, the 20-day-old capsule increased germination percentage and germination index. However, the germination percentage and the germination index of the 15-day-old capsule remained lower (Table 5 and Table 6). This finding was in accordance with Anderson's report (1967) that the age of the seed might play an important role in the rate of germination. And as in the report of Rao and Chua (1978), it stated that *S. premier* required 2 weeks between pollination to fertilization and 4 weeks for seed development, then fruit dehiscence in 6 weeks and percentage of seed sterility was 85.71.

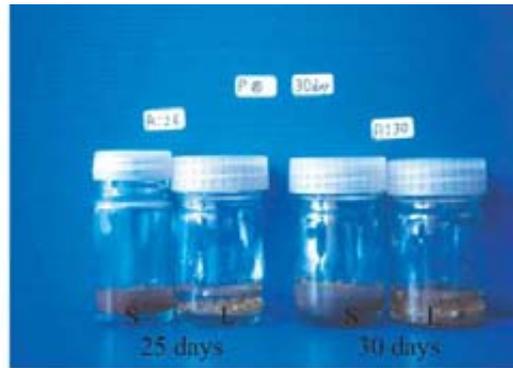


S. plicata Bl.

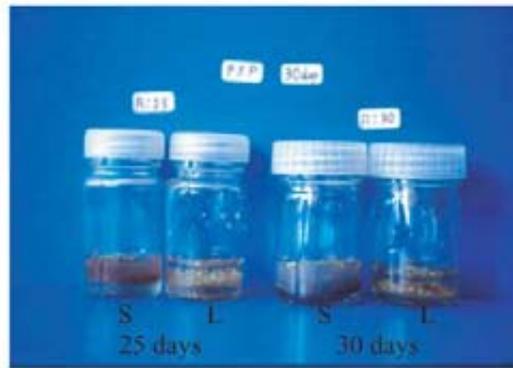
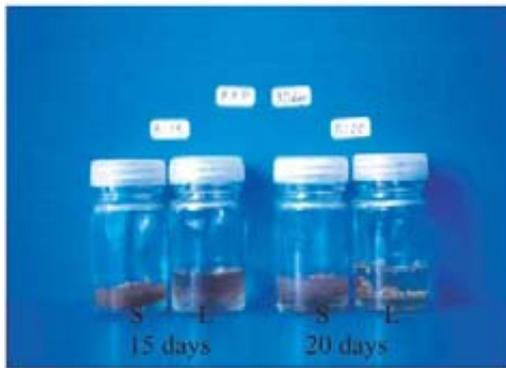


S. kimballiana (Hort.) Sander

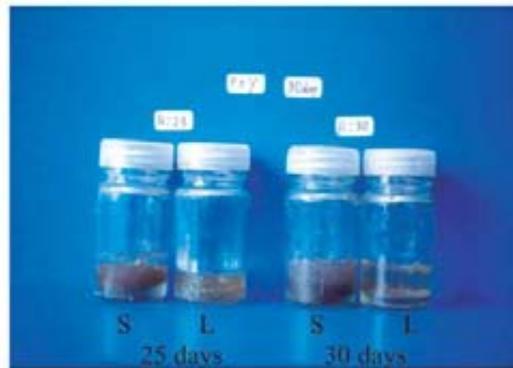
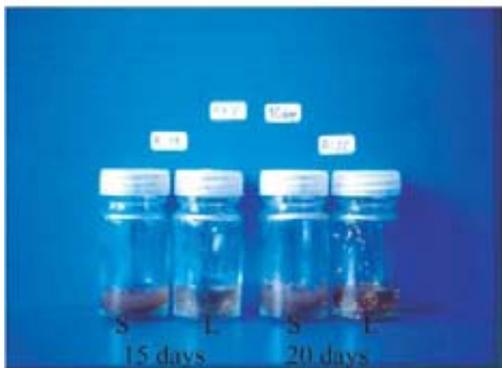
Figure 1 Flower characteristics of *S. plicata* Bl. and *S. kimballiana* (Hort.) Sander.



S. plicata self-pollination



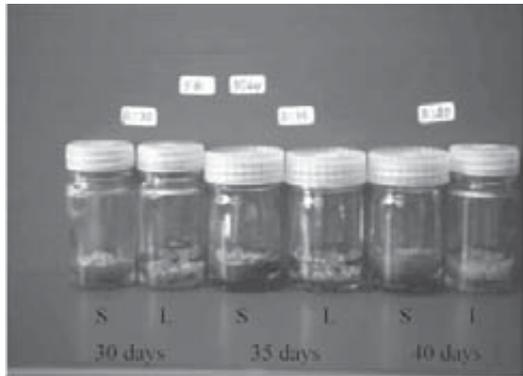
S. plicata intraspecific cross



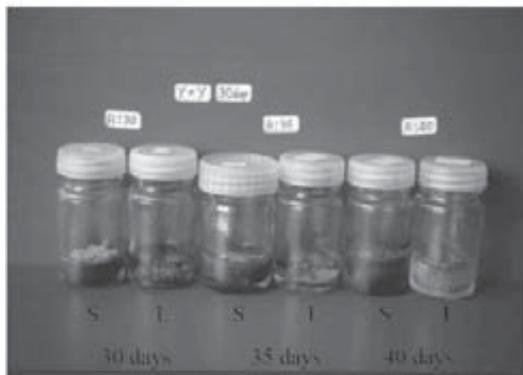
S. plicata interspecific cross

Figure 2 Protocorms of *S. plicata* Bl. on solid (S) and liquid (L) media at 1 month after germination of 15-, 20-, 25-, and 30-day-old capsule.

It seemed that high germination percentage and germination index of *S. plicata* was associated with high percentage of seed fertility. It was quite clear with the 15-day-old capsule. The results



S. kimbballiana self-pollination



S. kimbballiana intraspecific cross

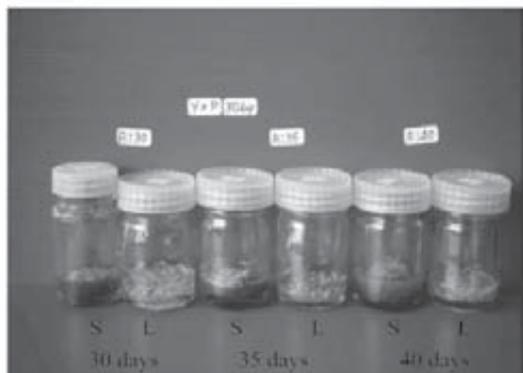


Figure 3 Protocorms of *S. kimbballiana* on solid (S) and liquid (L) media at 1 month after germination of 30-, 35-, and 40-day-old capsule..

appeared to concur with the findings of Lucke (1971) who stated that orchid seeds could be sown when the seed was halfway between fertilization and ripening of the capsule, and seeds should not be sown *in vitro* before the capsule was about two-thirds ripe. However, his findings contradicted the findings of Rasmussen (1995), who reported that *in vitro* culture seeds taken from immature capsules generally germinated more readily than ripe seeds. Their different findings may be due to genetic differences or due to the differences in species used. However, Arditti (1982) reported that whenever possible, the culture of immature seeds from green capsules was preferable to that of mature seeds from ripe capsules because it saved time and was simple, and this was the only way to obtain germination.

The effects of capsule age on germination percentage and germination index of *S. kimbballiana* were not different. In addition, there were no differences between solid and liquid media (Table 7) in this regard.

The results of *S. plicata* and *S. kimbballiana* were consistent with the findings of Kongmanee (2001), who made a study on seed germination and seedling development of *Dendrobium scabrilingne* Lindl. *in vitro*. She indicated that seedlings cultured in liquid medium with normal concentration of modified VW had the best seedling development.

It was also noticed that the 25- and 30-day-old capsules of *S. plicata* and 30, 40-day-old capsule of *S. kimbballiana* were suitable for germination. They yielded high germination percentages and good development of seedlings.

Different concentrations of paclobutrazol did not significantly affect fresh weight, plant height, leaf number, leaf size, number and root length on hybrid seedlings of *S. kimbballiana* x *S. plicata* (Table 8).

It was reported that paclobutrazol reduced shoot growth, increased root to leaf ratio and tolerance to water stress (Swietlik and Miller, 1983), reduced internode elongation (Barrett and Bartuska,

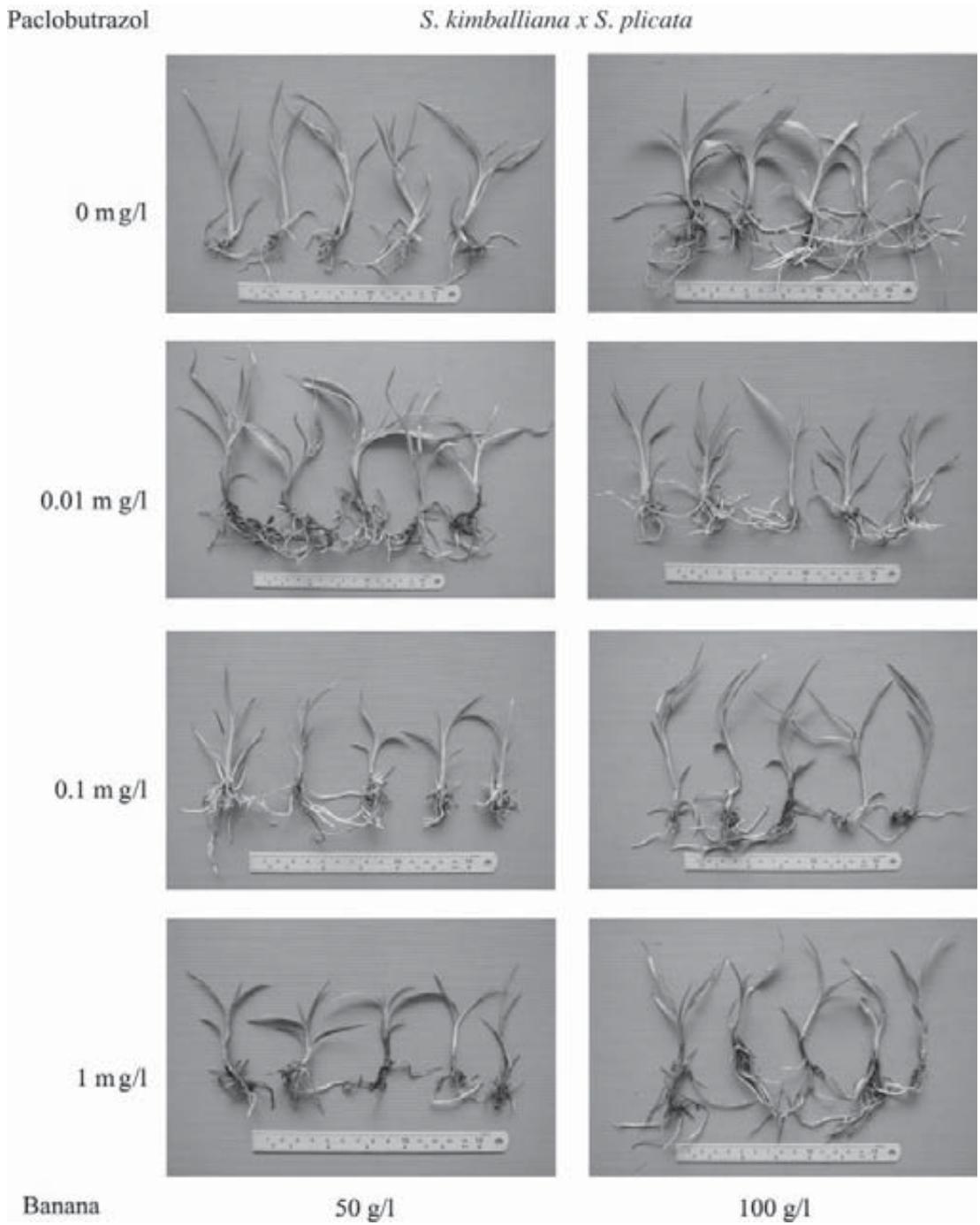


Figure 4 Seedlings of *S. kimballiana* (Hort.) Sander x *S. plicata* Bl, 4 months after subculture on solid media.

1982), and reduced leaf surface area (Wample and Culver, 1983). Kititrakunyanun (1999) also showed that when paclobutrazol concentration increased, the height, fresh weight and dry weight of the orchid plant decreased. The present finding was not consistent with these findings. It might be due to the use of low concentration of paclobutrazol. However, it seemed to agree with Chaithanu (1999) that media added with sugar and 0, 0.001, 0.01 or 1 mg/l paclobutrazol was not different in producing the numbers and sizes of tuber or root in all media in some terrestrial orchid, *Habenaria rhodocheila* Hance and *Pecteilis sagarikii* Seidenf.

Arditti (1968) reported that banana fruit tissue contained growth substances, which might enhance the growth of orchid seedlings. Moreover, Arditti (1982) also indicated that banana homogenate could enhance the growth of orchid seedlings or plantlets. In this experiment, 100 g/l blended banana yielded heavier fresh weight, taller plants, more leaves, longer leaves and more roots in interspecific cross of *S. kimballiana* x *S. plicata* (Table 8). Therefore, it was possible that though banana could enhance the seedling growth, the amount of banana used would depend on the orchid species to get satisfactory growth rate.

Even though the single effect of paclobutrazol and banana yielded clear results. The combination of these two treatments did not provide enough evidence to draw a general conclusion in this cross (Table 8 and 9). Although low concentration of paclobutrazol did not retard the plant growth, the combination of paclobutrazol and banana seemed to influence it. However, since paclobutrazol was one of the plant regulators, it should be applied in the exact amount. The amount lower or higher than a certain quantity could cause reverse effects. That was why some plant growth increased in single paclobutrazol treatment. The present results might not be very clear because there were only four concentrations of paclobutrazol used in this experiment. Therefore, it is necessary to extend the research with more

concentration levels of paclobutrazol. By doing so, it is hoped that the combined effects of paclobutrazol and banana could be found.

CONCLUSIONS

The study on seed germination and seedling development of *Spathoglottis* Bl. orchids could be concluded as follows:

1. Color, weight as well as size and fertile seeds gradually developed in *S. plicata* and *S. kimballiana*. The older the capsule age, the larger the seed and the higher the fertility rate. High germination percentage and germination index of *S. plicata* were associated with high percentage of seed fertility.

2. In general, seed germination of 25-day-old capsule of *S. plicata* and 30 days of *S. kimballiana* saved time and could reduce time of pollination because the number of fertile seeds, germination percentage and germination index at these ages were not much different from the older capsules.

3. The duration of the development of the seeds to protocorms (one month after sowing) depended on the *in vitro* culture media in both *S. plicata* and *S. kimballiana* where seeds swelled and turned green in about 17-22 days after germination in liquid medium which was 6 days faster than in solid medium. Therefore, seed germination could develop in liquid and solid media.

4. The 0, 0.01, 0.1 and 1 mg/l paclobutrazol supplement in modified VW medium did not significantly affect the seedling growth of *S. kimballiana*, while 100 g/l blended banana in media promoted seedling growth in fresh weight, plant height, leaf number, leaf size and root length.

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