

Handling and propagation of *Dendrobium* 'Iriana Jokowi' in Bogor Botanic Gardens, Indonesia

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Abstract. Rahayu EMD. 2016. *Handling and propagation of Dendrobium 'Iriana Jokowi' in Bogor Botanic Gardens, Indonesia. Nusantara Bioscience 8: 258-263.* *Dendrobium* 'Iriana Jokowi' is a new hybrid in Indonesia which has not been mass propagated yet. Mass propagation is necessary to ensure seedling availability. The orchid has been grown in the Orchids Greenhouse of Bogor Botanic Gardens since July 30, 2015. Handling and care for *Dendrobium* 'Iriana Jokowi', includes watering, provision of fertilizer, fungicides, and insecticides. Propagation has already been done is using seed culture. The seeds of *Dendrobium* 'Iriana Jokowi' were sown in four types of culture media, which are modified Vacin & Went (VW), modified Hyponex (HS), modified Knudson's C (KC), and Knudson's C (KCA). The seeds were able to germinate in all four tested media. The highest germination rate in the first 3 months after sowing (3 MAS) were found in KCA media, which are 93.21%. Germinated seed was then subcultured to four types of media in order to induce the growth of leaves and roots. The media used were T1A, T1V, HS, and KCA. After 5 months of planting (5 MAP), result showed that all four media were able to provide significant effect on the number of leaves and roots. The highest number of average leaves grown was 4.8 and found in T1A media. On the other hand, the highest number of average of roots grown was found in T1V media (4,2 roots).

Keywords: *Dendrobium* 'Iriana Jokowi', in vitro, seed culture

INTRODUCTION

Dendrobium 'Iriana Jokowi' is a hybrid *Dendrobium* created by Singapore Botanic Gardens (SBG). Creation of orchid hybrids in SBG were initiated by Prof. Eric Holttum in 1928. Until 2016, SBG has registered more than 500 hybrid orchids. As Singapore orchids gained fame, it became obvious that they should be used as agents to promote goodwill and foster closer ties between nations. From 1957, the Singapore Government began to honor State Visitors and other VIPs by naming selected orchid hybrids after them. This prized collection of "VIP Orchids" has become an important attraction of the National Orchid Garden. To date, the Gardens' has named over 200 VIP orchids. Examples of VIP orchids on display include *Dendrobium* Margaret Thatcher, *Dendrobium* Joe, and Jill Biden, *Papilionanda* William Catherine, *Renantanda* Akihito, *Papilionanda* Ban Ki-Moon Yoo Soon-Taek, and *Aranda* Zhu Rongji (Singapore Botanic Gardens 2016). By the time President Joko Widodo visited Singapore on July 29, 2015, the SBG gave one of their *Dendrobium* hybrids with *Dendrobium* 'Iriana Jokowi'. The Presidential Administration has then entrusted the care and propagation of the orchid to the Bogor Botanic Gardens in July 31 2015.

Dendrobium 'Iriana Jokowi' is a hybrid from various wild *Dendrobium* from Indonesia and other countries, along with hybrid *Dendrobium* (Irawati 2015, pers.com.). Wild *Dendrobium* distributed in Indonesia that becomes the parent of *Dendrobium* 'Iriana Jokowi' were *Dendrobium undulatum* R. Br., *Dendrobium phalaenopsis*

Fitzg., *Dendrobium schulleri* J.J. Sm., *Dendrobium stratiotes* Rchb.f., *Dendrobium mirbelianum* Gaudich., *Dendrobium lasianthera* J.J. Sm., *Dendrobium capra* J.J. Sm., *Dendrobium johannis* Rchb.f., *Dendrobium sutiknoi* P.O'Byrne, *Dendrobium taurinum* Lindl., and *Dendrobium violaceoflavens* J.J. Sm. Wild *Dendrobium* distributed in other countries that become the parent of *Dendrobium* 'Iriana Jokowi' is *Dendrobium tokai* Rchb.f. (Irawati 2015, pers.com.). The distribution of *Dendrobium tokai* includes Fiji and Tonga (Govaerts 2003) The flower of *Dendrobium* 'Iriana Jokowi' has twisted brownish yellow to reddish brown sepal, twisted brownish yellow to reddish brown tepal, and purple labellum.

Bogor Botanic Gardens was entrusted by the Presidential Administration to handle and propagate the orchid. Since only one specimen was available, therefore, seed culture was used as the mean of propagation. According to Vendrame et al. 2007, in vitro germination of hybrid seeds is a common practice among orchid growers. On the other hand, the seedling progenies are heterozygous and do not warrant true-to-type plants of hybrid cultivars (Martin and Madassery 2006; Chugh et al. 2009). Seed culture was already done to several *Dendrobium*, such as *Dendrobium* 'Second Love' (Ferreira et al. 2006), *Dendrobium* 'Sena Red', 'Mini WRL', 'Jaquelyn Thomas', and 'BFC Pink' (Vendrame et al. 2007), and *Dendrobium draconis* Rchb.f. (Nontachaiyapoom et al. 2011). Hence, protocols providing regeneration from various vegetative parts of mature plants are essential (Chugh et al. 2009). Some protocols have been used on the explant lateral shoot of *Dendrobium* 'Second Love' (Ferreira et al. 2006), leaves

of *Dendrobium* 'Sonia 17 and 28' (Martin and Madassery 2006), shoot-tip of *Dendrobium chrysotoxum* Lindl. (Roy et al. 2007), lateral shoots and terminal shoots of *Dendrobium* 'Zahra FR 62' (Winarto et al. 2013). The research from Ferreira et al. (2006) showed that *Dendrobium* 'Second Love' propagated using explant's lateral shoot did not exhibit any type of polymorphism. If the number of plants of *Dendrobium* 'Iriana Jokowi' has increased in Bogor Botanic Gardens, then the next propagation effort will be done from various explants from in vitro seedling or adult plant. This paper will discuss the handling and propagation of *Dendrobium* 'Iriana Jokowi' in Bogor Botanic Gardens.

The success of orchid's seed germination depends on the seed viability and media used for germination. The method seed for seed viability testing is through in vitro germination, either symbiotic or asymbiotic; germination in the wild, seed coloring with triphenyl tetrazolium chloride or fluorescein diacetate (Nontachaiyapoom et al. 2011; Dowling and Jusaitis 2012; Lemay et al. 2015; Seaton et al. 2015). Viable seed will grow and develop into protocorms. Developed protocorms from the petri dish will be subcultured to a new medium. Orchid protocorm can grow and develop into a plantlet if given enough and sufficient macro and micronutrients. The scope of the research is to observe the growth and development of *Dendrobium* 'Iriana Jokowi' plant in the Orchid Greenhouse of Bogor Botanic Gardens, testing various culture media suitable for the germination of *Dendrobium* 'Iriana Jokowi' seed, and test culture media for induction of leaves and roots on *Dendrobium* 'Iriana Jokowi' plantlets.

MATERIALS AND METHODS

The growth and development of *Dendrobium* 'Iriana Jokowi' in the Orchids Greenhouse of Bogor Botanic Gardens

The handling of *Dendrobium* 'Iriana Jokowi' done includes, watering, providing fertilizer, fungicide and insecticide spraying. Watering was done twice a day, while fertilization and spraying (fungicide and insecticide) were done once a week. The growth and development of *Dendrobium* 'Iriana Jokowi' after six months and one year of handling are displayed in a table. The propagation of *Dendrobium* 'Iriana Jokowi' was started by pollinating the orchid's flower. The pollination was done on August 11, 2015. Ripe fruit were harvested in December 22, 2015. The seed of *Dendrobium* 'Iriana Jokowi' were then sown in tissue culture media.

Seed germination test of *Dendrobium* 'Iriana Jokowi' in four different culture media

Mature capsules of *Dendrobium* 'Iriana Jokowi' were collected from Orchids Greenhouse of Bogor Botanic Gardens. The orchid capsule was soaked in 95% ethyl alcohol and flamed. These steps were repeated three times. Sterilized capsule then dissected longitudinally. The seeds were sown on the surface of the four media in the prepared petri dish. Seed germination was carried out in the culture

room at $25 \pm 2^\circ\text{C}$ and 80% relative humidity under white fluorescent tubes with a 16/8-h (day/night) photoperiod. Experiment for seed germination was using a completely randomized design with one factor, which was four media culture type. Germination media used were seed viability test media commonly used in tissue culture laboratory of Bogor Botanic Gardens (Puspitaningtyas and Handini 2014).

Media used were modified Vacin & Went (VW), modified Hyponex (HS), modified Knudson's C (KC), and Knudson's C (KCA). Modified Vacin & Went (VW) consisted of macro and micronutrients Vacin & Went with addition of active charcoal 1 g/L, bean sprout extract 100 g/L, tomato homogenate 100 g/L, coconut water 150 mL/L, dan NAA 10 mg/L. Modified Hyponex (HS) consisted of Hyponex fertilizer 0.5 g/L, potato homogenate 40 g/L, peptone 2 g/L, dan active charcoal 1 g/L. Modified Knudson's C (KC) consisted of macro and micronutrients Knudson C with addition of coconut water 150 mL/L, bean sprout extract 150 g/L, and active charcoal 1 g/L. Knudson's C (KCA) only contained macronutrient and micronutrient Knudson's C.

Each treatment consisted of four repetitions, with each repetition consisted of 100-200 sown seed. Seed germination and protocorm developmental stages were examined using the stereomicroscope attached with a digital camera (Olympus). Seed morphology before and after germination was observed. Germination percentage on 3 months after sowing (3 MAS) was counted based on the number of seeds germinate out of the 100-200 sown seed. The number of seed with intact seed coat, seed with enlarged embryo; and seed with enlarged embryo and seed coat ruptured (germinated) were counted and analyzed with ANOVA. If significant differences were found, then Duncan Post Hoc Test will be done in order to see the best media. Germinated seed that turned into protocorm were then subcultured to the leave and root growth induction media.

Induction of roots and leaves from *Dendrobium* 'Iriana Jokowi' protocorms

Protocorms of *Dendrobium* 'Iriana Jokowi' were subcultured to the media for growth induction of leaves and roots. Four media used were: T1A, T1V, HS, and KCA. Media T1A consisted of Growmore fertilizer 0.5 g/L, peptone 2g/L, banana homogenate 20 g/L, and active charcoal 1 g/L. Media T1V consisted of Vacin & Went media with the addition of banana homogenate 20 g/L, and active charcoal 1g/L. Media HS were modified Hyponex (HS) which contained Hyponex fertilizer 0.5 g/L, potato homogenate 40 g/L, peptone 2 g/L, and active charcoal 1 g/L. Media KCA only consisted of the macro and micronutrients from Knudson's C media. Experiment for roots and leaves induction was using a completely randomized design with one factor, which was four media culture type. Each treatment consisted of three repetitions, and each repetition consisted of 30 protocorms. The number of leaves and roots are grown on 5 months after planting (5 MAP) were counted. The number of leaves and roots were then analyzed with ANOVA, if significantly

differences were found, then Duncan Post Hoc Test will be done in order to see the best media. The vigor and color of the plantlets were also observed.

RESULTS AND DISCUSSION

Growth of *Dendrobium* 'Iriana Jokowi' in the Orchids Greenhouse of Bogor Botanic Gardens

The orchid plant, *Dendrobium* 'Iriana Jokowi' handled by the Presidential Administration on July 31, 2015 was in a state of stress. Therefore, the plant was immediately treated with Atonik. The plant's roots were immersed in Atonik solution (2 mL/L) for 5 minutes. The purpose of Atonik immersion is to induce the growth of new roots and also as an anti-stress agent for the plant. On the other hand, the leaves of *Dendrobium* 'Iriana Jokowi' also had yellowish spot. This was probably due to fungal infection. Therefore, the plant was immediately treated with fungicide (Dithane M45 2 g/L) by spraying it to all of the surfaces of the leaves. Fungicide treatment was done once a week.

The fungus infection was thought to be from *Cercospora dendrobii* Burnett. According to Burnett (1975), this fungus disease is known to attack only species and hybrids of *Dendrobium*. The first symptoms are noted on the underside of the leaves as light yellow spots. The spots continue to enlarge in a circular or irregular pattern and may eventually engulf the whole leaf. With age, the spots become slightly sunken, purple-black in color with the advancing margin remaining yellow. Soon after infection takes place, a corresponding yellow-green area can be noted on the top surface of the leaf. Old spots are purplish black in color and may somewhat resemble spider mite damage. Heavily infected leaves usually fall from the plant prematurely. The means of controlling this fungal infection is by spraying the plant with benomyl at the rate of 1 tablespoon per gallon of water, plus a wetting agent and repeat treatment if needed (Burnett 1975). Therefore,

we used fungicide containing benomyl, which was Benstar with concentration of 2 g/L.

The *Dendrobium* 'Iriana Jokowi' in the Orchids Greenhouse showed good growth (Figure 1). After one year, this plant had seven stems with 47 leaves, and one flower stalk consisting of 14 flowers. Fungicide treatment was proven to be successful against *C. dendrobii* fungus, and the number of yellow spots gradually decreased (Figure 1). When the plant arrived in Bogor Botanic Gardens, it had 70 yellow spots, but after one year of treatment, the number decreased into 13 spots.

Seed germination test of *Dendrobium* 'Iriana Jokowi' in four different culture media

The general shape of the seed of *Dendrobium* 'Iriana Jokowi' is an elongated ellipsoid. The seeds length varies between 0.2-0.55 mm and width between 0.09-0.1 mm (Figure 2A). The embryo is located in the center of the seed (Figure 2B). Each of the seed of *Dendrobium* 'Iriana Jokowi' consisted of testa and embryo, without endosperm (Figure 2B). Therefore, the seed will need germination media with specific composition to support its development to become a new individual.

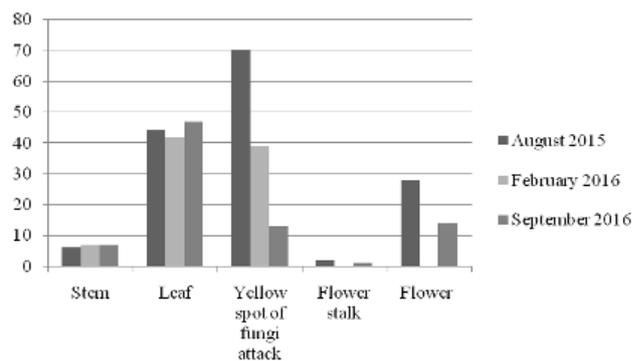


Figure 1. Graphic showing growth of *Dendrobium* 'Iriana Jokowi' after 1 year of treatment in Bogor Botanic Gardens.

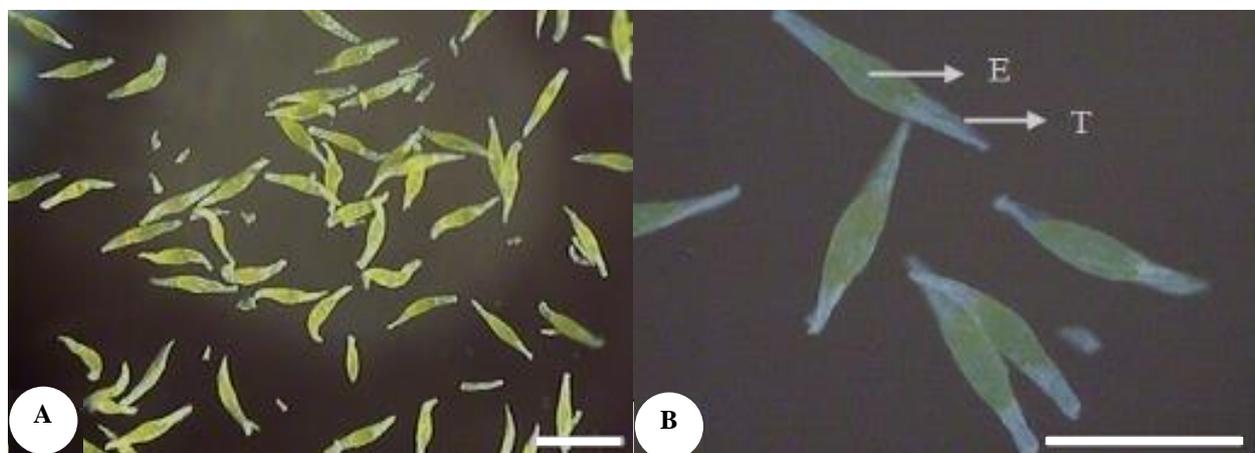


Figure 2. A. The shape of the seed of *Dendrobium* 'Iriana Jokowi' (magnified to 40x). B. The seed of *Dendrobium* 'Iriana Jokowi' showing the testa and embryo, without endosperm (magnified to 100x). E = Embryo, T = Testa. Bars = 0.5 mm

The result showed that the seeds of *Dendrobium* 'Iriana Jokowi' are able to germinate well on all four tested media (Table 1). This showed that all media are able to provide the macro and micronutrients needed for the seeds to germinate. The seeds started to germinate since 2 weeks after sowing (data not shown). According to Nontachaiyapoom et al. (2011) showed that almost 100% of *Dendrobium draconis* seeds in all treatments germinated at two weeks after sowing but the further development of these seeds varied greatly among treatments. Observation on three months after sowing (3 MAS) showed that germination rate was between 53.92 to 93.81% (Table 1). The highest germination rate observed in KCA media (93.81%) and HS media (92.86%). The high germination rate was probably due to the seeds was harvested during the right time. Zeng et al. (2012), stated that fruit harvesting during the right moment will cause a high germination rate because in this period the embryo fully formed and the testa might not be lignified, allowing it to be permeable to water and nutrients. This is probably because of efficient protein mobilization during rehydration and an undeveloped embryonic envelope. It is also because of the cuticular layer was not fully formed and suspensor cells were vacuolated, thereby enabling functional nutrition uptake (Zeng et al. 2012; Zhang et al. 2013).

According to Nontachaiyapoom et al. (2011), there are six stages in the development of seeds and protocorms of orchids. Those stages are: 0) no germination, seed with intact seed coat, 1) enlarged embryo, seed coat ruptured, 2) globular embryo, rhizoid present, 3) appearance of protomeristem, 4) emergence of first leaf, and 5) elongation of first leaf and further development. A seed is considered to be germinated if the seed coat is ruptured by the enlargement of embryo (Nontachaiyapoom et al. 2011). Observation after 3 MAS of *Dendrobium* 'Iriana Jokowi', indicated that the seed's growth and development stages were in line with Nontachaiyapoom et al. (2011) (Figure 3).

The mean number of germinated seeds after 3 MAS differ significantly among the four tested media (Table 1). The average number of seeds germinated in KCA media was 117.50 seeds, and HS media (123.50 seeds) differ significantly with VW media (68.75 seeds). Knudson's C media also been used to sow the seed of *Dendrobium chrysotoxum* (Roy et al. 2007). Hyponex is a commercial fertilizer used in culture medium for orchid seed germination and plantlet regeneration from protocorm-like bodies (plbs) (Thepsithar et al. 2009). HS media contains macronutrients NPK (25:5:20), but the average number of germinated seed was relatively the same with KCA media. Based on the observation, it was demonstrated that fertilizer (Hyponex) supplemented with potato homogenate, pepton, and active charcoal can be used as the nutrients in *Dendrobium* 'Iriana Jokowi' seed germination culture medium.

Simplification of a medium is one of the major goals of commercial growers, and the use of complex additives is important. The addition of potato homogenate in culture media enhances the growth and development of *Phalaenopsis gigantea* Blume protocorms (Murdad et al. 2010).

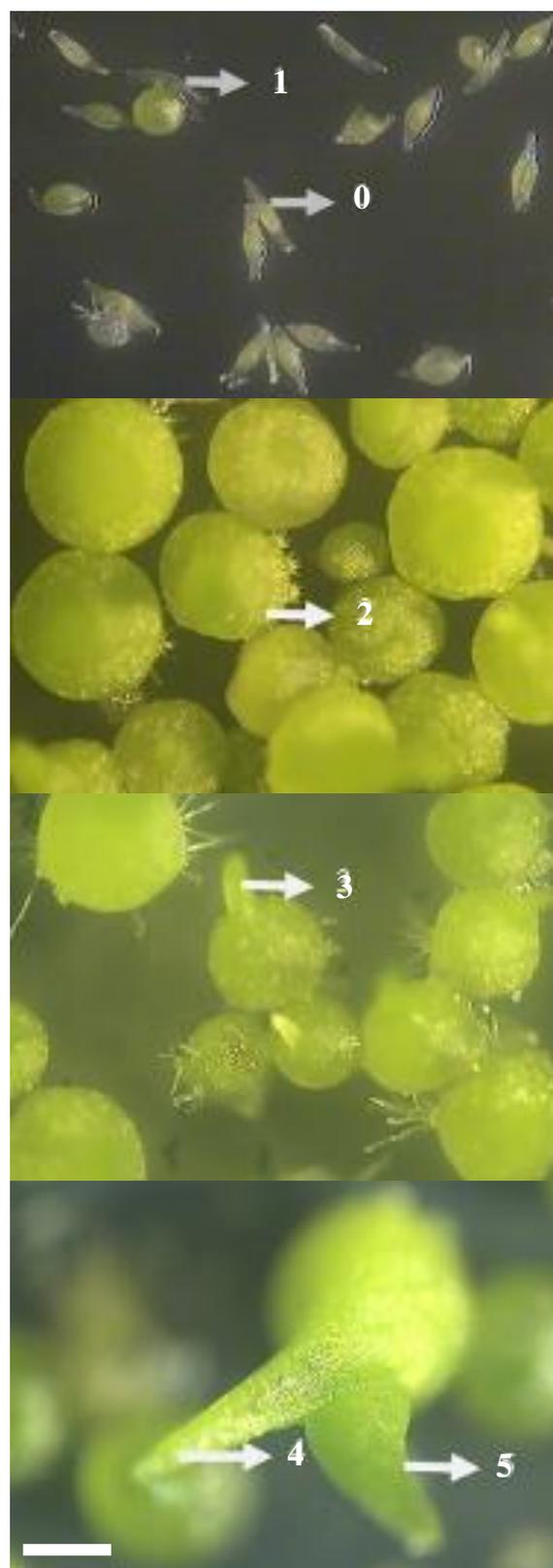


Figure 3. The stages of development of seeds and protocorm of *Dendrobium* 'Iriana Jokowi'. From left to right: Stage 0) no germination, seed with intact seed coat. Stage 1) Enlarge embryo, seed coat ruptured. Stage 2) Globular embryo, rhizoid present. Stage 3) Appearance of protomeristem. Stage 4) Emergence of first leaf. Stage 5) Elongation of first leaf and further development. Magnification of 40x. Bar = 0.5 mm.

Table 1. The germination rate and the average of seed growth of *Dendrobium* 'Iriana Jokowi' in four types of germination media on three months after sowing (3 MAS).

| Media | Germination rate (%) | Mean | | |
|-------|----------------------|----------------------------|--------------------------|--|
| | | Seed with intact seed coat | Seed with enlarge embryo | Seed with enlarge embryo, seed coat ruptured |
| VW | 53.92 | 56.00 ^a | 2.75 ^a | 68.75 ^b |
| HS | 92.86 | 9.00 ^b | 0.50 ^{ab} | 123.50 ^a |
| KC | 75.38 | 31.00 ^{ab} | 1.75 ^{ab} | 100.25 ^{ab} |
| KCA | 93.81 | 10.00 ^b | 0.00 ^b | 117.50 ^a |

Note: Values followed by different letters within a column are significantly different at $P < 0.05$ according to DMRT.

Table 2. Growth of plantlets of *Dendrobium* 'Iriana Jokowi' on the roots and leaves induction media on five months after planting (5 MAP).

| Media | Mean number of leaves | Mean number of roots | Plantlets posture |
|-------|-----------------------|----------------------|-------------------|
| T1A | 4.80 ^a | 3.50 ^b | green, vigour |
| T1V | 4.18 ^b | 4.20 ^a | green, vigour |
| HS | 4.12 ^b | 1.86 ^c | green, vigour |
| KCA | 4.00 ^b | 2.18 ^c | green, vigour |

Note: Values followed by different letters within a column are significantly different at $P < 0.05$ according to DMRT.

Potato homogenate contains polyamine and biosynthetic enzyme that affects growth and development of plant cells, especially to nucleic acid replication and cell division in mitosis. Moreover, potato consists of useful carbohydrate, sugar, protein, and vitamin for plant growth. Addition of potato homogenate in orchid culture medium promoted seed germination and enhanced growth of seedling (Arditti and Ernst 1993). Peptone is the product from digestion of protein by acid or enzyme such as meat casein and gelatin. Peptone was used as a source of organic nitrogen (Arditti and Ernst 1993). The advantages gained from this research is the efficient procedure for seed sowing of *Dendrobium* 'Iriana Jokowi' to minimize numbers of chemicals used and production cost.

Induction of roots and leaves from *Dendrobium* 'Iriana Jokowi' protocorms

Protocorms of *Dendrobium* 'Iriana Jokowi' at four months after sowing (4 MAS) were subcultured to leaves and roots induction media. The result on five months after planting (5 MSP) showed that the protocorms have grown into plantlets which had green leaves, roots, and vigorous look (Table 2 and Figure 4). The highest mean number of leaves (4.8 leaves) was observed on plantlets planted in T1A media (Table 2). While the highest mean number of roots (4.2 roots) was observed on plantlets planted in T1V media (Table 2).

T1A media consisted of Growmore fertilizer enriched with peptone, banana homogenate, and active charcoal.

Growmore fertilizer used contains NPK (20:20:20). T1V media consisted of Vacin & Went media with addition of banana homogenate and active charcoal. The addition of organic materials, such as peptone and banana homogenate seems to be able to complete the nutrients in the culture media, making the plantlet of *Dendrobium* 'Iriana Jokowi' grew well. Peptone was used as a source of organic nitrogen (Arditti and Ernst 1993). Research by Nhut et al. (2008) showed that the addition of 2 g/L peptone in the MS medium was most sufficient for shoot regeneration and it has an important role in maintaining shoot growth while NAA promoted rooting of avocado. Avocado shoots may die before rooting in the medium without peptone. This is a simple and low-cost medium for avocado tissue culture without the need for plant growth regulators.

According to Vyas et al. (2009), banana homogenate is known to promote growth of orchid seedlings. It contains minerals, IAA, GA₇, zeatin, zeatin riboside, and 2iP. Cell division-inducing compounds present in banana fruit may be responsible for its enhancing effect on orchid embryo development and differentiation. Vyas et al. (2009) also observed that the addition of banana homogenate in Knudson's C medium resulted in faster differentiation of *Dendrobium lituiflorum* Lindl. to form leaves and roots than in control media. Results observed by Zeng et al. (2012) shown that the addition of banana homogenate to culture media was the most suitable for plantlet growth of *Paphiopedilum wardii* Sumerh. in vitro since it resulted in the tallest shoots, most leaves and moderate length of root. Zhang et al. (2013) also observed similar outcome in the culture of *Cypripedium macranthos* Sw. which showed that the organic supplements improved the growth of seedlings in vitro as compared to the control. This effect was primarily observed on the addition of banana homogenate that appeared to have a significant influence on the growth of the seedlings of *C. macranthos*, in the form of increase in the number of roots and shoots developed.

HS and KCA media that were previously good for seed germination of *Dendrobium* 'Iriana Jokowi', turned up to be not effective in producing better plantlet growth. Result showed that HS and KCA media produce lower roots and plantlets number compared to T1A and T1V. The same result also observed in the culture media of *P. wardii*, where certain conditions optimal for seed germination of *P. wardii* appeared not to be similar to those required for optimal further growth and development of plantlets (Zeng et al. 2012). According to Zhang et al. (2013), organic supplements have been shown to stimulate seed germination and seedling growth of many orchids. However, their effects are complex and may vary depending on species, the types of explants or the developmental stages.

In conclusion, modified Hyponex and modified Knudson's C media can be used in seed germination of *Dendrobium* 'Iriana Jokowi'. While the better media used for induction of roots and leaves were modified Growmore and modified Vacin & Went. The addition of organic materials, such as peptone and banana homogenate can increase the growth of leaves and roots of *Dendrobium* 'Iriana Jokowi'.

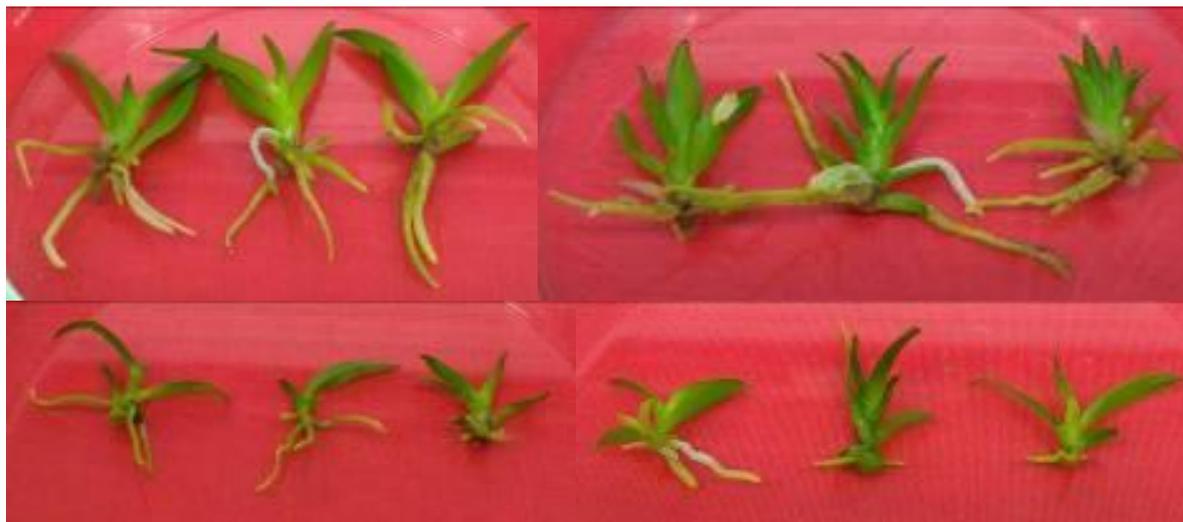


Figure 4. Plantlets of *Dendrobium* 'Iriana Jokowi' on five months after planting (5 MAP) in four testing media. From left to right: T1A, T1V, HS, and KCA media

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