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REGULAR ARTICLE

A STUDY ON THE USE OF ORGANIC ADDITIVES ON THE PROTOCORM-LIKE BODIES (PLBS) GROWTH OF *PHALAENOPSIS VIOLACEA* ORCHID

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SUMMARY

The potential of different concentrations of various banana cultivars extracts, papaya extract, tomato extract and coconut water (0, 10, 20, 30%) to the control (without carbon source and plant growth regulator free medium) for reliable proliferation of *Phalenopsis violacea* protocorm-like bodies (PLB) under in vitro condition. The results indicated that organic extracts were taken up from the media as shown by the increased in percentage of PLB proliferation rate. Maximum growth of PLB was obtained in half-strength Murashige and Skoog (MS) semi-solid medium supplemented with 10% of Mas (AA) banana pulp extract. However, it has been noticed that at a higher concentrations of organic extract tend to be inhibitory to the PLB proliferation which could be due to the osmotic stress. PLBs grown in half-strength MS supplemented with papaya extract had the lowest PLB proliferation rate among the different organic extracts. It can be concluded that, adding Mas (AA) banana pulp extract at concentration of 10% can be a potential replacement of sucrose in the media. Therefore, the short time length of in vitro culture and its high efficiency makes addition of suitable organic extracts well suited for mass propagation of *Phalenopsis violacea* orchid.

Keywords: *Phalaenopsis violacea*, Organic extract, Protocorm-like bodies (PLB).

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1. Introduction

Phalaenopsis orchids, which includes cultivars of *Phalaenopsis* and its intergeneric hybrid with *Doritis*, *Doritaenopsis*, is one of the most important orchids and most popular epiphytic monopodial orchid, which is grown for commercial production of cut flowers and potted plants (Belarmino and Mii, 2000; Chai et al., 2002 ; Tokuhara and Mii, 2003). *Phalaenopsis violacea* are important parent's varieties to produce novel *Phalaenopsis* hybrids with special fragrance. It is known for its beautiful flowers in terms of large size, form, colour and known to be originated from Borneo Island.

In vitro techniques for micropropagation of orchids have been widely used for commercial purpose. Micropropagation of *Phalaenopsis* through the formation of protocorm-like bodies (PLB) has been reported in recent years for regeneration of plant for breeding, microprogation and to produce target material for *Agrobacterium*-mediated transformation

(Arditti and Ernst, 1993; Tokuhara and Mii, 2003). Therefore, there is a need to establish a highly efficient PLB proliferation system to meet the demand for *Phalaenopsis* PLB in both research and industrial field. Although PLB can grow rapidly on a wide range of tissue culture media, addition care should be taken on the nutritional requirements, growth patterns and plantlet regeneration. This is due to the fact that media provides the mechanical support for the explant and it may be limited in specific media (Islam and Ichihashi, 1999; Aktar et al., 2008). The media and its formulation are very important to maximize orchid's vigor in the tissue culture condition. Balanced nutrient availability for quality medium and low cost is required to attain sustainable protocols in orchids.

Many undefined supplements such as organic additives were employed in early tissue culture media and now it is a common practice to improve the growth of orchids *in vitro*. A large number of complex additives like taro extract, coconut water, banana pulp, potato extract, peptone, tomato juice, slap honey, apple extract and beef extract can be very effective in providing undefined mixture of organic nutrients and growth factors. Therefore, the present study was undertaken to evaluate the proliferation of PLB following the supplementation of organic additives to the tissue culture media considering the low PLB formation rate and long time consumption for PLB formation. Thus, suitable organic additives are needed to be identified for large scale utilization in this orchid tissue culture.

2. Materials and Methods

Preparation of plant material

PLB of *Phalenopsis violacea* was previously initiated from healthy mature plant through shoot tip culture. The PLBs were maintained by subculturing every four weeks in fresh half-strength MS media solidified with 3g/L gelrite. The pH was adjusted to 5.70, before autoclaving. Newly developed healthy PLBs which are uniform and green were used as explants for the following experiments.

Preparation of organic extracts

Banana pulp, tomato and papaya were peeled and cut into about $1cm^3$ sections. A 50g of diced, fresh materials were homogenized with half-strength MS liquid media for 2 minutes in kitchen blender. Coconut water was extracted from tender coconut and filtered with Whatman filter paper No.1 to remove unwanted debris. Fruits and tender coconuts were purchased from Tesco Hypermarket located in Sungai Dua, Penang. All organic extracts were prepared fresh and immediately added to half-strength MS medium as required.

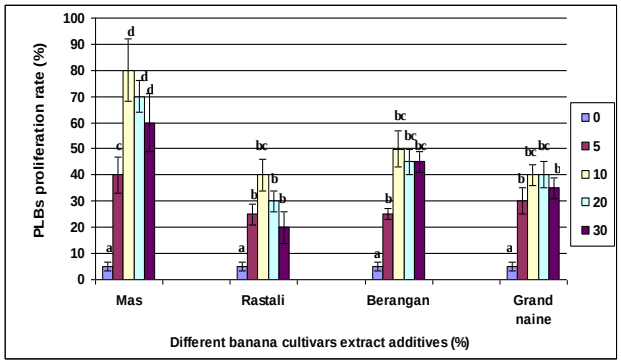
Media and culture conditions

Half strength MS semi-solid medium was used as the basal medium (BM) together with 0 (control), 10, 20, and 30% of different banana cultivars [Mas (AA), Rastali (AAB), Berangan (AAA) and Grand naine (AAA)] pulp extract, papaya extract, tomato extract and coconut water were added. Total of 0.1g of green and uniform PLBs were transplanted on BM supplemented with different concentration of organic extract additives. After preparing the media pH was adjusted to 5.8 with digital pH meter by adding 0.1 N NaOH or 0.1 N HCl. BM was supplemented with 3g/L gelrite to solidify the media. The media was autoclaved with $1.16kg/cm^2$ of pressure at 121^oC for 20 minutes. The autoclaved media was poured into new Petri dishes under sterile condition. Each treatment consisted of 3 replications with each contain 8 samples. Cultures were maintained in a growth chamber and allowed to grow at 25 ± 1^oC under 16 hours photoperiod of 1250 lux provided by fluorescent tubes to evaluate their effects on PLB proliferation. The experiment was laid out in Completely Randomized Design (CRD).The data were collected and recorded after 12 weeks on fresh weight of PLBs. Data were analyzed using one-way ANOVA and the differences contrasted using Duncan's multiple range test.

3. Results and Discussion

The growth of PLBs was stimulated on half-strength MS semi-solid medium supplemented with bananas, tomato, coconut water and papaya extracts. Response of PLBs in terms of growth varied with the type and quantity of extract in MS medium. Mas (AA), Rastali (AAB), Berangan (AAA), and Grand naine (AAA) banana pulp extract added to half-strength MS at 5, 10, 20 and 30% significantly enhanced *Phalaenopsis violacea* PLBs growth compared to that in control (5%) and other non-banana organic additives (Fig. 1).

Fig 1: Effect of different banana cultivars organic additives and concentrations on the growth of *Phalaenopsis violacea* PLBs. The results indicate the mean standard error (± SE) of 3 independent experiments with 10 replicates for each treatment concentration, the experiment was repeated thrice. Data were analyzed using one-way ANOVA and the differences contrasted using Duncan's multiple range test. Different letters indicate values are significantly different ($p<0.05$).



It appears from the present study, among the different banana cultivars extract additives of different concentration, 10% of Mas (AA) banana pulp extract added to half-strength MS was the best treatment. The present results agreed with the report of Aktar et al. (2008) who stated that the interaction between half-strength MS and Sabri banana pulp showed superior effect on fresh weight of *Dendrobium* PLBs. It could be due to the presence of higher percentage of sucrose, fructose and glucose concentrations in banana pulp extract and higher nitrate, sulphate and relatively lower phosphate content of half-strength MS medium (Aktar et al., 2008).

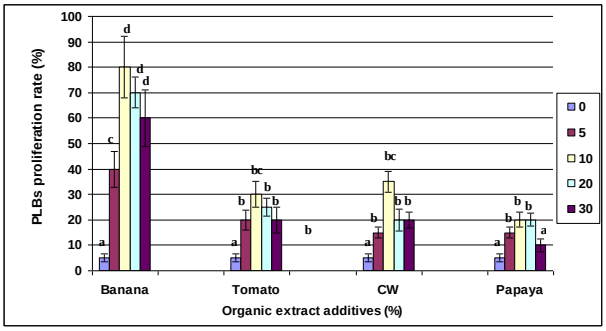
The stimulatory effect of banana pulp could be due to its ability to stabilize the pH of the medium. It is also notable that banana pulp can act as antacid to neutralize acidity condition. Apart from these, banana pulp extract contain higher level of iron, potassium, vitamin B6 and B12 and also trypthophan to promote PLBs growth. However higher concentration of banana pulp extract (20 and 30%) could be inhibitory to the *Phalaenopsis violacea* PLBs growth. Higher concentrations (20 and 30 %) of Mas (AA) banana pulp extract reduced the PLB proliferation rate from 80% to 70 and 60%. Among the four different cultivars of banana Mas (AA) pulp extract was exceptionally significant in promoting PLB growth at all concentrations even though it proved to be inhibitory at higher concentration (20 and 30%). Banana pulp extract of Rastali (AAB), Berangan (AAA) and Grand naine (AAA) appeared to enhance PLB growth and all were optimum at 10% concentration except for Grand naine (AAA) which had optimal effect at 20% concentration as well. The PLB proliferation rate was not significantly influenced by the three banana cultivars. For example, 5% pulp extract of all four different banana cultivars showed the lowest proliferation rate. Addition of 5% Mas (AA) banana pulp extract promoted the highest PLB proliferation rate, 40%. This is followed by Grand naine (AAA), Rastali (AAB) and Berangan (AAA) which scored PLB proliferation rate of 30, 25 and 25% respectively.

Among the 7 organic extracts tested including various banana cultivars, papaya extract recorded the lowest and none or less significant PLBs proliferation rate (Fig. 2). Based on the present results, 20 and 30% of papaya extract exert equal effect on PLB growth since the PLB proliferation rate was the same for both concentrations which was 20%. Furthermore, 30% papaya extract express inhibitory effect on PLB growth as it only scored 10% PLB proliferation rate. Therefore, it is less desirable to be incorporate into orchid micropropagation medium for the multiplication of PLB explants. It could be hypothesized that papaya extract contains elevated levels of phenolic compounds, organic acids and sterols which may induce cell

death and eventually inhibit the PLB proliferation rate. Furthermore, papaya contains very less amount of carbohydrate in the form of invert sugar, protein, minerals and vitamins per 100g compared to banana. Moreover, papaya contains riboflavin which stimulates rooting and suppresses the growth of callus unless the medium is supplemented with cytokinin and thiamine (Asano et al., 1996). Thus, it is less suitable for PLB proliferation purpose.

Coconut water and tomato extract supplemented into half-strength MS stimulated proliferation of *Phalaenopsis violacea* PLBs. However, higher concentrations (20 and 30%) of both tomato extract and coconut water tend to be inhibitory to PLB proliferation and were not significant at all except to that of control. Insertion of 10% coconut water to the half-strength MS medium scored 35% of PLB proliferation rate which is the highest as well as optimum level. This is followed by 20 and 30% of coconut water which promoted PLB proliferation to 20 % at both concentrations. Similar to coconut water, half-strength MS supplemented with 10% tomato extract appeared to be the optimum for PLB growth which scored 30% proliferation rate. Tomato extract at 5 and 30% produced the lowest PLB growth rate. It has been proven that coconut water is inhibitory towards growth of barley embryos and wheat embryo shoot apices (Li and Huang, 1996). Supplementation of coconut water also produces PLBs that were not uniform and smaller in sizes. Previous studies shows that coconut water supplemented to plant growth regulator free medium will cause the death of the PLBs. Similarly to this finding, coconut water does not improve PLB formation from callus of *Cymbidium* and *Cattleya* (Huan et al., 2004). Usually coconut water is added to tissue culture medium because it contains diphenyl urea, a growth factor which exhibits cytokinin-like responses. Natural dormancy inducing factors such as abscisic acid in coconut water or toxic substances released when coconut water is allowed to stand could also cause the inhibition of *Phalaenopsis violacea* PLBs. The effect of coconut water on PLB formation could be species dependent.

Fig 2: Effect of different organic additives and concentrations on the growth of Phalaenopsis violacea PLBs. The results indicate the mean standard error (+ SE) of 3 independent experiments with 10 replicates for each treatment concentration, the experiment was repeated thrice. Data were analyzed using one-way ANOVA and the differences contrasted using Duncan's multiple range test. Different letters indicate values are significantly different ($p<0.05$).



4. Conclusion

The above findings indicate that among the seven organic extract additives supplementation of 10% Mas (AA) banana pulp extract markedly enhanced and highly suitable for efficient PLB proliferation. This is followed by extracts of other banana cultivars pulp extracts, coconut water, tomato extract and papaya extract. Effects of organic extracts depend on plant sources, cultivars, and formulation of the materials used and basal medium composition. Supplementation of organic extracts to orchid culture medium is simple, and practical. Organic extract additives contain carbohydrates, protein, fat, vitamins, phenols, amino acids, fiber, hormones, sterols and organic acids at various levels. Thus, an extract being stimulatory or inhibitory towards the PLB proliferation is dependent upon the composition and its level in the extract. So analysis of constituents of banana pulp extract, coconut water, tomato juice and papaya juice is necessary for further studies in order to define a specific culture medium based on organic extract additives. Thus enable commercial orchid growers to reduce the labour cost, to improve culture media and to reduce the cost of *in vitro* culture of plants, to

shorten growth period and rapidly propagates *Phalaenopsis violacea* orchids.

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