IN VITRO PROPAGATION OF DENDROBIUM AND PHALAENOPSIS THROUGH TISSUE CULTURE FOR CONSERVATION

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ABSTRACT

The studies were focused on developing an efficient and effective propagation protocol for orchid species from genera Dendrobium and Phalaenopsis through tissue culture. The materials used were explants from adventive shoot tip, floral stalk buds and PLBs derived from seeds. The results indicated growth and development of adventive shoot tip explants of Dendrobium: a high survival percentage for explant with green color was shown by D. racianum, followed by D. laxiflorum, D. pseudococonantum, D. strebloceras, D. lineale, and D. veratrifolium. However, plantlets regeneration occurred only on D. pseudococonantum and D. strebloceras. Explant regeneration from seed derived protocorm-like bodies on D. spectabile occurred 40 days after inoculation transfer and subculture. High survival percentage of explant from floral stalk shoot was shown by P. amabilis. There were several plantlets surviving in acclimatisation. Explant regeneration from seed derived from protocorm-like bodies on P. hieroglypha occurred 40 days after inoculation and subculture. It was suggested that for ex situ conservation on certain species of Dendrobium and Phalaenopsis in the category of rare germplasms, tissue culture could be applied effectively and efficiently by using explant from adventive shoot tip, floral stalk buds and seed derived protocorm-like body explants for vegetative seed multiplication.

Keywords: orchid, conservation, species, in vitro culture

INTRODUCTION

Germplasms is a very valuable asset as raw materials in any orchid breeding programs. For example, orchids, such as Dendrobium and Phalaenopsis, which contain some species that are close to extinction, urgently require conservation. Indonesia with its climate and tropical rainforest is an ideal habitat for many orchid species. At present the existence of orchid germplasms in their natural habitat is at risk because of illegal selling, logging, and natural disaster. Their population is also drastically declining because of a lower rate of propagation in nature and overexploitation. One of the means for ex situ conservation is by propagation through in vitro culture.

Cloning technique by tissue culture resulted in vegetative propagation in mass number and the offspring genetically similar to the parental plant. It has made a possible choice for ex situ conservation in orchid. Many types of explants such as shoot tips (Sagawa and Kunisaki 1982; Malabadi et al., 2004; Malabadi et al., 2005), floral stalks (Lim-Ho and Lee, 1987; Young et al., 2000), protocorm-like bodies (Ishii et al., 1998; Lee and Lee, 2003; Huan et al., 2004), seed derived protocorm-like bodies (Chen and Chang, 2004) and protocorm-like bodies derived from mature seeds (Shimura and Koda, 2004) have been used as explants in tissue culture to produce plantlets.

Some Dendrobium and Phalaenopsis species were chosen to be employed in this study in order to investigate suitable explants material that is good for in vitro propagation. By using this method, valuable plants can be conserved and exploited.

MATERIALS AND METHODS

Plant materials used in this experiment were adventive shoot tips, floral stalk buds and seed-derived protocorm-like bodies (plb) from eight species of Dendrobium and four species of Phalaenopsis.

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Phalaenopsis. Dendrobium species used were from different sections: section Spatulata (Ceratobium) i.e. Dendrobium stratiotes and D. lasianthera; section Eleuteroglossum i.e. D. canaliculatum; section Latourea i.e. D. spectabile. Furthermore, some species from genus Phalaenopsis i.e. Phalaenopsis amabilis, P. amboinensis, P. hieroglypha and P. tetraspis were used.

Explants from adventive shoot tips of Dendrobium and floral stalk buds from Phalaenopsis were sterilized in the surface by using Clorox and Teepol. Each explant was placed on medium in 140 ml-volume culture bottle containing 30 ml solid medium. The media were VW, VW+0.5 mg l⁻¹BA, ½ MS and ½ MS+0.5 mg l⁻¹BA. Two months after cultured, explants were cut into pieces and subcultured into fresh medium containing similar medium with the addition of 2 mg l⁻¹ BA and 0.5 mg l⁻¹NAA. It was a descriptive experiment and because of insufficient number of explants, there was no replication used.

Another type of explant used in this experiment was four-month old PLBs derived from seeds. These seed-derived protocorm-like bodies (plbs) were longitudinally bisected. Ten explants were placed on medium as previously described. Regenerated plbs were subcultured into fresh medium containing similar medium with the addition of NAA. Cultures were maintained at temperature 25±2°C, light intensity of 30-40 µ mol m⁻² s⁻¹ and photoperiods of 16 hours in light and 8 hours in dark.

Observation was made, percentage of explant contamination, colour of explants, days of callus or plb initiation, percentage and number of explant development, and number of regenerated plantlets were recorded.

RESULTS AND DISCUSSION

Dendrobium Adventive Shoot Tip Explant

Results of experiments of in vitro plant regeneration of Dendrobium explants showed that a high percentage of shoot explants with green colour was obtained from D. racianum followed by D. lineale, D. pseudoconantum, D. strebloceras, D. laxiflorum and D. veratrifolium. Percentage of contamination on explants ranged from 0 to 100% (Table 1). In this experiment, shoot explant with green colour for eight species of Dendrobium ranged from 0 to 100%. From descriptive observation, it was shown that after cultured on medium, some explants of D. pseudoconantum turned into yellow and the lowest part of the adventive shoot tip explants of D. veratrifolium turned into white. It could be because these explants contained older tissues that made the growth and development (regeneration) slow and became yellow and white in colour. While explants of D. laxiflorum, D. pseudoconan-tum and D. strebloceras turned into brown, and explants of D. pseudoconantum and D. strebloceras turned into black. Explants with wide surface cut after several days in culture became brown or black in colour, and could not develop any further. Browning could also be a result of phenolic compound production (Figure 1).

Explants with green colour could develop further although not all could grow into new shoot. After 40 days of first subculture, green adventive shoot tip explants of D. pseudoconantum formed callus on medium containing ½ MS+BA+NAA. While D. streblo-ceras formed shoot on medium containing ½ MS+BA+NAA, plb and callus+plb on medium containing VW+BA+NAA. Of eight Dendrobium species, only D. pseudocon-natum and D. strebloceras were able to regenerate and continue to grow into plantlets (Figure 2).
Table 1. The growth and development of adventive shoot tip explants of *Dendrobium* species on *in vitro* culture

| Species         | Media      | Number of explants | Contamination (%) | Explant (%) with criterion of colour |
|-----------------|------------|--------------------|-------------------|-------------------------------------|
|                 |            |                    |                   | Green | Yellow | Brown | Black | White |
| *D. laxiflorum* | VW         | 1                  | 0                 | 100   | -      | -     | -     | -     |
|                 | VW+BA      | 1                  | 0                 | 100   | -      | -     | -     | -     |
|                 | ½ MS       | 1                  | 0                 | 100   | -      | -     | -     | -     |
|                 | ½ MS+BA    | 2                  | 0                 | 100   | 100    | -     | -     | -     |
| *D. pseudoconantum* | VW       | 2                  | 0                 | 100   | 100    | -     | -     | -     |
|                 | VW+BA      | 2                  | 0                 | 100   | 100    | -     | -     | -     |
|                 | ½ MS       | 2                  | 0                 | 100   | -      | 100   | -     | -     |
|                 | ½ MS+BA    | 3                  | 0                 | 100   | 100    | 100   | -     | -     |
| *D. canaliculatum* | VW       | 1                  | 100               | -     | -      | -     | -     | -     |
|                 | VW+BA      | 1                  | 100               | -     | -      | -     | -     | -     |
|                 | ½ MS       | 1                  | 100               | -     | -      | -     | 100   | -     |
|                 | 1/2MS+BA   | 2                  | 100               | -     | -      | -     | -     | -     |
| *D. strebloceras* | VW       | 2                  | 50                | 100   | -      | -     | -     | -     |
|                 | VW+BA      | 5                  | 40                | 100   | -      | 100   | 100   | -     |
|                 | ½ MS       | 4                  | 50                | 100   | -      | 100   | -     | -     |
|                 | 1/2MS+BA   | 4                  | 50                | 100   | -      | 100   | -     | -     |
| *D. sp. Maluku*  | VW         | 3                  | 100               | -     | -      | -     | -     | -     |
|                 | VW+BA      | 2                  | 100               | -     | -      | -     | -     | -     |
|                 | ½ MS       | 2                  | 100               | -     | -      | -     | -     | -     |
|                 | 1/2MS+BA   | 3                  | 100               | -     | -      | -     | -     | -     |
| *D. lineale*    | VW         | 2                  | 50                | 100   | -      | -     | -     | -     |
|                 | VW+BA      | 2                  | 50                | 100   | -      | -     | -     | -     |
|                 | ½ MS       | 2                  | 50                | 100   | -      | -     | -     | -     |
|                 | 1/2MS+BA   | 2                  | 50                | 100   | -      | -     | -     | -     |
| *D. veratrifolium* | VW       | 1                  | 0                 | -     | -      | -     | 100   | -     |
|                 | VW+BA      | 2                  | 50                | -     | -      | -     | 100   | -     |
|                 | ½ MS       | 1                  | 0                 | 100   | -      | -     | -     | -     |
|                 | 1/2MS+BA   | 2                  | 50                | 100   | -      | -     | -     | -     |
| *D. racianum*   | VW         | 1                  | 0                 | 100   | -      | -     | -     | -     |
|                 | VW+BA      | 1                  | 0                 | 100   | -      | -     | -     | -     |
|                 | ½ MS       | 1                  | 0                 | 100   | -      | -     | -     | -     |
|                 | 1/2MS+BA   | 1                  | 0                 | 100   | -      | -     | -     | -     |

The results of experiment, from which part of the method showed that number of plantlets of *D. pseudoconantum* regenerated on VW and VW+BA+NAA that survived in acclimatisation was 75 and 2, respectively. All plantlets regenerated on ½ MS and on ½ MS+BA+NAA died. While on *D. strebloceras* was 11 on VW, 26 on VW+BA+NAA, 11 on ½ MS, and 11 on ½ MS+BA+NAA (Table 2). The number of explant regeneration from seed derived protocorm-like bodies on *D. spectabile* was 22 calli and 10 plb on VW; 44 shoot on VW+BA; 44 plb and 44 shoot on ½ MS; 10 calli and 11 shoot on ½ MS+BA (Table 3.).
Figure 1. The growth and development of *Dendrobium* adventive shoot tip explant at 40 days after inoculation (A) *D. lineale*, (B) *D. pseudoconanthum*, (C) *D. strebloceras*, (D) *D. verratfolium*, on media (1) VW, (2) VW + BA, (3) ½ MS, (4) ½ MS + BA

Figure 2. (A) *D. strebloceras* and (B) *D. pseudoconanthum* plantlets after acclimatisation from adventive shoot tip explant from media (1) ½ MS (2) VW (3) VW (4) VW+BA+NAA
Table 3  Explant regeneration of seed derived protocorm-like bodies explants of *Dendrobium* species on *in vitro* culture 40 days after inoculation

| Species       | Media | Number of explant | Contamination (%) | Number of explant regeneration | Number per explant |
|---------------|-------|-------------------|-------------------|--------------------------------|-------------------|
|               |       |                   |                   | Callus  | PLB  | Shoot | PLB | Shoot |
| *D. spectabile* | VW    | 100               | 0                 | 2.2     | 10   | 0     | 26  | 0     |
|               | VW+BA | 100               | 10                | 0       | 0    | 44    | 0   | 4     |
|               | ½ MS  | 100               | 10                | 0       | 44   | 44    | 7   | 7     |
|               | 1/2MS+BA | 100           | 10                | 10      | 0    | 11    | 0   | 1     |
| *D. lasianthera* | VW    | 50               | 100               | 0       | 0    | 0     | -   | -     |
|               | VW+BA | 50               | 100               | 0       | 0    | 0     | -   | -     |
|               | ½ MS  | 50               | 40                | 0       | 0    | 0     | -   | -     |
|               | 1/2MS+BA | 50           | 20                | 0       | 0    | 0     | -   | -     |
| *D. ascipilanense* | VW    | 130              | 76.92             | 0       | 0    | 0     | -   | -     |
|               | VW+BA | 80               | 50.00             | 0       | 0    | 0     | -   | -     |
|               | ½ MS  | 90               | 55.56             | 0       | 0    | 0     | -   | -     |
|               | 1/2MS+BA | 50           | 0                 | 0       | 0    | 0     | -   | -     |

Although the existence of plant growth hormone was essential for callus induction, callus differentiation took place on ½ MS medium without addition of plant growth hormone. Plb regeneration process from callus and its germination did not depend on exogenous plant growth hormone (Ishii et al., 1998; Roy and Banarjee, 2003; Zhao, et.al., 2008). It differed from embryonic callus of many species which needed the addition of specific plant growth hormone for somatic embryogenesis (Huan et al., 2004; Luo et al., 1999; Chengalrayan et al., 2001). In callus induction, synthesis system of endogenous hormone could be triggered and the rate of hormone raised allowing the cells to proliferate and differentiate on medium without exogenous plant growth hormone (Smith and Krikorian, 1990).

**Dendrobium** Seed-Derived Protocorm Like Bodies Explant

Table 3 shows that contamination was on plb explant *D. ascipilense* (0-76.92%) and *D. lasianthera* (20-100%).

Meanwhile, on *D. spectabile*, the contamination was 0-10%. Explant regeneration of seed - derived plb of *D. spectabile* 40 days after cultured had formed callus, plb, and shoot (Figure 3).

The number of explants regenerated after 40 days of inoculation from seed- derived protocorm like-body of *D. spectabile* was 22 calli and 10 plbs on medium VW; 44 shoots on VW+BA; 44 plbs and 44 shoots on ½ MS and 10 calli and 11 shoots on medium 1/2MS+BA. Some other plbs explants had not shown any further development (Table 3). As on *D. lasianthera* and *D. ascipilanense*, there was no significant development on day 40 after inoculation. The number per explant of plbs and shoots of *D. spectabile* after subculture was : plbs 26 on VW; 3 on VW+BA, 40 on ½ MS and 47 on ½ MS+BA; and shoots 38 on VW, 7 on VW+BA, 11 on ½ MS , 47 on ½ MS+BA (Table 4). However, none survived in acclimatisation.
In many plant species, callus plays an important part in the in vitro plant regeneration. In several orchid species, callus has also been induced successfully. Although at first orchid tissue culture did not focus on callus induction because the rate of growth was low and necrotic in the culture (Zhao et al., 2008). But recently, many lines having been produced on several orchid species were from callus (Lee and Lee, 2003; Lu, 2004). Actually, in order to obtain the same plant material, most of the time, callus was to be avoided since some characteristics might have changed. In this case, it will be better to obtain protocorm-like bodies.

Those calli succeeded to grow into plantlet via indirect protocorm like bodies for mass production. Callus induction from protocorm segment was enhanced by growth hormone like BA. It was reported that BA succeeded in inducing callus on D. fimbriatum (Roy and Banarjee, 2003), and D. candidum (Zhao et al., 2008). Plant regeneration from callus culture on orchid is usually through middle phase protocorm-like body.

On D. candidum, different development of granules globular callus came from inside or outside callus that formed cells with solid cytoplasm and little vacuoles (Zhao, et al., 2008). It was the characteristic of embryonic cells (Eady et al., 1998; Li et al., 2001; Nikam et al., 2003). Those granules could develop into plbs and the plbs in the suitable condition would develop into plantlets. While the other plbs could proliferate further and formed secondary plbs, this was a common characteristic of many orchid species (Wimber, 1963; Arditti and Ernst, 1993).

Those calli succeeded to grow into plantlet via indirect protocorm like bodies for mass production. The condition during acclimatization might not meet its requirement, which might explain the death of plantlets at acclimatitation.

**Phalaenopsis Floral Stalk Buds Explant**

Data show that P. amabilis had a high percentage of survival of explants from floral stalk buds (Figure, 4). Table 5 shows that at first culture, contamination percentage ranged from 0 to 100%, while explant life percentage ranged from 0 to 75%.
Figure 4. *P. amabilis* floral stalk bud explant regeneration at 60 days after inoculation on media (A) 1/2 MS (B) 1/2 MS+BA+NAA (C) VW (D) VW+BA+NAA

Table 5. Plant regeneration of *Phalaenopsis* explants from flower stalk buds on *in vitro* culture

| Species         | Cultured | Subcultured | Number of plantlets (10 months after first subcultured) |
|-----------------|----------|-------------|--------------------------------------------------------|
|                 | Media    | Number of explant | Days after inoculation | Contamination (%) | Explant survival (%) | Media |                                        |
| *P. amabilis*   | VW       | 3            | 4               | 0              | 50                  | VW    | 5                                        |
|                 | VW+BA    | 3            | 4               | 0              | 100                 | VW+BA+NAA | 25                                        |
|                 | 1/2 MS   | 3            | 4               | 0              | 50                  | 1/2 MS | 7                                        |
|                 | 1/2 MS+BA | 4          | 4               | 0              | 75                  | 1/2 MS+BA+NAA | 10                                        |
| *P. amboinensis*| VW       | 1            | 3               | 0              | 50                  | VW    | -                                        |
|                 | VW+BA    | 1            | 3               | 0              | 50                  | VW+BA+NAA | -                                        |
|                 | 1/2 MS   | 1            | 3               | 0              | 50                  | 1/2 MS | -                                        |
|                 | 1/2 MS+BA | 2          | 3               | 0              | 50                  | 1/2 MS+BA+NAA | -                                        |
| *P. tetraspis*  | VW       | 2            | 3               | 50             | 50                  | VW    | -                                        |
|                 | VW+BA    | 2            | 3               | 100            | 0                   | VW+BA+NAA | -                                        |
|                 | 1/2 MS   | 2            | 3               | 50             | 50                  | 1/2 MS | -                                        |
|                 | 1/2 MS+BA | 2          | 3               | 50             | 50                  | 1/2 MS+BA+NAA | -                                        |
Table 6. Shoot initiation and length of Phalaenopsis explants from floral stalk buds on in vitro culture

| Species            | Media | Cultured Shoot initiation (days after inoculation) | Shoot length 60 days after inoculation (cm) | Subcultured Shoot initiation (days after subcultured) | Shoot length 30 days after subcultured (cm) |
|--------------------|-------|--------------------------------------------------|------------------------------------------|---------------------------------------------------|-----------------------------------------|
| *P. amabilis*      | VW    | 3.                                               | 0.97                                     | -                                                 | -                                       |
|                    | VW+BA+NAA | 7                                               | 0.47                                     | 4                                                 | 1.30                                    |
|                    | ½ MS   | 5                                               | 0.20                                     | -                                                 | -                                       |
|                    | ½ MS+BA | 5                                               | 0.78                                     | 9                                                 | 0.54                                    |
| *P. amboinensis*   | VW    | -                                               | -                                        | -                                                 | -                                       |
|                    | VW+BA+NAA | -                                              | -                                        | -                                                 | -                                       |
|                    | ½ MS   | 3                                               | 0.20                                     | 0                                                 | 0                                       |
|                    | ½ MS+BA+NAA | -                                             | -                                        | -                                                 | -                                       |
| *P. tetraspis*     | VW    | -                                               | -                                        | -                                                 | -                                       |
|                    | VW+BA+NAA | -                                              | -                                        | -                                                 | -                                       |
|                    | ½ MS   | 1                                               | 0.10                                     | 0                                                 | 0                                       |
|                    | ½ MS+BA+NAA | 2                                             | 0.40                                     | 0                                                 | 0                                       |

Remarks: - = explant death; 0 = explant alive but no development

Data of acclimatisation shows that number of plantlets regenerating on VW, VW+BA+NAA, ½ MS, and ½ MS+BA+NAA which survived during acclimatisation was 5, 25, 7, and 10, respectively (Figure 5).

The mean of percentage of survival of explants of *P. amboinensis* and *P. tetraspis* was around 50%. *Phalaenopsis tetraspis* and *P. amboinensis* are *Phalaenopsis* species with a short stalk, while *P. amabilis* had a long stalk.

Technically, there were different difficulties in preparing explant from those two types of floral stalk buds. It was more difficult to prepare explant from a type of short flower stalk. Besides, it also grows slowly on in vitro culture and none grew any further.

There was shoot initiation on *P. amabilis*, *P. amboinensis* and *P. tetraspis* at first culture. At subculture, shoot initiation took place only on *P. amabilis*. Thirty days after subculture, the shoot length was 1.30 cm on VW+BA+NAA and 0.54 cm on ½ MS+BA+NAA. There was no further growth on *P. amboinensis* and *P. tetraspis*.
In Vitro Propagation of Dendrobium and Phalaenopsis

Phalaenopsis Seed - Derived Protocorm-like Bodies Explant

Table 7 shows that there was good growth and development of explant from seed-derived protocorm-like-bodies 40 days after inoculation. The percentage of life explants of P. hieroglypha was 100% and explants regenerating into plbs were 12.22% on 1/2MS+BA and 7.78% on VW+BA.

It had been reported that callus could be formed from seed-derived protocorm with a frequency of 50% (Lu, 2004), from plbs segment 53% (Huan et al., 2004), shoot tips 66.70% (Roy and Banarjee, 2003), and root tips 25% (Chen and Chang, 2000). After several subculturing, the number of callus would replicate three to five times in a month and the average number of protocorm-like bodies was 90.7 per callus culture (Lu, 2004), 134 per 0.01 g fresh weight callus 134 per 0.01 g (Huan et al., 2004), 32.5 per mass callus (Roy and Banarjee, 2003), and 29.1 per 9 mm2 mass callus (Chen and Chang, 2000).

In this experiment, the percentage of the plb formation from explants of P. hieroglypha was 8.89% on VW, 1.11% on VW+BA, 6.67% on ½ MS and 3.33% on ½ MS+BA (Figure 6). After subculturing, the percentage of plbs formation was 5% plbs and 0.56% for the shoot formation (Figure 7).

Table 7. Explant regeneration of Phalaenopsis seed-derived protocorm-like bodies 40 days after cultured

| Species       | Media  | Days of PLB initiation | Percentage of PLB from explant (%) | Days of shoot initiation | Percentage of shoot from explant (%) | Number of plbs per explant |
|---------------|--------|------------------------|-----------------------------------|--------------------------|--------------------------------------|----------------------------|
| P. hieroglypha| VW     | 12                     | 8.89                              | 0                        | 0                                    | 1.41                       |
|               | VW+BA  | 14                     | 1.11                              | 0                        | 0                                    | 1.22                       |
|               | ½ MS   | 17                     | 6.67                              | 0                        | 0                                    | 1.11                       |
|               | ½ MS+BA| 15                     | 3.33                              | 0                        | 0                                    | 0.11                       |

Table 8. Explant regeneration of Phalaenopsis explant protocorm-like bodies after first subcultured

| Species       | Days of plb initiation | Percentage of plb from explant (%) | Days of shoot initiation | Percentage of shoot from explant (%) | Number of plbs per explant |
|---------------|------------------------|-----------------------------------|--------------------------|--------------------------------------|----------------------------|
| P. hieroglypha| 15                     | 5.00                              | 2                        | 0.56                                  | 1.02                       |
The percentage of plb formation was 8.89\% on VW, 1.11\% on VW+BA, 6.67\% on ½ MS and 3.33\% on ½ MS+BA. At the subcultured, the percentage of plb formation was 5\% and the percentage of shoot formation was 0.56\%.

Table 8 shows that after subcultured, explants of protocorm-like bodies of Phalaenopsis formed plb. However, there was no plantlets of P.hieroglypha surviving during acclimatization. The explant development was slow and the size was too small to be able to develop into normal plantlets.

In this experiment only plbs formed from seed-derived protocorm-like bodies of P. hieroglypha and there was no plantlets surviving during acclimatization. The regeneration process was slow and the plantlets size was small. It could be due to the genotypes or the concentration of growth hormone used in this experiment. However, further studies are required.

CONCLUSIONS AND SUGGESTIONS

CONCLUSIONS

There were two Dendrobium species that continued to regenerate from adventive shoot tip explant. The number of plantlets of D. pseudoconantum regenerating on VW, VW+BA that survived during acclimatisation was 75 and 2 respectively. No plantlets regenerating on ½ MS and ½ MS+BA of survived during acclimatisation. The number plantlets of D. strebioceras regenerating on VW was 11.26 on VW+BA, 11 on ½ MS, and 11 on ½ MS+BA. These plantlets survived during acclimatisation. Explant regeneration from seed - derived protocorm-like bodies was only performed by D. spectabile. The number of plbs per explant of D. spectabile after subcultured on VW; VW+BA; ½ MS; ½ MS+BA was 26, 3, 40, and 47. Shoot formation on VW, VW+BA, ½ MS and ½ MS + BA was 38, 7, 11, and 47 respectively.

Explant regeneration from floral stalk buds only P. amabilis. The percentage of survival plantlets of P. amabilis in acclimatisation was 62.5\% on VW, 83.33\% on VW+BA+NAA, 77.77\% on ½ MS and 83.33\% on ½ MS+BA+NAA. Survival percentage of explant from protocorm-like bodies on P. hieroglypha was 100\%. The percentage of plb formation on VW; VW+BA, ½ MS, and ½ MS+BA was 8.89\%; 1.11\%; 6.67\% and 3.33\%. Formation of plbs and shoot was 5\% and 0.56\% respectively.

SUGGESTIONS

It was suggested that for ex situ conservation on certain species of dendrobium and phalaenopsis in the category of rare germplasms, tissue culture could be applied by using explant from adventive shoot tip, floral stalk buds and seed-derived protocorm-like body explant for vegetative seed multiplication. Each species needed specific treatment and environmental condition in acclimatization. Initial explants used should be in a great number to minimize the risk of explant survival during the in vitro culture and acclimatisation.
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