IN VITRO SEED GERMINATION, SEEDLING GROWTH AND ACCLIMATIZATION Of *Dendrobium* hybrids (Orchidaceae)

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ABSTRACT

This present study was conducted to investigate effects of basal media and peptone on in vitro seed germination, and effects of basal media and activated charcoal on in vitro seedling growth of *Dendrobium* hybrids (Orchidaceae). Mature *Dendrobium* hybrids seed pods, 3 months after pollination, were surfaced sterilized. The seeds were aseptically sowed on two basal media (Hyponex or Vacin and Went (VW), with or without addition of 2 g/l peptone). Growth of protocorms were recorded at 60 days after seed sowing. Protocorms of 2.5 month old, which were approximately 0.7-1 cm in length, were germinated on two basal media with or without 2 g/l activated charcoal (AC). All media for seed germination as well as for seedling growth were enriched with 2% sucrose, 15 % coconut water, 5 % banana homogenate and solidified with 7 g/l agar powder. Seedling growth was recorded after 4 month of cultures. Results showed that best germination media for *Dendrobium* seeds was VW + 2g/l peptone, followed by Hyponex + peptone. The media without peptone resulted in lowest protocorm growth. The best medium for in vitro seedling growth was Hyponex + AC followed by VW + AC, whereas VW without AC resulted in the lowest seedling growth. After 4 months of cultures, most of the seedlings were 3.5-4.5 cm in size, with 3-6 primary roots and 4-5 leaves. Furthermore, eight month-old in vitro grown *Dendrobium* seedlings were successfully acclimatized on shreded fern stem potting medium in shaded green house, with survival rate of 95%.

Keywords**:** *Dendrobium,* seed germination, seedling growth, media, in vitro.

INTRODUCTION

Dendrobium is the second largest genus of Orchidaceae which consists of more than 1000 species of most popular orchids all over the world. The color range of *Dendrobium* is varied from white, yellow, lavender or combination of these colors. Some of its hybrids are brilliant red or dark purple or have exotic shape and colors. Many of *Dendrobium* hybrids which predominate markets have more than one infloresence (sprays) per plant, bloom more than twice annually and suitable for either cut flower arrangement or potted flowers (Rentoul, 2003).

Dendrobium can be propagated through seeds or vegetatively through the formation of protocorm-like bodies. Propagation by seeds is the only way to obtain new qualified hybrids. The tiny seeds of *Dendrobium* generally consist of embryos coated by thick net-like testa without endosperm. These seeds hardly germinate in ex vitro condition except in symbioses with mycorhiza. Unsymbioses seed germination can be done in vitro by sowing seeds aseptically on nutrient medium consisting inorganic and organic components, as well as sucrose as a source of energy. Successful seed germination, however needs suitable media formulation as well as organic supplements added in the media. Knudson C, Vacin & Went (VW) are media formulation generally used fo orchid seed germination (Sagawa, 1991, Mc Kendrick, 2000). However, since many orchid growers are interested in breeding and in producing seedling more easily, and many of them use very small and limited laboratory, experiment to study the use of suitable complete fertilizer as a major nutrient component for in vitro seed germination compared to standard media formulations is needed. Organic supplements such as banana homogenates, coconut water casein hydrolysate, peptone or tryptone are fequently added to basal media to increase germination and growth of protocorms (Pierik, 1987, George, 1996). Peptone is a source of organic nitrogen consisting of pancreatic

digest amino acids. Peptone consists of various amino acids (arginine, aspartate acid, sisteine, glutamate acid, glysine, histidine, iso leusin, leusine, lysine, metionine, fenilalanine, threonine, triptofan, tyrosine and valine), vitamines (nicotinic acid, pyridoxine, biotin and thiamine) and phosphates (Arditti dan Ernst, 1992). Activated charcoal can be used as non-selective absorbent which might have positive effects on growth of seedlings (George, 1996). This present study was conducted to investigate effects of basal media (Vacin & Went vs Hyponex) and peptone on in vitro seed germination, and the effects of basal media (Vacin & Went vs Hyponex) and activated charcoal on in vitro seedling growth of *Dendrobium* hybrids (Orchidaceae).

MATERIALS AND METHODS

The experiments were conducted in Plant Science Laboratory, Department of Agronomy, Faculty of Agriculture The University of Lampung during June 2006-June 2007. Mature *Dendrobium* hybrid seed pods were harvested at 3 months after pollination and surfaced sterilized by soaking the pods in 30% commercial bleach (1.6% NaOCl) followed by three times rinses with sterile water. After that, the pods were dip in 96% ethanol and burnt until the flame gone. The pod was excised and seeds were sowed aseptically on two basal media (VW or Hyponex), with or without addition of 2 g/l peptone). This experiment was conducted in completely randomized design with 5 replicates. Each experimental unit consisted of one- 350 ml culture bottle containing 30 ml medium. On the surface of medium, a certain amount of *Dendrobium* seeds were sowed. Growth of protocorms represented by fresh weight of 100 protocorms and percentage of protocorms with leaf primordia were recorded at 60 days after seed sowing.

For seedling growth experiment, protocorms of 2.5 month old which were approximately 0.7-1 cm in length, were transferred to two different basal media with (VW or 2 g/l Hyponex) or without 2 g/l activated charcoal (AC). This experiment was conducted in completely randomized design with 5 replicates. Each experimental unit consisted of one culture bottle containing ten protocorms. All media for seed germination as well as for seedling growth were enriched with 2% (w/v) sucrose, 15 % (v/v) coconut water, 5 % (w/v) banana homogenate and solidified with 0.7% (w/v) agar powder. The pH of medium was adjusted to 5,7, then autoclaved at 1.1 kg/cm2 (121^0C) for 20 min. All cultures were incubated at 25 ± 2 ^{0}C under continuous light provided by fluoresencent light of about 2000 lux intensity. Seedling growth, represented by fresh weight of seedlings and number of roots was recorded after 4 month of cultures.

RESULTS AND DISCUSSION

Seed Germination experiment.

Dendrobium seed germination and subsequent protocorm growth, which were represented by 100 protocorm fresh weight and percentage of protocorms with leaf primordia, were affected by basal media formulation as well as addition of 2 g/l peptone. The best germination media resulting in the highest fresh weight of 100 protocorms was VW + 2g/l peptone, followed by Hyponex + peptone. The media without peptone resulted in lowest protocorm fresh weight. In addition, effects of peptone on the growth of *Dendrobium* protocorm growth was dependent upon basal medium used for seed germination. Addition of peptone to Vacin & Went leaded to the increase of protocorm fresh weight by 80% (from 324.09 to 585.07 mg), whereas addition of peptone to Hyponex medium increased protocorm fresh weight only by 15 % (363.98 to 418.99 mg) (Figure 1).

The case is almost the same with percentage of protocorms with leaf primordia. Addition of peptone to Vacin & Went leaded to the increase of percentage of protocorm with leaf primordia by 150% (i.e. from 27.39% to 68.60%), while addition of peptone to Hyponex resulted in the increase of the parameter by 77% (i.e. from 19,39 to 34.33%) (Figure 2). Figure 3 showed the size and

appearance of protocorms grown in the four media tested. Most protocorms in VW + peptone medium were larger in size and had leaf primordia. Protocorms from Hyponex medium without addition of peptone, on the other hand, were smallest and hardly grew their leaf primordia.

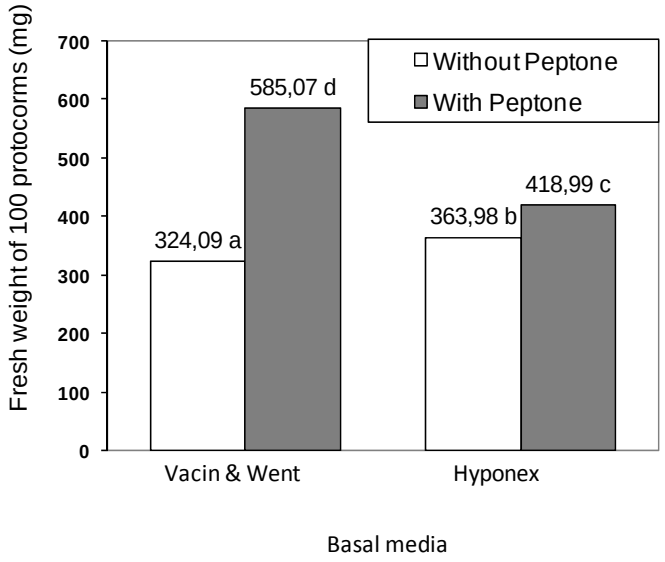

Figure 1. Effects of basal media (VW vs Hyponex) with or without addition of 2g/l peptone on fresh weight of 100 protocorms on 8 weeks after seed sowing. Mean values followed by different letters are statistically different according to LSD test (P<0.05).

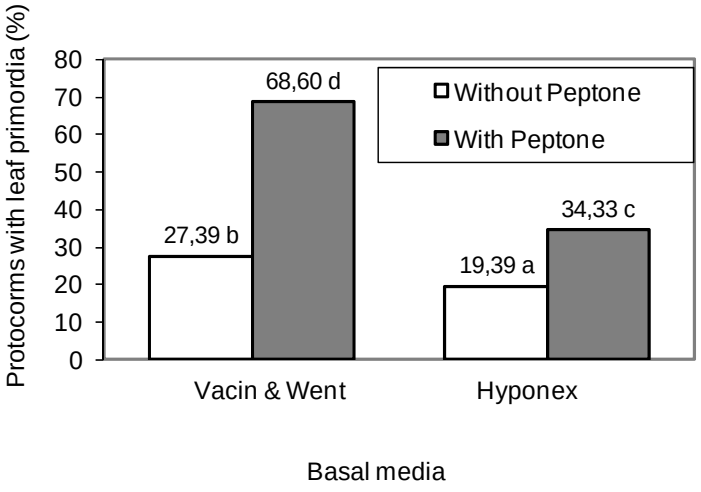

Figure 2. Effects of basal media (VW vs Hyponex) with or without addition of 2g/l peptone on percentage of protocorms with leaf primordia on 8 weeks after seed sowing. Mean value followed by different letters are statistically different according to LSD test (P<0.05).

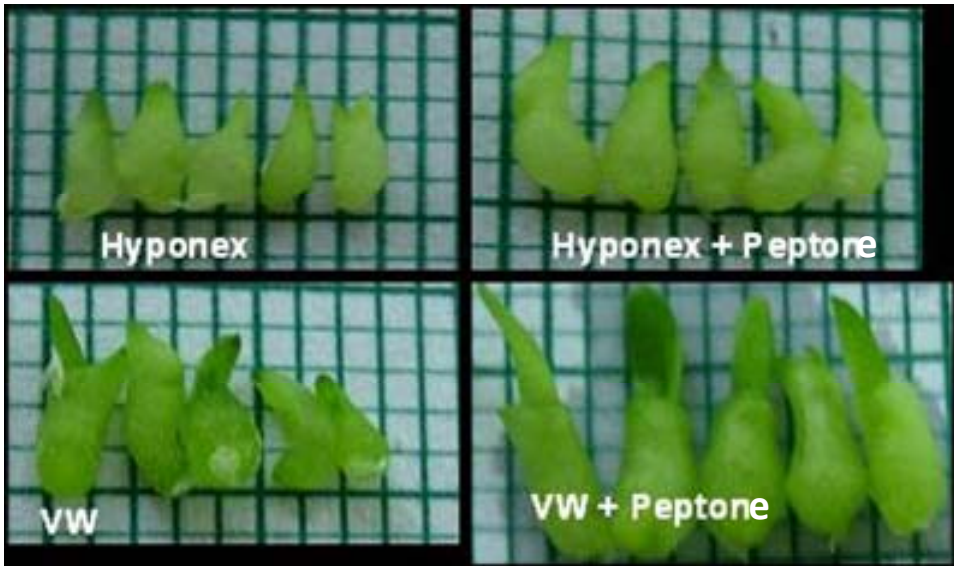

Figure 3. Appearance of protocorms in different basal media with or without addition of peptone. Protocorms in peptone-containing media are larger in size and having larger leaf primordia.

Seedling Growth Experiment.

Results of this experiment showed that Hyponex was better than VW and addition of activated charcoal (AC) into both basal media increased growth of *Dendrobium* seedling, represented by fresh weight and number of roots of seedling. Figure 4 showed that without addition of AC, Vacin & Went or Hyponex gave the same fresh weight of seedling. However, with addition of AC, Hyponex resulted in the highest fresh weight, followed by VW + AC. Without addition of AC, Hyponex medium was better than VW for root growth of seedling and addition of AC to Hyponex also resulted in even higher number of roots compared to VW + AC. In other words, the best medium for in vitro *Dendrobium* seedling growth was Hyponex + AC followed by VW + AC, whereas VW without AC resulted in the lowest seedling growth. Appearance of seedling grown in the four media tested was shown in Fig. 6. Seedlings grown in medium with AC (Fig.6b and Fig.6d) appeared more vigorous with larger and thicker leaves. After 4 months of cultures or 6 month after seed sowing, most of the seedlings were 3.5-4.5 cm in size, with 3-6 primary roots and 4-5 leaves.

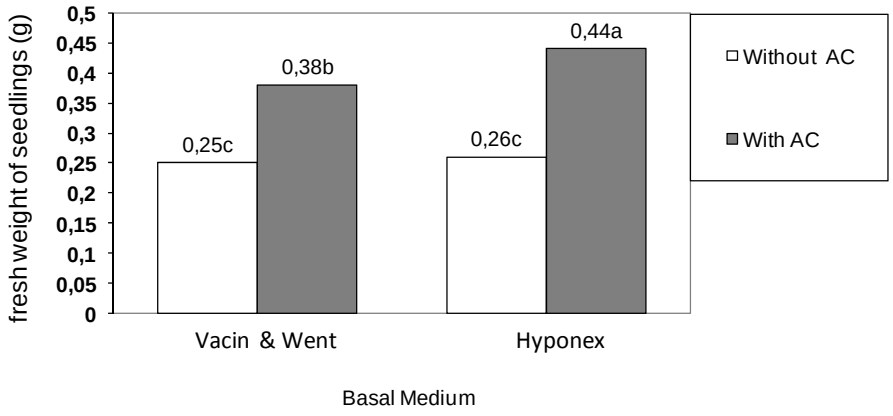

Figure 4. Effects of basal media and activated charcoal (AC) on fresh weight of *Dendrobium* seedling grown in vitro.

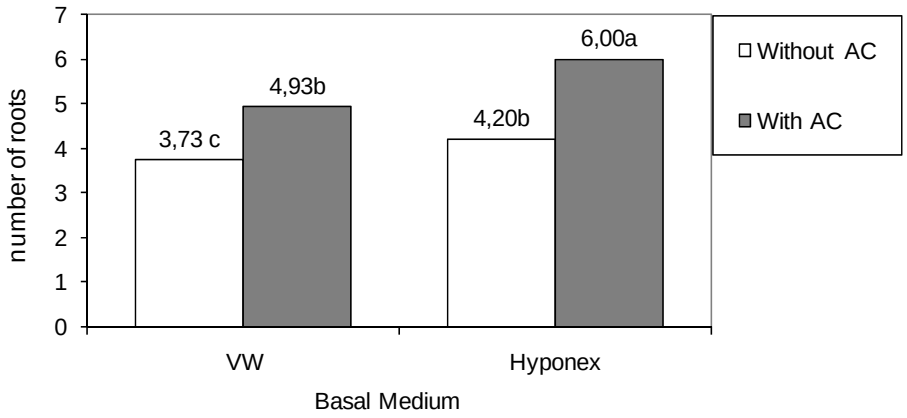

Figure 5. Effects of basal media and activated charcoal (AC) on the number of roots of *Dendrobium* seedling grown in vitro.

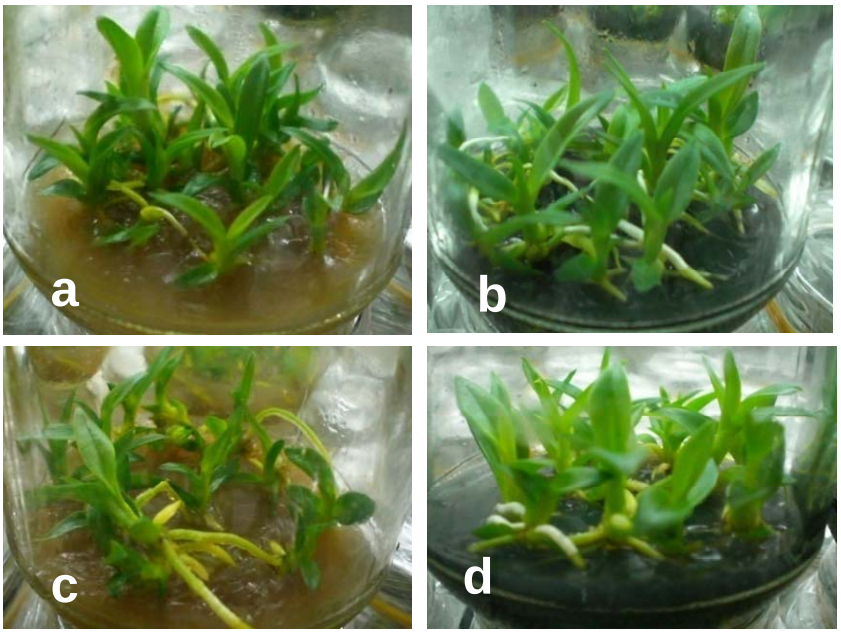

Figure 6. Appearance of *Dendrobium* seedling grown in vitro on medium (a) Vacin & Went; (b) Vacin & Went + activated charcoal; (c) Hyponex; and (d) Hyponex + activated charcoal, at 6 months after seed sowing.

The seedlings were subcultured on the same medium, and at 8 months of cultures, most of them were more than 6 cm length, have more than 5 roots and ready for acclimatization. Acclimatization was conducted by transfering seedlings to shreded fern stem potting media and placed in a shaded greenhouse. After 4 months, the survival rate of acclimatized seedlings were 95%.

Dendrobium seeds require artificial medium containing essential nutrients, sucrose and organic supplements to germinate in vitro (George, 1996). Balanced media are required for orchid seed germination as well as seedling development in vitro (Arditti, 1992). In this experiment, results showed that addition of 2 g/l peptone into both Vacin & Went and Hyponex medium significantly increased protocorm growth. The best medium for *Dendrobium* seed germination was VW +

peptone, followed by Hyponex + peptone. This results indicates that the use Hyponex fertilizer for nutrient medium components if enriched with peptone, although was not as good as VW+ peptone, resulting in better protocorm growth compared to VW or Hyponex alone. Results of this experiments also proved that peptone was a useful organic nitrogen source for *Dendrobium* seed germination and seedling growth in vitro. In previous experiment, Ramadiana *et al.* (2007) found that addition of 2 g/l peptone into ½ MS, VW or Knudson C increased percentage of *Phalaenopsis amabilis* seed germination in vitro. In other experiments, it was also found that addition of 1-2 g/l peptone to ½ MS medium increased 100 protocorm fresh weight and percentage of protocorms with leaf primordia. The higher the proportion of protocorms with leaf primordia compared to grobular stage (without leaf primordia) at 8 weeks after seed sowing means the higher protocorm growth rate at certain treatment. Globular protocorms are those that have not grown their leaf primordia yet. It appeared that peptone, which consists of of various amino acids (arginine, aspartate acid, sisteine, glutamate acid, glysine, histidine, iso leusin, leusine, lysine, metionine, fenilalanine, threonine, triptofan, tyrosine and valine), vitamines (nicotinic acid, pyridoxine, biotin and thiamine) and phosphates (Arditti dan Ernst, 1992), was a beneficial organic nitrogen source for *Dendrobium* protocorm growth.

Results of the second experiments showed that addition of 2 g/l activated charcoal (AC) into VW or Hyponex medium resulted in increased *Dendrobium* seedling growth. The best seedling growth was obtained in Hyponex + AC medium, followed by VW + AC medium. George (1996) reviewed that finely-devided activated charcoal is often used advantageously to media at different stages of tissue cultures. The positive effects of AC might be due to its potential to absorb toxic substance which may be present in media ingredients, produced as a results of autoclaving, or exuded by cultured tissue. Syaputri (2009) found that addition of 2 g/l AC along with 100-150 g/l banana homogenate into Grow More medium resulted in the best *Dendrobium* seedling growth.

CONCLUSION

Results of this experiment showed that best germination media for *Dendrobium* seeds was VW + 2g/l peptone, followed by Hyponex + peptone. The media without peptone resulted in lowest protocorm growth. The best medium for in vitro seedling growth was Hyponex + AC followed by VW + AC, whereas VW without AC resulted in the lowest seedling growth. After 4 months of cultures, most of the seedlings were 3.5-4.5 cm in size, with 3-6 primary roots and 4-5 leaves. Furthermore, eight month-old in vitro grown *Dendrobium* seedlings were successfully acclimatized on shreded fern-stem potting medium in a shaded green house, with survival rate of 95%.

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