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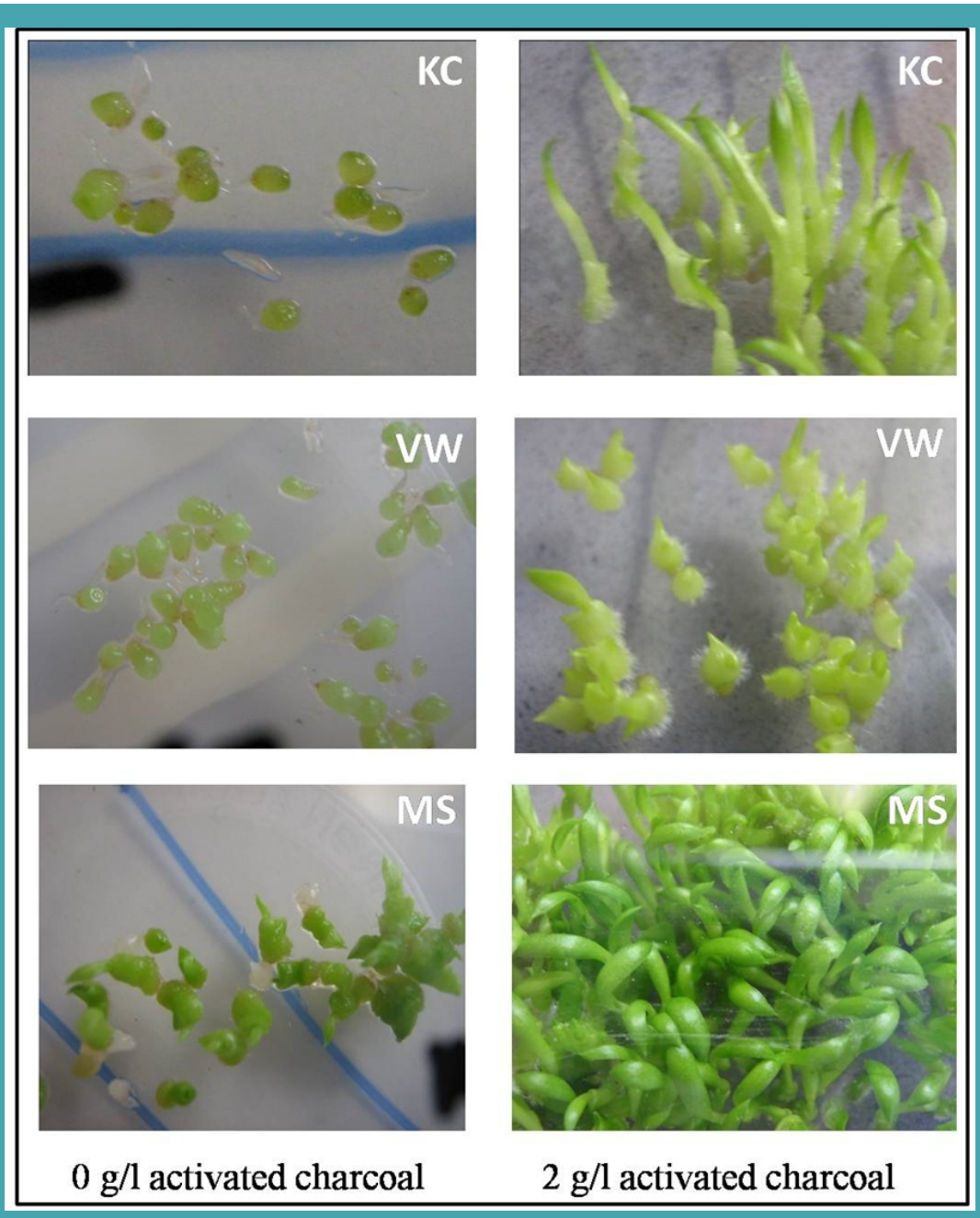

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Keterangan foto cover depan: Pertumbuhan komparatif dan perkembangan *D. taurulinum* (Comparative growth and development of *D. taurulinum*), sesuai dengan makalah pada halaman 49.

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THE EFFECT OF CULTURE MEDIA AND ACTIVATED CHARCOAL ON ASYMBIOTIC SEED GERMINATION AND SEEDLING DEVELOPMENT OF A THREATENED ORCHID *Dendrobium taurulinum* J.J. Smith IN VITRO [Pengaruh Media Kultur dan Arang Aktif pada Perkecambahan Biji dan Perkembangan Seedling Anggrek Langka *Dendrobium taurulinum* J. J. Smith *in vitro*]

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ABSTRACT

Seed germination and seedling development are an initial and crucial stage in the plant growth and development. Many factors influence seed and seedling development. The aim of the present study was to investigate the effects of different culture media (KC, VW, and MS) and the concentration of activated charcoal (0 g/l and 2 g/l) on asymbiotic seed germination and seedling development of a threatened orchid, *Dendrobium taurulinum*. Results showed that germination occurred regardless of culture media type, however the percentage of seed germination was higher on media with 2 g/l activated charcoal (> 90 %) than on media without activated charcoal (0 g/l). After 12 weeks asymbiotic seed culture, seeds sown on media with 2 g/l activated charcoal grew and developed more rapidly to leaf-bearing protocorms (seedlings) compared to media without activated charcoal.The highest occurrence of advanced seedlings (stage 4, 89.77%) was observed on MS medium with 2 g/l activated charcoal.

Key words: threatened orchid, seed germination, seedling development, conservation, *Dendrobium taurulinum*

ABSTRAK

Perkecambahan biji dan perkembangan *seedling* merupakan tahapan inisiasi penting dalam pertumbuhan dan perkembangan tanaman. Banyak faktor mempengaruhi perkecambahan biji dan perkembangan *seedling*. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh dari media kultur (KC, VW, dan MS) dan konsentrasi arang aktif (0 g/l dan 2 g/l) terhadap perkecambahan biji secara asimbiotik dan perkembangan *seedling* anggrek langka, *Dendrobium taurulinum*. Hasil penelitian ini menunjukkan bahwa biji berkecambah pada semua jenis media, tetapi, persentase perkecambahan biji pada media dengan 2 g/l arang aktif lebih tinggi (> 90 %) dibandingkan dengan persentase perkecambahan biji pada media dengan 0 g/l arang aktif. Setelah 12 minggu kultur biji secara asimbiotik, biji yang tumbuh pada media dengan 2 g/l arang aktif tumbuh dan berkembang lebih cepat menjadi *seedling* dibandingkan dengan pada media tanpa arang aktif. *Seedling* pada fase 4; fase pada tahapan pertumbuhan seedling lebih lanjut banyak dijumpai tumbuh (89.77%) pada media MS dengan 2 g/l arang aktif.

Kata kunci: anggrek yang terancam, perkecambahan biji, perkembangan *seedling*, konservasi, *Dendrobium taurulinum*

INTRODUCTION

Dendrobium taurulinum is an epiphytic orchid endemic to Seram Island, Moluccas, Indonesia. This species predominantly occurs in lowlying lands (Cribb, 1986). Many orchid species in Indonesia are experiencing population decline as a result of habitat destruction, forest degradation, and overcollection. These anthropogenic processess can be harmful for the survival and the sustainability of orchids in the natural habitat. This lead to the setting of conservation priorities for threatened orchid species in Indonesia. *Dendrobium taurulinum* (Figure 1) is one of 44 orchids that is nationally listed as a threatened species for conservation priority (Risna *et al.*, 2010).

Conservation programs for *Dendrobium taurulinum* should be formulated to protect this species from becoming extinct. Propagation plays a significant role in the conservation of threatened species to generate a large number of seedlings for reintroduction or reinforcement to increase the population size. Asymbiotic seed culture techniques are often and widely used in the propagation of orchids (Rubluo *et al.,* 1993; Ramsay and Stewart, 1998; Kauth *et al.,* 2006; Sarasan *et al.,* 2006; da Silva, 2013).

Asymbiotic seed culture is adopted in the propagation of orchids based on the biological characteristic of orchids. Orchids highly depend on mycorrhizal fungi to support seed germination and seedling development. For some orchids, this mycorrhizal dependency remains to the adult stage (entire life of orchids). Orchid seeds are tiny and lack of nutrient reserves (Arditti and Ghani, 2000). For germination, orchid seeds require external nutrient supply. This is fulfilled by forming symbiotic associations with compatible mycorrhizal fungi in natural habitats, as mycorrhizal fungi serve as suppliers of nutrients such as carbon source, vitamins and other essential nutrients (Smith and Read, 1997; Batty *et al.*, 2001;

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Figure 1. *Dendrobium taurulinum* flower (photo by: Destario Metusala)

Rasmussen, 2002, Brundrett *et al*., 2003, Swarts *et al*., 2010). In the laboratory, agar media containing essential nutrients have been developed including KC (Knudson C; Knudson, 1946), VW (Vacin and Went, 1949), and MS (Murashige and Skoog, 1962) that support orchid seed germination by acting as a substitute for nutrients supplied by mycorrhizal fungi. Orchid seeds are sown on these nutrient-containing media in aseptic culture, which is termed asymbiotic seed culture.

Asymbiotic seed germination protocols have been established for many orchid species. The protocols of orchid seed germination are species-specific, where each species require a specific protocol that can be different to other species (Pedroza-Manrique and Mican-Gutierrez, 2006; Stewart and Kane, 2006; Johnson *et al.*, 2007; Avila-Diaz *et al.*, 2009; Vyas *et al*., 2009; Vasudevan and Staden, 2010; Roy *et al*., 2011; Paul *et al*., 2011). The addition of activated charcoal in culture media has shown to promote seed germination of many orchids (Ernst, 1974; Arditti, 2008; Znaniecka *et al*., 2005; Hossain, 2008; Pacek-Bienik *et al*., 2010; Hossain *et al*., 2010; Shin *et al*., 2011). The aim of the present study was to examine various basal germination media with and without addition of activated charcoal to develop an efficient asymbiotic seed germination and seedling development protocol for *Dendrobium taurulinum* to support conservation of this threatened species.

MATERIALS AND METHODS

Seed source and sterilization

Mature capsules (dehisced capsules) were collected from *Dendrobium taurulinum* plants in the green house of Purwodadi Botanic Garden-Indonesian Institute of Sciences. Seeds from dehisced capsules (signifying that the embryos were completely developed and mature) were placed in an envelope and kept in ambient temperature. The seeds were processed for asymbiotic seed germination treatments the next day. The seeds were placed in folded filter papers, and surface sterilised in 1 % v/v sodium hypochlorite (NaOCl) solution for 30 minutes and rinsed three times in sterile distilled water.

Asymbiotic seed germination media and activated charcoal treatments

Three asymbiotic seed germination media: Knudson C (KC), Vacin and Went (VW), and Murashige and Skoog (MS) were screened for their effectiveness in promoting asymbiotic seed germination and seedling development (Table 1:Table 4). The effect of the addition of activated charcoal (®Brataco) (0 g/l and 2 g/l) into media on the asymbiotic germination and seedling development was also examined.

The pH of all media was adjusted to 5.5 with NaOH and HCl prior to autoclaving at 121° C 1.15 psi for 20 minutes. Surface sterilised seeds were inoculated onto the surface of sterile germination media in 9 cm diameter Petri plates containing ca. 25 ml media using a sterile bacterial inoculating loop. An average of 60 seeds were sown onto each plate. Three replicate plates were prepared for each treatment. Plates were sealed with a thermoplastic film (ParafilmÒ). Seed cultures were incubated at 25ºC and under 12 h light / 12 h dark photoperiod.

Germination scoring and statistical analysis

Seed germination (%) and seedling development stage (scale of 0 - 4; Table 2) were recorded after 12 weeks. Percentage of seedlings in each stage was calculated for each treatment by dividing the number of germinated seeds in each stage by the total number of seeds x100. Germination was considered to have occurred only when protocorms were

Table 1. Nutrient composition of germination media used for asymbiotic seed germination of *Dendrobium taurulinum* (modified from Dutra *et al*, 2008)

	KC	VW	MS
Macronutrients (mM)			
Ammonium	7.63	7.63	20.63
Nitrate	8.47	5.20	39.44
Calcium	4.24	1.96	3.01
Chlorine	-	-	6.03
Magnesium	1.02	1.02	1.51
Potassium	1.84	7.036	20.07
Phosphate	1.84	3.13	1.25
Sulphate	5.93	5.93	1.64
Sodium	-	-	0.002
Micronutrients (µM)			
Boron	-	-	100
Cobalt	-	-	0.1
Iron	100	100	100
Copper	-	-	0.1
Manganese	34	0.034	100
Iodine	-	-	5
Molybdenum	-	-	1
Zinc	-	-	30
Organic nutrient			
Coconut water	150 ml	150 ml	150 ml
Sucrose	20 g	20 g	20 g

KC- Knudson C; VW – Vacin & Went, MS-Murashige & Skoog

Table 2. Seed germination and description of early to late stages of seedling development during *in vitro* culture of *Dendrobium taurulinum*.

No	Stage	Description
1	Stage 0	Ungerminated seeds
2	Stage 1	Protocorm
3	Stage 2	Protocorm with the initiation of leaf primordium and rhizoids
4	Stage 3	Plantlet with first leaf
5	Stage 4	Plantlet with 2 leaves

present (stage 1) (Fig.1 and Fig. 2). Total percentage of seed germination was calculated by dividing the total number of germinated seeds at stages 1 - 4 inclusive by the total number of seeds x 100. Stage 0 represented ungerminated seeds. Data were analyzed using Analysis of Variance (ANOVA) MINITAB 14. Significant differences between treatments were determined by Tukey's test at $\alpha = 0.05$

RESULTS

Developmental stages from seeds to seedlings

Records of the growth and development of seeds of *Dendrobium taurulinum* into seedlings in *asymbiotic* culture showed the sequence of developmental stages (Fig. 2; Table 2). Seed germination was marked by the morphological development of seeds into a green spherical structure (protocorms) (stage 1). Further development was signified by the

development of leaf primordia and rhizoids that emerged from the apical and basal parts of the protocorms, respectively (stage 2). Advanced seedling development occured where a leaf primordium developed and converted into the first leaf (stage 3) and developed further into plantlets with 2 leaves (stage 4). The stages of growth and development of *D. taurulinum* seeds into seedlings were similar to other orchids as described in (Ramsay *et al.,* 1986; Johnson *et al*., 2007; Dutra *et al*., 2008; Vasudevan and van Staden, 2010; Stewart and Kane, 2010).

Asymbiotic seed germination media and activated charcoal treatments

After 12 weeks asymbiotic culture, seeds germinated on all media treatments (KC, VW, and MS) with or without activated charcoal (0 g/l and 2 g/l), with the total seed germination ranged from 81,2 % to 99,3 % (Fig. 3).However, there was varied response in terms of seed germination and seedling developmental stage between culture media with or without activated charcoal (0 g/l and 2 g/l) (Table 3).

Early developmental stages (1 and 2) showed typical appearance of protocorms and protocorms with leaf primordia and were more prevalent in culture media with 0 g/l activated charcoal than in 2 g/l activated charcoal (Table 3). A high percentage of stage 1 was observed in KC and VW with 0 g/l activated charcoal (respectively; 81.2 % and 87.8%),

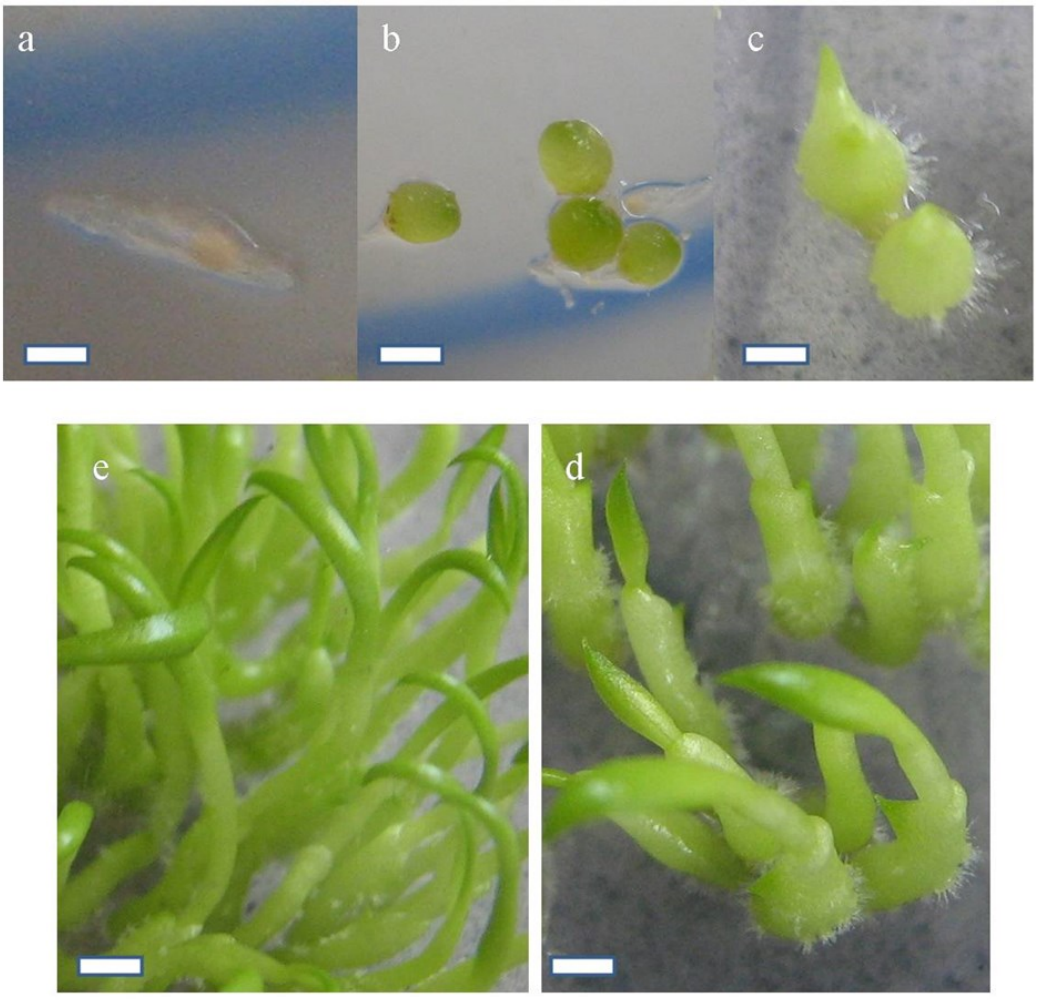

Figure 2. Asymbiotic seed germination and seedling development of *Dendrobium taurulinum* (a) Stage 0: ungerminated seeds (b) Germination, Stage 1: protocorms (c) Stage 2: protocorms with the initiation of leaf primordium and rhizords (d) Stage 3: protocorms with first green leaf (e) Stage 4: Plantlets with 2 leaves after 12 weeks of seed inoculation. Scale bars = 1 mm

while a high percentage of stage 2 was observed in MS with 0 g/l activated charcoal (64.8%). (Table 3)

Advanced seedling development (stages 3 and 4) showed the emergence of the first one to two leaves) and was more prevalent in culture media with 2 g/l activated charcoal (Table 3). Media KC with 2 g/l activated charcoal supported the highest percentage of stage 3 protocorms (93.2 %). However the most advanced seedling development (stage 4) only occured in MS with 2 g/l activated charcoal with a high (89.8%) observed. (Table 3).

It can be seen clearly that *Dendrobium taurulinum* seeds grew and developed more rapidly on media with 2 g/l activated charcoal than on media with no activated charcoal (Figure 4). After 12 weeks asymbiotic seed culture, seeds on media without activated charcoal only developed into stage 1 and 2 protocorms. Seeds sown on KC without activated charcoal only reached stage 1, while VW and MS without activated charcoal reached stage 2 (Figure 5). Seeds on media with 2 g/l activated charcoal developed into advanced stage protocorms (3 and 4) more often that on media without activated charcoal. KC and VW media with 2 g/l activated charcoal could reach stage 3 after 12 weeks seed culture, while the most advanced seedlings with two leaves (stage 4) were only found on MS with 2 g/l activated charcoal, while on KC and WV (with 2 g/l activated charcoal) only developed into seedlings with first leaf (Figure 4).

Total percentage of seed germination was calculated from the total of percentage of stage 1 to

Table 3. Comparative effects of culture media and activated charcoal on *in vitro* seed germination and seedling development stage percentage (%) after 12 week asymbiotic seed culture of *D. taurulinum*.

	Activated charcoal 0 g/l				Activated charcoal 2 g/l			
Culture Media	Stage 1 (%)	Stage 2 (%)	Stage 3 (%)	Stage 4 (%)	Stage 1 (%)	Stage 2 (%)	Stage 3 (%)	Stage 4 (%)
KC	81.2±4.6 (b)	0 (a)	0 (a)	0 (a)	0 (a)	6.1±6.12 (a)	93.2±6.8 (b)	0 (a)
VW	87.8±2.7 (b)	0 (a)	0 (a)	0 (a)	10.3±0.9 (b)	74±3.18 (b)	7.2±0.65 (a)	0 (a)
MS	13.3±7.8 (a)	64.8±9.84 (b)	9.6±2 (b)	0 (a)	2.3±2.3 (a)	0.9±0.88 (a)	3.2±3.2 (a)	89.8±10.2 (b)

Note: Different letters indicate significantly different. KC (Knudson C), VW (Vacin and Went), MS (Murashige and Skoog)

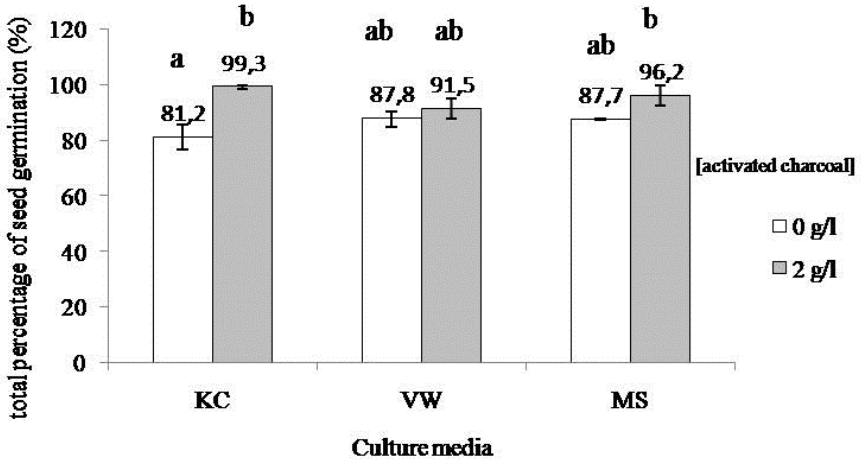

Figure 3. The effect of culture media and activated charcoal on the total percentage of seed germination of *Dendrobium taurulinum* ± SE (Standart Error). Different letters above columns indicate signifycantly different. KC (krudson C), VW (Vacin and Went), MS (Murashige and Skoog)

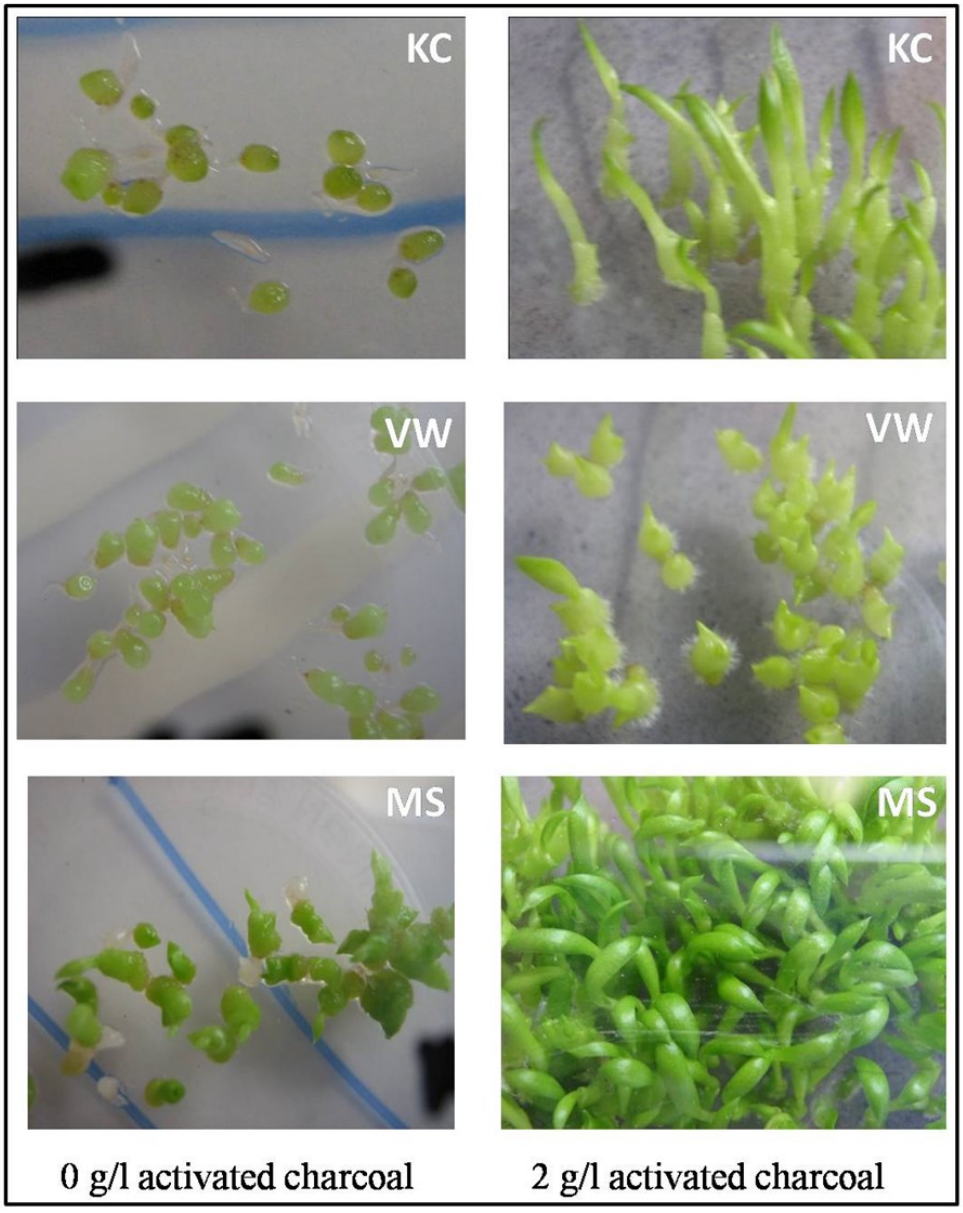

Figure 4. Comparative growth and development of *D. taurulinum* seeds on media KC, VW and MS with the addition of activated charcoal (0 g/l and g/l) after 12 weeks seed culture *in vitro*

stage 4, excluding stage 0 (ungerminated seeds). The result showed that the addition of 2 g/l activated charcoal in all culture media resulted in higher germination (92.18 - 99.32 %) than media without activated charcoal (81.2 - 87.78 %) (Figure 3).

DISCUSSION

Asymbiotic seed germination has been applied for propagation and conservation of many threatened orchids. In the present study, an efficient protocol for seed germination and seedling development of a threatened Indonesian orchid, *Dendrobium taurulinum* was developed. Orchid seeds lack of nutrient reserves, thus, exogenous nutrients are required to support seeds to germinate (Johnson *et al.,* 2011). A variety of culture media including KC (Knudson C), VW (Vacin and Went), and MS (Murashige and Skoog) that contain a variety of composition and

concentration of nutrients has been used in the present study as the substitute of nutrients supplied by mycorrhizal fungi in the natural habitat. Culture media containing activated charcoal are known to have beneficial effects with *in vitro* culture of orchids (Ernst, 1974; Arditti, 2008; Znaniecka *et al.,* 2005; Thompson *et al.,* 2006; Hossain, 2008; Pacek-Bienik *et al.,* 2010; Shin *et al.,* 2011).

In the present study, seeds germinated in all culture media with or without activated charcoal (0 or 2 g/l). However, there was variation in seed germination response and developmental stages between these treatments. Activated charcoal appears to support greater seed germination in all culture media (> 90 %) compared to treatments without activated charcoal. These results confirm results in other studies showing that activated charcoal has an important role in the seed germination of many other orchid species (Ernst, 1974; Arditti, 2008; Znaniecka *et al.,* 2005; Thompson *et al.,* 2006; Hossain, 2008; Pacek-Bienik *et al.,* 2010; Shin *et al.,* 2011). Znaniecka *et al* (2005) reported that the addition of activated charcoal in culture media resulted in the highest percentage of seed germination of *Encyclia* aff. *oncidioides*. Hossain (2008) also demonstrated that the percentage of seed germination of *Epidendrum ibaguense* increased on media supplemented with activated charcoal. Pacek-Bienik *et al* (2010) also reported that asymbiotic seed germination and seedling development of *Zygostates grandiflora* was better on media supplemented with activated charcoal. Shin *et al* (2011) also reported the significant effect of the activated charcoal to increase the percentage of seed germination of *Calanthe* hybrids. In contrast to the promotive effect of the activated charocoal on seed germination and seedling development that have been widely reported, Pierik *et al*., (1988) reported that activated charcoal had no effects on seed germination and slightly inhibited seed germination of *Paphiopedilum ciliore*, but positively increased further seedling development.

After 12 weeks asymbiotic seed culture, different responses in developmental stages between treatments was also observed in the present study. While most seeds still initiated early growth and development in media treatments without activated charcoal, the formation of a high percentage of advanced developmental protocorm stages (marked with the emergence of leaves at stages 3 and 4) was observed in the media treatments with activated charcoal. This suggests that activated charcoal is able to accelerate seedling growth and development. The presence of activated charcoal in the media also enhanced the development of leaves and roots in *Zygostates grandiflora* (Pacek-Bienik *et al.*, 2010). Roy *et al* (2011) also reported the advantage of the addition of activated charcoal in media for better seedling growth and development of *Vanda coerulea.* The promotive effect of activated charcoal is related to the ability of activated charcoal (i) to absorb toxic metabolites and phenolic compounds that decreased toxic metabolites and phenolic compounds in the culture media. (ii) to remove the inhibitory substances from the culture media (iii) to release substances naturally present in AC that promote growth (Thomas, 2008; Thompson *et al*., 2006; Mensuali Sodi *et al*., 1993).

Although all media treatments containing activated charcoal stimulated germination to the advanced seedling stage with the formation of leaves, the most advanced seedlings (with the development of two leaves; stage 4) were only observed on MS media with 2 g/l activated charcoal, while seedlings on KC and VW (with 2 g/l activated charcoal) only showed first leaf or stage 3 protocorm development. This might be due to the composition and concentration of macronutrients and micronutrients in MS medium being more complex and at higher concentrations compared to KC and VW media. The concentration of ammonium and nitrate in MS medium is higher (20.63 and 39.44 mM) compared to KC (7.63 and 8.47 mM) and VW (7.63 and 5.20 mM) media. Altogether, the availability of nitrogen in MS medium is much greater (60.07 mM) compared to KC (16.1 mM) and VW (12.83 mM) media. Nitrogen is a critical nutrient compound required for plant growth and development (Raghavan and Torrey, 1964; Dijk and Eck, 1995a, 1995b). MS media also contains more complex micronutrients including (B, Co, Fe, Cu, Mn, I, Mo, and Zn) compared to KC and VW media that only contain two compounds of micronutrients (Fe and Mn). Micronutrients are also essential for growth and development of plants.

The suitability of MS medium for seed germination and seedling development has also been reported for other orchids: such as *Satyrum nepalense* (Mahendran and Bai, 2009), *Laelia speciosa* (Avila Diaz *et al.*, 2009), *Dendrobium chrysanthum* (Rao and Barman, 2014), *Dendrobium hookerianum* (Paul *et al.,* 2011), *Cymbidium mastersii* (Mohanty *et al.,* 2012). Other media compositions tested for orchid seed germination and seedling development, such as Hydro-Coljap medium (for *in vitro* germination and growth tof *Odontogossum gloriosum;* Pedroza-Manrique and Mican-Gutierrez, 2006), PhytoTechnology Orchid Seed Sowing Medium (for *in vitro* growth of *Eulophia alta* (Johnson *et al.*, 2007), VW Medium for *Bletia purpurea* (Dutra *et al.,* 2008), indicate species specificity as regards orchid seed germination and seedling development.

The present study describes a simple and efficient protocol for seed germination and seedling development of *Dendrobium taurulinum* using media MS with the addition of 2 g/l activated charcoal to generate a large number of seedlings in a relatively short period.

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Pedoman Penulisan Naskah Berita Biologi

Berita Biologi adalah jurnal yang menerbitkan artikel kemajuan penelitian di bidang biologi dan ilmu-ilmu terkait di Indonesia. Berita Biologi memuat karya tulis ilmiah asli berupa makalah hasil penelitian, komunikasi pendek dan tinjauan kembali yang belum pernah diterbitkan atau tidak sedang dikirim ke media lain. Masalah yang diliput, diharuskan menampilkan aspek atau informasi baru.

Tipe naskah

1. **Makalah lengkap hasil penelitian (*original paper*)**
   Naskah merupakan hasil penelitian sendiri yang mengangkat topik yang *up-todate*. Tidak lebih dari 15 halaman termasuk tabel dan gambar. Pencantuman lampiran seperlunya, namun redaksi berhak mengurangi atau meniadakan lampiran.
2. **Komunikasi pendek (*short communication*)**
   Komuniasi pendek merupakan makalah hasil penelitian yang ingin dipublikasikan secara cepat karena hasil termuan yang menarik, spesifik dan baru, agar dapat segera diketahui oleh umum. Artikel yang ditulis tidak lebih dari 10 halaman. Hasil dan pembahasan boleh digabung.
3. **Tinjauan kembali (*review*)**
   Tinjauan kembali merupakan rangkuman tinjauan ilmiah yang sistematis-kritis secara ringkas namun mendalam terhadap topik penelitian tertentu. Hal yang ditinjau meliputi segala sesuatu yang relevan terhadap topik tinjauan yang memberikan gambaran '*state of the art*', meliputi temuan awal, kemajuan hingga issue terkini, termasuk perdebatan dan kesenjangan yang ada dalam topik yang dibahas. Tinjauan ulang ini harus merangkum minimal 30 artikel.

Struktur naskah

1. **Bahasa**
   Bahasa yang digunakan adalah bahasa Indonesia atau Inggris yang baik dan benar.
2. **Judul**
   Judul harus singkat, jelas dan mencerminkan isi naskah diikuti oleh nama dan alamat surat menyurat penulis. Nama penulis untuk korespondensi diberi tanda amplop cetak atas (*superscript*).
3. **Abstrak**
   Abstrak dibuat dalam dua bahasa, bahasa Indonesia dan Inggris. Abstrak memuat secara singkat tentang latar belakang, tujuan, metode, hasil yang signifikan, kesimpulan dan implikasi hasil penelitian. Abstrak berisi maksimum 200 kata, spasi tunggal. Di bawah abstrak dicantumkan kata kunci yang terdiri atas maksimum enam kata, dimana kata pertama adalah yang terpenting. Abstrak dalam bahasa Inggris merupakan terjemahan dari bahasa Indonesia. Editor berhak untuk mengedit abstrak demi alasan kejelasan isi abstrak.
4. **Pendahuluan**
   Pendahuluan berisi latar belakang, permasalahan dan tujuan penelitian. Sebutkan juga studi terdahulu yang pernah dilakukan.
5. **Bahan dan cara kerja**
   Pada bagian ini boleh dibuat sub-judul yang sesuai dengan tahapan penelitian. Metoda harus dipaparkan dengan jelas sesuai dengan standar topik penelitian dan dapat diulang oleh peneliti lain. Apabila metoda yang digunakan adalah metoda yang sudah baku cukup ditulis sitasi dan apabila ada modifikasi harus dituliskan dengan jelas bagian mana dan apa yang dimodifikasi.
6. **Hasil**
   Sebutkan hasil-hasil utama yang diperoleh berdasarkan metoda yang digunakan. Apabila ingin mengacu pada tabel/grafik/diagram atau gambar uraikan hasil yang terpenting dan jangan menggunakan kalimat 'Lihat Tabel 1'. Apabila menggunakan nilai rata-rata harus menyebutkan standar deviasi.
7. **Pembahasan**
   Jangan mengulang isi hasil. Pembahasan mengungkap alasan didapatkannya hasil dan apa arti atau makna dari hasil yang didapat tersebut. Bila memungkinkan, bandingkan hasil penelitian ini dengan membuat perbandingan dengan studi terdahulu (bila ada).
8. **Kesimpulan**
   Menyimpulkan hasil penelitian, sesuai dengan tujuan penelitian, dan penelitian berikut yang bisa dilakukan.
9. **Ucapan terima kasih**
10. **Daftar pustaka**
    Tidak diperkenankan untuk mensitasi artikel yang tidak melalui proses peer review. Apabila harus menyitir dari "Laporan" atau "komunikasi personal" dituliskan 'unpublished' dan tidak perlu ditampilkan di daftar pustaka. Daftar pustaka harus berisi informasi yang *up to date* yang sebagian besar berasal dari *original papers*. Penulisan terbitan berkala ilmiah (nama jurnal) tidak disingkat.

Format naskah

1. Naskah diketik dengan menggunakan program Word Processor, huruf New Times Roman ukuran 12, spasi ganda kecuali Abstrak. Batas kiri -kanan atas-bawah masing-masing 2,5 cm. Maksimum isi naskah 15 halaman termasuk ilustrasi dan tabel.
2. Penulisan bilangan pecahan dengan koma mengikuti bahasa yang ditulis menggunakan dua angka desimal di belakang koma. Apabila menggunakan bahasa Indonesia, angka desimal menggunakan koma (,) dan titik (.) bila menggunakan bahasa Inggris. Contoh: Panjang buku adalah 2,5cm. Lenght of the book is 2.5 cm. Penulisan angka 1-9 ditulis dalam kata kecuali bila bilangan satuan ukur, sedangkan angka 10 dan seterusnya ditulis dengan angka. Contoh lima orang siswa, panjang buku 5 cm.
3. Penulisan satuan mengikuti aturan *international system of units*.
4. Nama takson dan kategori taksonomi merujuk kepada aturan standar termasuk yang diakui. Untuk tumbuhan *International Code of Botanical Nomenclature* (ICBN), untuk hewan *International Code of Zoological Nomenclature* (ICZN), untuk jamur *International Code of Nomenclature for Algae, Fungi and Plant* (ICFAFP), *International Code of Nomenclature of Bacteria* (ICNB), dan untuk organisme yang lain merujuk pada kesepakatan Internasional. Penulisan nama takson lengkap dengan nama author hanya dilakukan pada bagian deskripsi takson, misalnya pada naskah taksonomi. Sedangkan penulisan nama takson untuk bidang lainnya tidak perlu menggunakan nama author.
5. Tata nama di bidang genetika dan kimia merujuk kepada aturan baku terbaru yang berlaku.
6. Ilustrasi dapat berupa foto (hitam putih atau berwarna) atau gambar tangan (*line drawing*).
7. Tabel
   Tabel diberi judul yang singkat dan jelas dalam bahasa Indonesia dan Inggris, sehingga Tabel dapat berdiri sendiri. Tabel diberi nomor urut sesuai dengan keterangan dalam teks. Keterangan Tabel diletakkan di bawah Tabel. Tabel tidak dibuat tertutup dengan garis vertikal, hanya menggunakan garis horisontal yang memisahkan judul dan batas bawah.
8. Gambar
   Gambar bisa berupa foto, grafik, diagram dan peta. Judul ditulis secara singkat dan jelas. Keterangan yang menyertai gambar harus dapat berdiri sendiri, ditulis dalam bahasa Indonesia dan Inggris. Gambar dikirim dalam bentuk .jpeg dengan resolusi minimal 300 dpi dan terpisah dari badan tulisan atau dalam file yang berbeda.
9. Daftar Pustaka
   Sitasi dalam naskah adalah nama penulis dan tahun. Bila penulis lebih dari satu menggunakan kata 'dan' atau *et al.* Contoh: (Kramer, 1983), (Hamzah dan Yusuf, 1995), (Premachandra *et al.,* 1992). Bila naskah ditulis dalam bahasa Inggris yang menggunakan sitasi 2 orang penulis maka digunakan kata 'and'. Contoh: (Hamzah and Yusuf, 1995).

   a. Jurnal
      Nama jurnal ditulis lengkap.
      Premachandra GS, H Saneko, K Fujita and S Ogata. 1992. Leaf Water Relations, Osmotic Adjustment, Cell Membrane Stability, Epicu-tilar Wax Load and Growth as Affected by Increasing Water Deficits in Sorghum. *Journal of Experimental Botany* **43,** 1559-1576.

b. Buku
   Kramer PJ. 1983. *Plant Water Relationship*, 76. Edisi ke-(bila ada). Academic, New York.
c. Prosiding atau hasil Simposium/Seminar/Lokakarya.
   Hamzah MS dan SA Yusuf. 1995. Pengamatan Beberapa Aspek Biologi Sotong Buluh *(Sepioteuthis lessoniana)* di Sekitar Perairan Pantai Wokam Bagian Barat, Kepulauan Aru, Maluku Tenggara. *Prosiding Seminar Nasional Biologi XI,* Ujung Pandang 20-21 Juli 1993. M Hasan, A Mattimu, JG Nelwan dan M Litaay (Penyunting), 769-777. Perhimpunan Biologi Indonesia.
d. Makalah sebagai bagian dari buku
   Leegood RC and DA Walker. 1993. Chloroplast and Protoplast. In: *Photosynthesis and Production in a Changing Environment.* DO Hall, JMO Scurlock, HR Bohlar Nordenkampf, RC Leegood and SP Long (Eds), 268-282. Champman and Hall. London.
e. Thesis dan skripsi.
   Keim AP. 2011. Monograph of the genus *Orania* Zipp. (Arecaceae; Oraniinae). University of Reading, Reading. [PhD. Thesis].
f. Artikel online.
   Artikel yang diunduh secara online mengikuti format yang berlaku misalnya untuk jurnal, buku atau thesis, serta dituliskan alamat situs sumber dan waktu mengunduh. Tidak diperkenankan untuk mensitasi artikel yang tidak melalui proses *peer review* atau artikel dari laman web yang tidak bisa dipertangung jawabkan kebenarannya seperti wikipedia.
   Forest Watch Indonesia[FWI]. 2009. Potret keadaan hutan Indonesia periode 2000-2009. *http://www.fwi.or.id*. (Diunduh 7 Desember 2012).

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Untuk setiap penelitian yang melibatkan hewan sebagai obyek penelitian, maka setiap naskah yang diajukan wajib disertai dengan '*ethical clearance approval*' terkait *animal welfare* yang dikeluarkan oleh badan atau pihak berwenang.

Lembar ilustrasi sampul
Gambar ilustrasi yang terdapat di sampul jurnal Berita Biologi berasal dari salah satu naskah. Oleh karena itu setiap naskah yang ada ilustrasi harap mengirimkan ilustrasi dengan kualitas gambar yang baik disertai keterangan singkat ilustrasi dan nama pembuat ilustrasi.

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