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Growth Medium for Intergeneric Hybrids between *Phalaenopsis* 2166 and *Vanda* 'saint valentine'

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Jalan dr. Soeparno no. 63 Grendeng Purwokerto 53122
Telepon (0281) 638794 Faksimile (0281) 631700
Email: biologi@unsoed.ac.id Laman: http://bio.unsoed.ac.id

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Murni Dwiati, Triani Hardiyati, Agus Hery Susanto[1], Titi Chasanah, and Dian Palupi

Faculty of Biology, Jenderal Soedirman University, Indonesia

E-mail: murnidw@yahoo.co.id

Abstract. A study on the growth of plbs of intergeneric hybrids between *Phalaenopsis* 2166 and *Vanda* 'saint valentine' on four different in vitro culture media was conducted. This study was aimed to find out the best growth medium for intergeneric hybrids between *Phalaenopsis* 2166 and *Vanda* 'saint valentine', and to obtain medium producing the best roots for the hybrids. The media used were MS, NP, VW, and Knudson. The results showed that all four in vitro culture media were suitable for the hybrid's growth. In addition, medium NP (New Phalaenopsis) produced the best and most extended root length. Hence, it is recommended to use the media for growing plbs of the intergeneric hybrids between *Phalaenopsis* 2166 and *Vanda* 'saint valentine' before acclimatization.

1. Introduction

Phalaenopsis is an orchid genus that commonly possesses flowers of soft colors but highly distinctive and varied patterns, e.g., spots, sandy spots, lines, and dots. Vanda, on the other hand, is another one known to have bright color flowers. Thus, hybrids produced by crossing between Phalaenopsis and Vanda are expected to have flowers with a bright color of various patterns.

Intergeneric crosses between *Phalaenopsis* and *Vanda* have been reported to successfully produce seedlings [1]. In general, F1 generation seedlings resulting from such crosses tend to phenotypically show the traits of female parents. Reciprocal crosses for the same characters, including leaf shape, leaf thickness, and growth rate, make it clearer that female parent's phenotypes are more visible in the seedlings obtained. As well, crosses between *Phalaenopsis* 2166 with pink flowering of maroon red spots as female parents and *Vanda* 'saint valentine's' with bright red flowers as male parents have been reported to successfully produce fruits developing in *Phalaenopsis*. These hybrid fruits resemble *Phalaenopsis* fruits [2].

To develop the hybrid plbs of the intergeneric crosses between *Phalaenopsis* 2166 and *Vanda* 'saint valentine's,' a very specific medium is needed. Some media have been reported to grow particular orchid species, e.g., MS for *Cymbidium devonianum* Paxt. [3], Knudson for *Cymbidium elegans* (Lindl.) and *Coelogyne punctuata* Lindl., Nitsch for *Cymbidium iridioides* D. Don and KC, VW and MS for *Aerides rosea* ex Lindl. [4]. NP was found suitable for *Phalaenopsis* and *Doritacenopsis* [5], while VW was the best medium for *Vanda coerulea* Lindl. and *Vanda* ters [6]. The content of each medium varies greatly, both in nutrients, sugar as a carbon source, and water. Plb depends mostly on the in vitro culture medium to mechanically support the plb stand. These medium types and formulation are essential to obtain maximum growth and development of orchid plbs so that much better vigor of orchid seedlings will result [6].

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To support the growth and development of their plbs, the seeds of intergeneric hybrids between *Phalaenopsis* 2166 and *Vanda* 'saint valentine's' should be grown onto suitable media, i.e., NP or VW, which is known as the best growing medium for both parents or the female parent only. Therefore, this study aimed to find out the best in vitro culture medium for growing plbs of intergeneric hybrids between *Phalaenopsis* 2166 and *Vanda* 'saint valentine'.

2. Methods

The experiment was arranged in a Randomized Block Design employing four types of media, i.e., MS (Murashige & Skoog), NP (New Phalaenopsis), VW (Vacint & Went), and Knudson. There were five blocks as replications, i.e., planting date, in which all treatments were placed randomly, giving rise to 20 experimental units. Parameters examined included leaf number, leaf length and width, leaf shape, leaf tip, root number, root length, and longest root length. Light intensity, air temperature, and humidity around the culture shelf were also measured as supporting data.

½ MS, NP, VW, and Knudson media were prepared. Activated charcoal was added in the media to absorb phenol compounds produced by plbs of *Phalaenopsis*. The culture bottle containing the treatment medium was put into an autoclave and sterilized (a pressure of 0.15 MPa at 121º C for 20 minutes).

Plbs were subcultured and prepared twice. Planting was carried out inside LAF under sterile conditions. The mouth of the bottle was pre-heated with Bunsen fire. The already sterilized scalpels and tweezers were heated first. Plbs from culture bottles were taken using tweezers and then placed in sterile Petri dishes, having been coated with filter paper. These plbs were separated one by one so that they were appropriately separated, after which they were taken using sterile tweezers and planted in a culture bottle containing a growing medium. The mouth of the bottle was heated on a Bunsen fire and covered with aluminum foil and a wrapper and tied using a rubber band. Bottles having been filled with plbs were labeled corresponding to the respective treatment and planting date. These were then placed on a culture rack. The data obtained were analyzed using ANOVA. Further analysis employing the Tukey test was carried out when the treatment showed a significant effect.

3. Results

In vitro culture media showed no significant effects on leaf number, leaf length, leaf width, and root number of plbs. The absence of differences does not mean that the plbs could not grow well on the media. Instead, they all grew well, but no different growth among the media was observed.

Then, visually, the hybrids developing from the plbs showed strong vigor with sufficiently thick leaves, fresh green, and moderately healthy roots. Overall, the intergeneric hybrids between *Phalaenopsis* 2166 and *Vanda* 'Saint Valentine's' had rounded green, dark leaves with a lower surface of dark purple and the upper surface of dark green. The tip of the leaf tended to be rounded except for hybrids grown in VW medium that had lanceolate leaves with an upper surface of bright green and a lower surface of light purple (Figure 1).

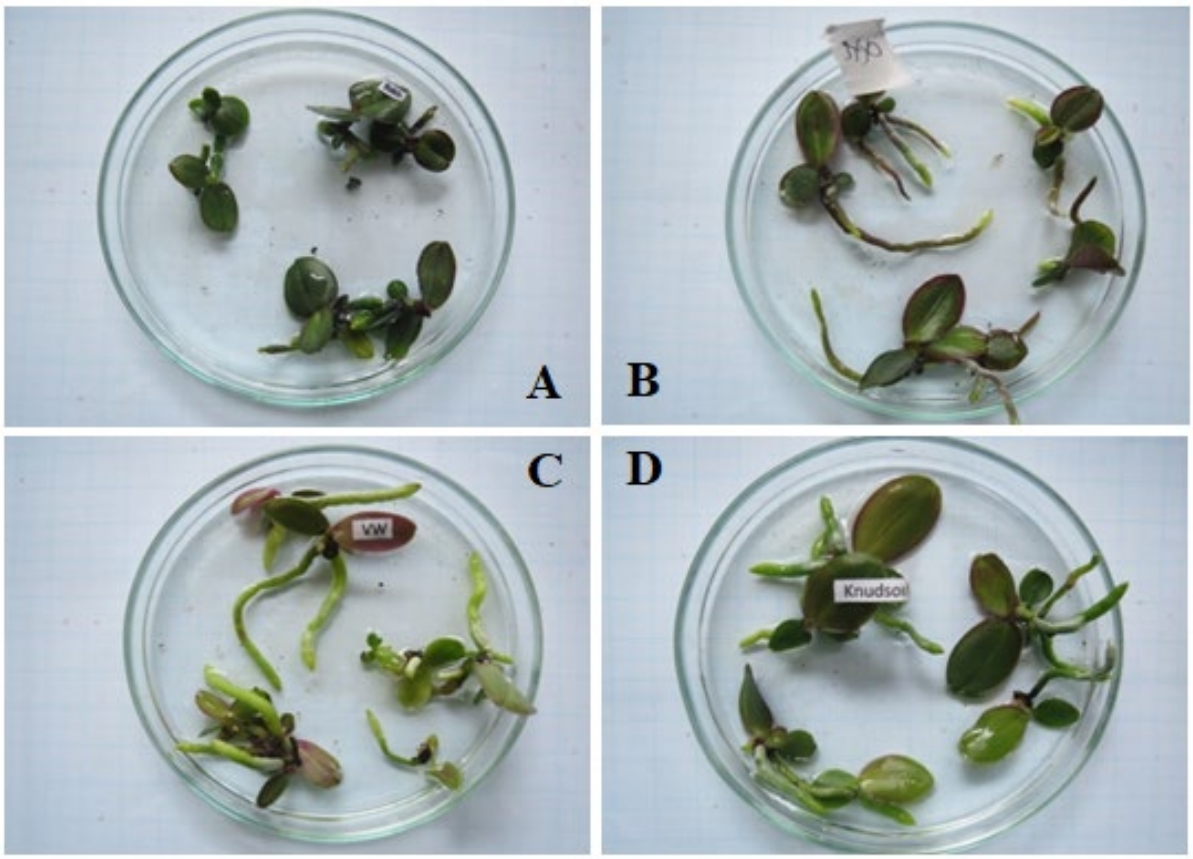

Figure 1. Vigour of intergeneric hybrids between *Phalaenopsis* 2166 and *Vanda* 'saint valentine' (A: MS medium, B: NP medium, C: VW medium, D: Knudson medium).

Significant effects of the media on root length and the longest root of the intergeneric hybrids between *Phalaenopsis* 2166 and *Vanda* 'saint valentine' were detected. Further analysis using the Tukey test proved that NP and Knudson were found as the best media for root length. Similarly, NP medium was the more suitable one for obtaining the most extended root length (Table 1).

Table 1. The effects of *in vitro* culture media on root lenght and longest root length of intergeneric hybrids between *Phalaenopsis* 2166 and *Vanda* 'saint valentine'

Medium	Root Length (mm)	Longest Root Length (mm)
NP	23.6 a	40.0 p
Knudson	21.9 a	24.3 q
VW	15.6 b	30.0 pq
MS	12.7 b	21.7 q

Note: Numbers followed by different letters show significant differences at 0.05 level of Tukey test

4. Discussion

Phalaenopsis plb requires ½ MS inorganic nutrients [7]. This condition is sufficient to grow *Phalaenopsis* plb. Observation on the growth of plbs of *Aranda, Cattleya*, and *Dendrobium* orchids in three types of in vitro culture media showed that the medium of ½ MS was found as the best medium

for the growth of plbs of intergeneric hybrids among the orchids [8]. Correspondingly, [9] reported that the ½ MS was the best medium in stimulating the growth of *Cypripedium formosum* plbs.

The differences in the vigor and appearances among the intergeneric hybrids grown in various media were caused by differences in nutrients, elements available, and the completeness of each element contained in the medium. Plant vigor depends largely on root formation, which is initiated by carbohydrate metabolism resulting in the formation of new cells. Roots serve to absorb nutrients available in the culture medium, and one of the elements mostly required for root formation is phosphor [10]. It explains why the hybrids grown in NP show the best vigor in comparison to those in the other three media. The highest phosphor content is found in NP, i.e., 462.7 mg/L, followed by VW, Knudson, and MS having phosphor content of 450 mg/L, 250 mg/L, and 170 mg/L respectively.

[3] stated that the MS medium was very good for growing *Cymbidium devonianum* Paxt plbs. This medium can accelerate plb growth three weeks faster in comparison to B5 medium. Because the media used for growing plbs of intergeneric hybrids between *Phalaenopsis* 2166 and *Vanda* 'saint valentine' were the last ones prior to the acclimatization process, an in vitro culture medium capable of producing sufficiently long roots and longest root length was needed, so that the acclimatization process would occur properly. One of the conditions that determine the success of the acclimatization process is that the roots should be strong and long enough readily adhering to the acclimatization medium. This would finally enable high adaptability of the hybrids to a new drier environment.

It was found that the intergeneric hybrids between *Phalaenopsis* 2166 and *Vanda* 'saint valentine' were able to grow well on chopped fern media after the acclimatization process. However, hybrids that are most adaptable to the environment are those grown in Knudson and NP media. Hybrids grown in both media show rapid growth. As well, their roots adapt quickly to the media. It is demonstrated by the broader, fresher, new leaf formations in comparison to those of hybrids grown on ½ MS and VW media. Long roots of hybrids are proven to assist the acclimatization process. In addition, hybrids grow and form new leaves quickly, and are responsive to leaf fertilizer.

5. Conclusion

It can be concluded that all the four media can be used for growing plbs of intergeneric hybrids between *Phalaenopsis* 2166 and *Vanda* 'saint valentine'. NP (New Phalaenopsis) medium produces the best longest root and root length of the hybrids.

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