sucipto hariyanto <sucipto-h@fst.unair.ac.id>

2795108: Revision requested

3 messages

Isabel Marques <alby.belza@hindawi.com> Mon, Apr 27, 2020 at 10:44 PM
To: sucipto-h@fst.unair.ac.id

Dear Dr. Hariyanto,

In order for your submission "Organic Compounds: Contents and Its Role to Improve Seed Germination and Protocorm Development in Orchids" to International Journal of Agronomy to proceed to the review process, there needs to be a revision.

Reason & Details: "Please take attention to the reviewer comments, which should have been addressed on your response. The response letter should provide details of how changes were made, answering point-by-point the comments raised. "

For more information about what is required, please click the link below.

MANUSCRIPT DETAILS

Kind regards,
Alby Belza
International Journal of Agronomy

sucipto hariyanto <sucipto-h@fst.unair.ac.id> Wed, Apr 29, 2020 at 10:20 AM
To: Isabel Marques <alby.belza@hindawi.com>

Dear Alby Belza,

Thank you for your information about our manuscript, we will revise as soon as possible.

Best regards,
Sucipto Hariyanto
[Quoted text hidden]

---

sucipto hariyanto <sucipto-h@fst.unair.ac.id> Sat, May 9, 2020 at 10:25 PM
To: Isabel Marques <alby.belza@hindawi.com>

Dear Isabel Marques,

We hereby provide information that we have submitted our revised manuscript with the response letter via system at 9 May 2020
Thank you for your attention and cooperation.

Best regards,
Sucipto hariyanto

On Mon, Apr 27, 2020 at 10:44 PM Isabel Marques <alby.belza@hindawi.com> wrote:
[Quoted text hidden]

2795108 : Author Feedback Needed

4 messages

Evangeline Anand <evangeline.anand@hindawi.com> Thu, May 21, 2020 at 7:15 PM
To: sucipto-h@fst.unair.ac.id
Cc: edy-s-w-u@fst.unair.ac.id

Dear Dr. Hariyanto,

This is regarding the manuscript “2795108” titled “Organic Compounds: Contents and Its Role to Improve Seed Germination and Protocorm Development in Orchids" submitted to the journal “International Journal of Agronomy”.

While proceeding with the final stage of the Review process we found that the following queries need to be addressed.

1.The resolution of all figure is insufficient. We require higher-resolution and better-quality versions which include clear labels. Each figure should be a separate ps, eps, fig, ai, Visio, wmf, emf, Word, Excel, PowerPoint, opj, or PDF file which can be edited. Please note that jpg, bmp, png, and tif files cannot be edited by default.

2.Please upload Revised editable version of the manuscript under main manuscript section. (Doc format)

An early reply would be appreciated.

Best Regards,
Evangeline Anand,
Quality Check Team.

---

sucipto hariyanto <sucipto-h@fst.unair.ac.id> Fri, May 22, 2020 at 8:08 AM
To: Evangeline Anand <evangeline.anand@hindawi.com>

Dear Evangeline A.

Thank you for your information, We will upload our revised editable manuscript as soon as possible.
We still double check the manuscript.

Best regards,
Sucipto Hariyanto
[Quoted text hidden]

---

sucipto hariyanto <sucipto-h@fst.unair.ac.id> Fri, May 22, 2020 at 2:32 PM
To: Evangeline Anand <evangeline.anand@hindawi.com>

Dear Evangeline,
We inform you that we have just uploaded 4 files via systems, which are:
1. Cover letter
2. Main manuscript (doc. file)
3. Figure (ppt.file)
4. Table (doc. file

We also attach 4 files
Thank you for your attention and cooperation.

Best regards,
Sucipto Hariyanto

[Quoted text hidden]

4 attachments

Cover letter May 22.doc
27K

Main article.doc
224K

Fig.1.pptx
116K

Table 1.Review 21052020.doc
160K

Evangeline Anand <evangeline.anand@hindawi.com> Sat, May 23, 2020 at 11:08 AM
To: sucipto hariyanto <sucipto-h@fst.unair.ac.id>

Dear Dr. Hariyanto,

Thank you for your response.

Best Regards,
Evangeline Anand,
Quality Check Team.
[Quoted text hidden]

sucipto hariyanto <sucipto-h@fst.unair.ac.id>

Files requested - Organic Compounds: Contents and Its Role to Improve Seed Germination and Protocorm Development in Orchids

1 message

Quality Checking Team <support@hindawi.com> Sat, May 23, 2020 at 10:55 AM
To: sucipto-h@fst.unair.ac.id

Hindawi

Dear Dr. Sucipto Hariyanto,

Regarding your manuscript Organic Compounds: Contents and Its Role to Improve Seed Germination and Protocorm Development in Orchids 2795108, we would be grateful if you could please address the following:

Additional Comments:

This email is just for your information - no action is needed. We noticed that the manuscript was uploaded as a supplementary file - we have corrected this on your behalf, so please take no further action

Once these are taken care of we can continue with the process.

Kind regards,
Quality Checking Team

sucipto hariyanto <sucipto-h@fst.unair.ac.id>

2795108: Galley Proofs

1 message

International Journal of Agronomy <production.b@hindawi.com> Thu, Jun 4, 2020 at 5:03 PM
To: sucipto-h@fst.unair.ac.id
Cc: edy-s-w-u@fst.unair.ac.id

Dear Dr. Sucipto,

I am pleased to let you know that the first set of galley proofs of your Review Article 2795108 titled "Organic Compounds: Contents and Its Role to Improve Seed Germination and Protocorm Development in Orchids," is ready. You can apply your corrections directly to the manuscript with the Online Proofing System (OPS).

Using the OPS, you can quickly and easily make corrections directly to your galley proofs and submit these corrections with a single click.

https://ops.hindawi.com/author/2795108/

If a new corresponding author is added, they must log into their manuscript tracking system account and add their ORCID ID. Any additional ORCID IDs added on during proofing will also need to be updated on that author's account. Delays can occur if this isn't done.

To expedite the publication of your manuscript, please send us your corrected galley proofs within three days.

Please ensure that you read the proofs thoroughly and make all necessary corrections at this stage. A second round of proofs may be requested only for checking essential changes or major revisions.

Best regards,

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****
Hindawi Production Team
Hindawi
https://www.hindawi.com
****

REVIEWER RECOMMENDATIONS AND COMMENTS & RESPONSES TO REVIEWER COMMENTS

May 22th 2020

Editor-in-Chief International Journal of Agronomy

I wish to resubmit an article review entitled " Organic Compounds: Contents and Its Role to Improve Seed Germination and Protocorm Development in Orchids**”**, which we have revised according to the reviewer’s suggestion.

Attachment file:

1. Main manuscript (word file)
2. Figure 1 (ppt. file)
3. Table 1 (word file)

I will abide by all the rules that apply in the Internatonal Journal of Agronomy.

Thank you for your consideration of this article.

Sincerely,

Dr. Sucipto Hariyanto
Department of Biology
Faculty of Science and Technology
Universitas Airlangga
Campus C Unair, Mulyorejo, 60115 Surabaya, Indonesia

Reviewer 1.

A.Comments for the author

Review Reports	The results of correction in accordance with the reviewer recommendation
The study reviewed the role organic contents that influences seed germination, protocorm development and seedling growth of different species. This paper showing that an important collective source of information in the field of orchid cultivation and agronomy for orchid conservation efforts and ornamental purposes. However, the source of information has not fulfiled the requirements of orchid cultivars.	In the manuscript we have informed the orchid cultivar, i.e. 1. *Calanthe hybrids* [38] (Bulduseong x Hyesung and Chunkwang x Hysung). Recently, these hybrids have been cultivated in South Korea and are popular in Japan and Korea. Their flowers are big, beautiful, fragrans, and their color attractives. 2. *Vanda pumela*, these species have been cultivated and have great medicinal value in Nepal [40]. 3.*Spattoglottis plicata*, in Bangladesh this species many … in the home garden as ornamental orchid commodity [47]. 4. *Phaius tankervielae* var alba, these species have been cultivated as ornamental and traditional medicine [41]. And there are still many example in our manuscript.

B.Major Issues

Review Report	The results of correction in accordance with the reviewer recommendation
There are two aspects of the review outcomes were missing in the whole manuscript. 1. Authors fail to add the information on the number of days/weeks/months on orchids seed germination by the effect of different organic contents.	The articles that we have reviewed are not all that have duration of germinating data. The following information about the duration of seed germination at addition organic compound and has been added to the manuscript: **Reference 31:** The fastest germination (14 days) and highest germination rate (93%) was found in ½ MS medium supplemented with CW 150 $mL.L^{-1}$ **Reference 34:** The fastest germination (65 days), optimum seed germination rate of 65.33% and seedling formation rate of 35.67% achieved on ½ MS medium containing NAA 0.5 $mg.L^{-1}$ and AC 1.0 $g.L^{-1}$ supplemented with 100 $mL.L^{-1}$ CW. **Reference 81:** David et al. (2015) also reported that the supplemented TJ on KC medium promoted rapid germination of seed (23 days) than without TJ (28 days). **Reference 93:** Addition of 0.50% YE significantly enhanced (85.9%) seed germination and shortened germination time to 23 days.

2. It would be appreciable if authors add the information of some orchid success germination and growth development under field conditions by the effect of organic contents, it may vary significantly. Therefore it is important to elucidate or add those information and datasets would bring interesting outcomes and useful for the conservation efforts and orchid cultures.	The success of seed germination of orchid by the effect of organic contents have been shown on **key result obtained**. The articles that we have reviewed are not all that have ex-vitro survival data of orchid plant. The following information about the plant survival rate on ex-vitro condition and has been added to the manuscript: **Reference 31:** Of the fourty five plantlets transplanted to soil and fourty survived. **Reference 32:** Complete seedlings were then transferred in clay pots with mixture media of brick pieces, sphagnum moss, pine bark and charcoal pieces (1:!:1:1), where 70% seedling survived **Reference 34:** Zeng et al. [34] also reported that plantlets 5 cm in height were then transplanted into pots with mixture media (1:2;1) (v/v/v) shattered fir bark : stone for orchid : sieved peat, after 180 days in a greenhouse, 92.33% of plantlets survived. **Reference 35:** Survival of *Renanthera imschootiana* plantlets (95%) was found after transplanting for 60 days into pots with media sphagnum moss in greenhouse. **Reference 41:** Plantlets with 2-3 cm in height were transplanted into plastic pots with mixture media of coconut fibre and sphagnum moss (3:1 v/v) and acclimated in greenhouse. The surviving ratio of *Dendrobium lasianthera* were more than 90%. **Reference 42:** The healthy *in vitro* plantlets with 2 to3 leaves were individually grown in pots with mixture peat moss: brick pieces : charcoal pieces (0.25:1:1) with 90% plantlets survived. **Reference 43:** The well-rooted plantlets were transferred to pots containing mixture composed of saw dust, coconut coir, humus and coal pieces at 1;1;1;2 (w/w) with 80% survival in outside environment. **Reference 45:** The plantlets regenerated via *in vitro* seed germination processes were transferred into pots with mixture charcoal, broken brick pieces and/or tree fern roots, showed 80% survival. **Reference 61:** Plants regenerated by *in vitro* germination processes were successfully growth in greenhouse conditions for 180 d and the survival rate was 88.5%.

	Reference 64: Plants regenerated via asymbiotic seed germination processes were successfully acclimatized in greenhouse conditions and the survival rate was more than 85%. **Reference 81:** David et al. (2015) also reported that the supplemented TJ on KC medium promoted rapid germination of seed (23 days) than without TJ (28 days). Plantlets regenerated via *in vitro* seed germination process were successfully acclimatized in greenhouse conditions. **Reference 93:** The seedlings approximatelly 6.5 cm in height were adapted at the greenhouse environment and continued to grow developed into healthy plantlets.
3. Authors may include conditions.	I am agree, and have been added on manuscript.
4. Please also include the effect of medium condition on orchid germination under field conditions and their data on seedling development if possible.	Have been added on manuscript

C. Minor Issues

1. The authors should survey the kinds of literature up to 2019

Review Report	The results of correction in accordance with the reviewer recommendation
The authors should survey the kinds of literature up to 2019	We have added three articles publish up to 2019 on manuscript, as follows. 1. S. Maharjan, S. Pradhan, B.B. Thapa, B. Pant, "*In Vitro* Propagation of Endangered Orchid, *Vanda pumila* Hook.f. through Protocorms Culture," *American Journal of Plant Sciences*, vol. 10,pp.1220-1232,2019. http://www.scirp.org/journal/ajps. 2. T. Punjansing, M. Nakkuntod, S. Homchan, P. Inthima, A. Kongbangkerd, "Influence of Organic Supplements on Shoot Multiplication Efficiency of *Phaius tankervilleae* var.*alba*, " *International Journal of Agricultural and Biosystems Engineering*, vol.13, no. 4, pp. 105-109, 2019. 3. E.S.W. Utami, and S. Hariyanto, "*In Vitro* Seed Germination and Seedling Development of a Rare Indonesian Native

	Orchid *Phalaenopsis amboinensis* J.J.Sm," *Scientifica*, vol. 2019, pp.1-6, 2019. https://doi.org/10.1155/2019/8105138.

2. References are not appropriate format. Kindly follow the journal format and style

Page	Row	Written	After revision
15	14	*Plant Cell, Tissue and Organ Cultur,*	*Plant Cell Tissue and Organ Culture*
	22	*Plant Cell, Tissue and Organ Culture*	*Plant Cell Tissue and Organ Culture*
		… With ..	… with …
16	1	*Plant Cell Tiss Org Cult,*	*Plant Cell Tissue Organ Culture*
	5	*Proc. Natl. Acad. Sci., India,*	*Proceeding of the National Academy of Science India*
	10	*Asian Pasific Journal of Reproduction*	*Asian Pacific Journal of Reproduction*
	17	*Amer. J. Bot*	*American Journal of Botany*
	18	doi.org/10.6165/tai.2004.49(2).124.	doi.org/10.1002/j.1537-2197.1995.tb12682.x
17	5	*Acta Agron*	*Acta Agronomica*
	15	*Am. J. Bot*	*American Journal of Botany*
	21	*Journal. of Phytol,*	*Journal. of Phytology*
	27	*Hort Sci*	*Horticultural Science*
18	3	*Plant Biotechnol Rep*	*Plant Biotechnology Reports*
	6	*African Journal of Biotechnology,*	*African Journal of Biotechnology*
		http://doi: 10.5897/AJB11.4001	deleted
	11	*J. Plant Biotechnol*	*Journal of Plant Biotechnology*
	19	*PLOS,* vol.	*PLoS One*, vol.
	25	*J. Trop. Plant Physiol*	*Journal of Tropical Plant Physiology*
19	25	*Sci Hortic,* vol. 94, no.1	*Scientia Horticulturae,* vol. 115, no.2
	29	*Plant Cell Tissue Organ Cult*	*Plant Cell Tissue and Organ Culture*
20	4	*Journal Bangladesh Agril. Univ,*	*Journal Bangladesh Agricultural University*
	11	*In Vitro Cell. Dev. BiolPlant,*	*In Vitro Cellular and Developmental Biology-Plant,*
	22	*J. Agric. Food. Chem,*	*Journal of Agricultural and Food Chemistry*
	30	*Gayana Bot,*	*Gayana Botanica*
22	2	*Hort Science*	*Horticultural Science*
	3	http://hortsci.ashspublications.org/content/34/2/233.full.pdf.	https://doi.org/10.21273/HORTSCI.34.2.233

3. **Kindly check the spelling of marked words in the in the pdf file of the manuscript**

   Yes, we have double checked and have been proofread by professional proofread (proofread certificate attached).

4. **Please check the language before the submission of the final version**

Yes, already checked and have been proofread by professional proofread (proofread certificate attached).

5. **Kindly make sure that only 6 organic contents are available on orchid seed germination through the literature survey. It seems less in numbers.**

Review Report	The results of correction in accordance with the reviewer recommendation
Kindly make sure that only 6 organic contents are available on orchid seed germination through the literature survey. It seems less in numbers.	Have been added at no. 10 i.e **yeast extract.**

Reviewer 2.

A.Comments for the author

The manuscript "Organic Compounds: Contents and Its Role to Improve Seed Germination and Protocorm Development in orchids." contains a review of the effect of various organic compounds during germination, the development of protocols and PLBs in various genera and species of orchids. Although the bibliographic compilation is quite extensive, it does not provide new conclusive information, it only emphasizes the stimulating effect that these compounds can present in these processes of growth and development of these plants. As mentioned by the authors, this effect is widely known and the use of these compounds is also wide.	We have been revised
In general, the observed responses could be due more to the response of the species or genus and also perhaps of the base medium used, rather than the organic compound used. The summary of results shown in Table 1 exemplifies this very well. The authors do not mention anything in this regard, nor do they show whether any of these compounds is better than another, in a certain combination or together with a certain type of medium. After this analysis it is not clear to me what is novelty in this bibliographic review	We have been revised

B. General comments

The abstract does not comply with the journal regulations. Neither in the recommendations to the authors nor in several articles reviewed, these had this structure, nor the manuscript template, which appears as a guide.	The abstract have adjusted to the guideline of International Journal of Agronomy.
The method mentioned with the abstract is not described in the body of the manuscript. It is not clear what was the analysis method used and if indeed, it allowed the authors to consider all the available and updated literature in the research area.	We have added it to manuscript.
According to the international system of units (SI), concentrations should be noted as e.g: g L-1, instead in several papers reviewed in the journal, I observed different ways of showing it. Therefore, I make an observation to the editorial group, so that they suggest how to write down the concentrations.	I am agree … we have revised
I'm not native to the English language but I noticed several problems writing and grammar, I suggest that the manuscript is reviewed by native person.	I am agree …. We have proofread by professional proofread/native person (attaced).
In general, throughout the text, many acronyms are used for the compounds being studied, the culture media, the phytohormones, etc. This causes the thread of the manuscript to be lost in several parts, they should use only the necessary acronyms or perhaps only the most common ones in the area and those of the compounds that are being analyzed.	I am agree …we have been revised
Several culture media mentioned throughout the review, are placed only with their acronyms and without their reference authors, this restricts the complete understanding of the document only to experts in orchid cultivation. They must put the full name of the culture media and their bibliographic citations Similarly, the full names of phytohormones should be placed the first time they are mentioned. Throughout the text, several phytohormones are placed only in acronyms.	Ok, we have been revised

C. Specific comments

page 2, paragraph 2, line 1: it says "ad", they should say "and"	Ok
Table 1: The description of the table should be self-explanatory on its own. The compounds, media and phytohormones that are mentioned in the table with acronyms must be defined.	Ok, we have been revised
Page 4 line 9. says: "..resulted in workshop seed germination", modify by: "... resulted in higher seed germination"	I am sorry …. We did not find that sentence.
At point 6. It says: potato, it should say "potato homogenate". Synchrony must be maintained throughout the text, at first described as potato homogenate.	Ok, we have been revised
page 12, last paragraph: the effects described for the CHT are a little out of the general context reviewed throughout the manuscript for the rest of the compounds analyzed. This information should be excluded and based only on the results related to seed germination and protocorm development or PLB	Ok, we have revised
Fig 1: like the table, the description must be self-explanatory. Acronyms must be defined, they should be used in the same way that they are used throughout the text, for example; some are with their names but not complete (potato, banana), others with initials (CW). It is not known what the description in the box means, although it seems obvious it must be explained somewhere (description of the figure) or at least referred to in the text where the nutrient contributions of each compound are listed.	I am agree …. We have been revised
The yellow letters inside the figure are not noticeable.	Ok …. We have changed it to red
Review the format of the references, some lack details, e.g. in 1, a comma is missing, in 9 there is a word in red, etc.	Ok …. We have been revised

Review Article

Organic Compounds: Contents and Its Role to Improve Seed Germination and Protocorm Development in Orchids.

Edy Setiti Wida Utami, Sucipto Hariyanto*

Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, 60115, Indonesia

*Corresponding Author: Sucipto Hariyanto, Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Mulyorejo (Kampus C Unair). Surabaya, Post Code 60115, Indonesia. Tel: +6282139070704; E-mail: sucipto-h@fst.unair.ac.id

In nature, orchid seed germination is obligatory following infection by mycorrhizal fungi, which supplies the developing embryo with water, carbohydrates, vitamins, and minerals, causing the seeds to germinate relatively slowly and at a low germination rates. The non-symbiotic germination of orchid seeds found in 1922 is applicable to *in vitro* propagation. The success of seed germination *in vitro* is influenced by supplementation with organic compounds. Here, we review the scientific literature in terms of the contents and role of organic supplements in promoting seed germination, protocorm development, and seedling growth in orchids. We systematically collected information from scientific literature databases including Scopus, Google Scholar, and Proquest, as well as published books and conference proceedings. Various organic compounds, i.e. coconut water (CW), peptone (P), banana homogenate (BH), potato homogenate (PH), chitosan (CHT), tomato juice (TJ), and yeast extract (YE) can promote seed germination, growth, and development of various orchids. They also stimulate seedling development, the formation of protocorm-like bodies (PLBs), plantlet growth, and multiple shoot formation. The addition of organic compounds to culture media, individually or in combination, accelerates seed germination and seedling development. Different types and concentrations of organic nutrients are needed for the success of *in vitro* cultures, depending on the species and genotype.

.

1. Introduction

Orchid, member of the Orchidaceae, are one of the largest and most diverse families of flowering plants, consistsing of 763 genera and more than 28,000 accepted species [1]. Orchids are found in various habitats, primarily (70%) attached to tree trunks in forests as epiphytes, growing in the shade and comprising almost two thirds of the world's epiphytic flora, the remaining 25% are terrestrial, while 5% are found on various support systems [2, 3].

Orchid seeds have limited food storage tissue, which is needed for germination and protocorms development, making seed germination in natural conditions is relatively low (< 5%) [4, 5]. Under natural conditions, mature seeds depend on compatible mycorrhizal fungi for germination and early development [6,7]. Therefore, *in vitro* orchid seed germination is a crucial aspect in propagation and conservation programs. Seeds cultured *in vitro* can develop into complete seedlings without the aid of fungi, which is a suitable approach for commercial orchid production [8-12].

Initially, *in vitro* seed germination used mycorrhizal fungi isolated from various natural environments to stimulate germination and was known as "symbiotic seed germination". The addition of organic nutrients to *in vitro* culture was meant to stimulate seed germination. In 1922, Knudson [13] successfully developed a method to stimulate protocorm production in orchids by culturing the seeds *in vitro* and sprinkling them on sterile nutrient media plus sucrose. This technique was known as "asymbiotic seed germination" because it did not involve mycorrhizal fungi. Both approaches were effective.

2. The Orchid Seed

Orchid seeds are the smallest of those produced by flowering plants and are therefore called "dust seeds" [14]. However, despite their microscopic structure, they exhibit a wide variety in shape, size, color, weight, and testa (cell number, size, ornamentation) and embryo characteristics.

According to Molvray and Kores [15], orchid seeds can be crescent, broadly ellipsoid, filamentous, spindle-shaped, fusiform, oblong, irregular, or clavate. Verma et al. [16] established that ovoid, filiform, and spatulate-shaped seed are present in *Androcorys monophylla, Goodyera biflora* and *Platanthera clavigera*, respectively.

Seed color also varies, including orange yellow in *Dendrobium formosum* and *Dendrobium densiflorum*, brownish yellow in *Dendrobium hookerianum*, yellow in *Cymbidium bicolor*, white in *Eria dalzellii*, pale yellow in *Liparis elliptica* and *Pholidota pallida*, golden yellow in *Bulbophyllum mysorense*, and light yellow in *Coelogyne breviscapa* [16, 17]. Molvray and Kores [15] showed that seed size varied from 150 to 6,000 µm and weight ranged from 0.31 to 24 µg.

The cellular organization of seed is simple and consists of an undifferentiated mass of embryonal cells and a rudimentary endosperm, covered within a transparent testa [18]. According to Arditti and Ghani [14], orchid embryos are relatively small and simple, generally oval or spherical in shape, and sometimes consist of only a few cells, mostly without an endosperm.

3. The Role of Organic Nutrient Supplements

The development and regeneration of *in vitro* cultured plant tissues can be improved by adding a variety of organic nutrients [19-21]. These may include coconut water (CW), peptone (P), potato homogenate (PH), banana homogenate (BH), chitosan (CHT), tomato juice (TJ), and yeast extract (YE). Organic nutrients are a source of vitamins, amino acids, fatty acids, carbohydrates, peptides and growth factors, which all facilitate growth [22]. Few organic nutrients have been studied for their contents and role in improving seed germination and development of the protocorm in orchids using *in vitro* models (Table 1, Fig. 1). Some of these studies are discussed in the following sections.

4. Coconut Water

CW is a colorless liquid endosperm obtained from *Cocos nucifera*. It is often added to culture media containing auxin for the rapid induction of propagation and cell growth. The use of CW in tissue culture was first attempted by Van Overbeek et al. [23, 24], who reported that adding it to the culture medium was essential for the development of young *Datura stramonium* embryos.

CW contains soluble sugars as a natural source of carbon, as well as amino acids and vitamins, such as thiamin, pyridoxine, ascorbic acid, and minerals [25, 26]. It also consists of various organic ions such as phosphorus, magnesium, potassium, calcium, iron, and manganese [27, 28], all of which facilitate germination [29]. Furthermore, CW contains indoleacetic acid

(IAA), abscisic acid (ABA), gibberelic acid (GA), and zeatin [30], which are generally used as growth supplements in plant tissue culture.

Orchid seed germination is usually increased by adding CW to the medium (Table 1). For instance, Thomas and Michael [31] reported 93% germination of *Rhynchostylis retusa* seeds after the addition of 150 mL L^{-1} of CW to a half - strength Murashige and Skoog medium (½ MS; Murashige and Skoog [32]). Similar conclusions were made by Piri et al. [33], who stated that the addition of CW 150 mL L^{-1} to Mitra medium (M; Mitra et al. [34]) was most suitable for seed germination of *Acampe papilosa.*

According to Huh et al. [35], MS medium supplemented with CW enhances seed germination and protocorm formation of *Cypripedium macranthos*. In a study of different organic supplements, including CW, birch sap (BS), maple sap (MPS), BH, and P, at various concentrations, the use of 100 mL L^{-1} CW resulted in the highest germination rate (70.8%) and protocorm formation rate (74.2%). Zeng et al. [36] also established that ½ MS medium containing 0.5 mg L^{-1} α-naphthaleneacetic acid (NAA) and 1.0 g L^{-1} activated charcoal (AC) supplemented with CW, enhanced seed germination and seedling formation of *Paphiopedillum wardii.* Among the different of organic supplements, including CW, PH, BH, trypton (T), and P with various concentrations, 100 mL L^{-1} CW increased seed germination (65.33%) and had the highest seedling formation (35.67%) compared to other treatments, while Wu et al. [37] concluded that ¼ MS medium containing 0.5 mg L^{-1} NAA and 1 g L^{-1} AC, supplemented with 200 mL L^{-1} CW and 1 g L^{-1} P, after 75 days in culture, was suitable for seed germination and protocorm development of *Renanthera imschootiana*.

CW not only affects germination and protocorm development, but also plays an important role in the formation of protocorm-like bodies (PLBs), plantlet growth, and multiple shoot induction. According to Baque et al. [38], the hyponex medium, Kano [39], supplemented with CW effectively enhanced plantlet growth of ornamental orchid *Calanthe* hybrids. Of the various CW concentrations tested (0, 10, 30, 50, and 100 mL L^{-1}), a concentration of 50 mL L^{-1} significantly increased plantlet dry weight. Maharjan et al. [40] assayed the effect of different concentrations of CW (0, 50, and 100 mL L^{-1}) on shoot formation of medicinal orchid *Vanda pumila* protocorms and found that the number and length of shoot increased when cultured on ½ MS medium supplemented CW. Similar conclusions were made by Pudjansing et al. [41] who determined that the addition of CW (50 mL L^{-1}) in ½ MS medium containing 50 g L^{-1} PH was

best for promoting shoot multipication of *Phaius tankervilleae* var. alba. This result was supported earlier by Jainol and Jualang [42] observed the effects of different organic supplements (CW, TJ, BH, P, and YE), at various concentrations on multiple shoot formation in *Dimorphorchis lowii* and found that CW at 150 mL L^{-1} resulted in the highest number of shoots. Kaur et al. [43] investigated the effects of the addition of various organic supplements (BH, CW, and without organic additives as control) at various concentrations on the *in vitro* multiplication of protocorms in a medicinal orchid (*Dendrobium nobile*). In their study, M medium supplemented with 200 mL L^{-1} CW was most suitable for the enhancement of protocorms multiplication.

5. Peptone

P is occasionally used as a media supplement in orchid cultivation, facilitating explant growth and development. Numerous studies have shown that P supplements enhance germination (Table 1). For example, Utami et al. [44] evaluated the effect of different P concentrations (0, 1, 2, and 3 g L^{-1}) on seed germination and shoot formation of *Dendrobium lasianthera*, and observed at the 4, 8 and 12 weeks after cultured. Among the different concentrations of P tested, seed germination rate (100%) was obtained after 12 weeks of incubation in all treatments. However, they also reported that shoot formation was highest (84.0%) in the Vacin and Went medium (VW; Vacin and Went [45]) supplemented with 2 g L^{-1} P. Seed germination rates of *Epidendrum ibaguense* Kunth and ornamental orchid *Spathoglottis plicata* Blume were higher on M or Phytamax medium (PM; Phytamax^{R}, Sigma Chemical Co. USA) supplemented with 2 g L^{-1} P than on treatments that did not contain P [46, 47]. Similarly, Buyun et al. [48] found a seed germination rate of 100% for *Dendrobium parishii* with Knudson medium (KC; Knudson [49]) supplemented with 2 g L^{-1} P. According to Srivastava et al. [50], the seed germination rate of *Aerides ringens* increased significantly when cultured on KC medium containing 8.9 µM benzylamino purine (BAP) and supplemented with P. Among the different concentrations of P tested (0.25, 0.5 or 0.75 g L^{-1}), the highest germination rate (89.28%) was obtained in 0.5 g L^{-1} P as an additive after 30 days of incubation.

P is also known to stimulate the multiple shoot and protocorms formation. Devi et al. [51] identified that P improved the *in vitro* multiplication of protocorms of medicinal epiphytic orchid *Taprobanea spathulata*. Of the different concentrations tested, 5 g L^{-1} P was a suitable

concentration for the induction of multiple protocorms. Kaur and Bhutani [52] evaluated the effects of different organic additives (BH and P) at various concentrations on the *in vitro* multiplication of shoots in *Paphiopedilum venustum* and discovered that modified terrestrial orchid medium (BM-1; Van Waes and Debergh [53]) supplemented with P (1 g L^{-1}) was the most effective in stimulating multiple shoots, with three shoots per explant. In addition to promoting the formation of multiple shoots and protocorms, peptone promotes seedling growth [54] because it contains high levels of amino acids [55] and vitamins, including thiamin, biotin, pyridoxine, and nitrogen [56].

6. Potato Homogenate

Orchid seed germination was significantly affected by the presence of PH in the medium (Table 1). Islam et al. [57] evaluated the effect of PH on *in vitro* germination of *Vanda roxburgii* and showed that hyponex medium supplemented with PH increased the seed germination rate and promoted seedling development. Similarly, Bakar et al. [58] evaluated the effect of different organic supplements (PH, CW, and TJ) at various concentrations on seed germination of *Dimorphics lowii* and found that 10% (w/v) of PH resulted in the highest seed germination rates. Additionally, PH enhanced seedling growth of *Dendobium tosaense* [59] and stimulated the multiplication and growth of *Dendrobium officinale* shoots [60]. Besides, 75 g L^{-1} PH in VW medium containing 100 mL L^{-1} was most suitable for *in vitro* proliferation in *Bulbophyllum nipondii* pseudobulbs [61].

PH contains important vitamins, such as C, B1, and B6, and mineral elements, such as potassium, iron, and magnesium, as well as carbohydrates and amino acids, which facilitate seed germination [62, 63]. Miransari and Smith [64] reported that seeds consume large amounts of nitrogen during germination, and therefore, PH in the medium can influence germination rates.

7. Banana Homogenate

Seed germination and protocorm development were affected by BH in the media (Table 1). Pereira et al. [65] evaluated the effects of different organic additives (BH and TJ) on the germination of *Chloraea gavilu* and showed that Malmgren modified terrestrial orchid medium (MM; Malmgren [66]). supplemented with BH successfully improved seed germination.

Islam et al. [67] investigated the effects of different BH concentrations (0, 2.5, 5, 10, and 20 %; w/v) on the growth and development of PLBs *Dendrobium sp*, and showed that 1/2 MS medium supplemented with 10% BH was the most suitable for PLBs regeneration. According to Zeng et al. [68], plantlet formation of ornamental lithophytic orchid *Paphiopedilum hangianum* increased significantly when PLB were sub-cultured on HO_{26} medium (Zeng et al. [69]) containing 1 g L^{-1} P, 1 mg L^{-1} NAA and supplemented with BH. The same study concluded that Harvais medium (Harvais [70]) with various organic supplements effectively induced seedling growth of *Cypripedium macranthos*. Among the various organic supplements studied (CW, PH and BH), BH at a concentration of 25 or 50 g L^{-1} improved shoot and root number and shoot and root length [71]. Gonçalves et al. [72] found that the number of *Hadrolaelia purpurata* seedlings produced was highest on KC medium containing 90 g L^{-1} of BH. Furthermore, Utami and Hariyanto (73) observed the effect of supplementation with 150 mL L^{-1} CW with different BH concentrations (0, 5, 10, 15 g L^{-1}) on the plantlet development of *Phalaenopsis amboinensis* and found that the organic supplementation of CW together with BH had significantly increased the growth of plantlets. Banana has often been used as an organic additive in *in vitro* cultures because of its high levels of potassium, manganese, calcium, sodium, iron, zink, thiamin, riboflavin, niacin, pyridoxine, pantothenic acid, ascorbic acid, folic acid [74] and natural growth regulators such as zeatin, gibberellin and IAA [75, 76]. It is also rich in carbohydrates, supplying energy to heterotrophic plants during the early stages of *in vitro* cultivation [77].

8. Chitosan

CHT, a biopolymer derivate of chitin, is mostly found in the exoskeleton of arthropods and crustaceans [78] and has been applied in various fields, including agriculture [79].

Numerous studies have shown that orchid seeds germination is affected by the addition of CHT to the media (Table 1). Kananont et al. [80] studied the effects of various types of CHT, at different concentrations on the seed germination of various orchid species and found that none of the six CHT types tested (CHT polymers: P_{70}, P_{80}, P_{90} and CHT oligomers: O_{70}, O_{80}, O_{90}) at five concentrations (0, 10, 20, 40, and 80 mg L^{-1}) had a significant effect on seed germination in *Dendrobium bigibbum* var. compactum; however, the addition significantly increased germination in *Dendrobium formosum*. The highest germination rate (91.2%) was recorded using CHT type O_{80} at 10 mg L^{-1}. Restanto et al. [81] evaluated the effect of CHT on zygotic embryo

development of *Dendrobium* sp *in vitro* and observed that CHT significantly affected zygotic embryo growth and differentiation. Of the different CHT concentrations tested (0, 5, 10, 15, 20, and 25 mg L^{-1}), the PLB number, PLB fresh weight, and number of plantlets was highest using 15 mg L^{-1} of CHT. Nge et al. [82] also studied the effect of shrimp CHT at different concentrations (0, 5, 10, 15, 20 and 25 mg L^{-1}) on the meristematic bud growth of *Dendrobium phalaenopsis* and showed that VW medium supplemented with 15 mg L^{-1} CHT resulted in the highest fresh weight of PLBs. Additionally, the same study concluded that ½ MS liquid medium supplemented with CHT effectively induced PLB development of *Grammatophyllum speciosum*. Of the different concentrations of CHT tested (0, 5, 10, 15, 20, 25, 50 or 100 mg L^{-1}), a concentration of 15 mg L^{-1} resulted in the highest growth rate (4-fold increase) [83]. Samarfard and Kadir [84] investigated the effects of CHT on PLB proliferation in *Phalaenopsis gigantea* and among the six concentrations (0, 5, 10, 15, 20 and 25 mg L^{-1}) tested, 10 mg L^{-1} CHT in VW medium resulted in the highest fresh weight after 20 weeks of cultivation.

Chitosan is also known to stimulate plantlet growth and development under greenhouse conditions. Pitoyo et al. [85] studied the effects of CHT on the growth of *Gramatophyllum scriptum* plantlets and suggested that CHT had significant effects on some parameters, including plantlet height and leaf length. Kumari et al. [86], investigated the effects of different CHT concentrations (2.5, 5, 7.5 and 10 mg L^{-1}) on the growth and development of *Dendrobium* cv. Sonia 17. Of the four concentrations tested, 7.5 mg L^{-1} resulted in the highest number of spikes per plant, an increased flower diameter, an enhanced spike length and a higher number of florets per spike. Similarly, Charoenwattana and Petprapai [87] studied the effects of CHT on the growth of plantlets of *Dendrobium* orchids and reported that an optimal leaf number was achieved at a concentration of 100 mg L^{-1} within 10 weeks of transplanting. The increase in plantlet growth was allegedly under the influence of photosynthesis. According to Barka et al. [88], the addition of CHT derivative, i.e. chitogel, improved CO_2 fixation 1.5 fold and O_2 production 2 fold, indicating that CHT derivatives have the potential to increase photosynthesis, thereby increasing plant growth. According to Uthairatanakij et al. [89], the beneficial effect of CHT may induce by a signal to synthesize of plant hormones auxin and gibberellin.

9. Tomato Juice

TJ significantly influences the germination of orchids seed and subsequent protocorm development (Table 1). David et al. [90] investigated the effect of different complex additives (TJ, CW, P, and YE) at various concentrations on seed germination in *Vanda belvola in vitro*. In their study, KC medium supplemented with 15% (v/v) TJ was most suitable for promoting germination. Muthukrishnan et al. [91] also examined the effects of various organic supplements (TJ, CW, and PH) at different concentrations on seed germination in *Geodorum densiflorum* and showed that 1/2 MS medium supplemented with 5% (v/v) TJ resulted in the highest rate of seed germination and protocorm growth. Similarly, Gnasekaran et al. [92] reported that 20 and 30% (v/v) TJ improved PLB proliferation in *Vanda* Kasem's Delight more than the addition of papaya extract (PE) or PH.

Tomato juice contains carbohydrates, vitamins and minerals [93], glucose, and fructose [94], which benefit cellular division and support systems. Tomatoes also contain lycopene, a vigorous antioxidant with the ability to eliminate the formation of free radicals, repair injured cells, and suppress DNA oxidation [95]. Tomatoes also contain other antioxidants, such as α-carotene, β-carotene, and ascorbic acid [96-99]. According to Gnasekaran et al. [92], sugars and antioxidants play an important role in cell proliferation and the production of healthy PLBs.

10. Yeast Extract

YE is also an important source of amino acids and vitamins, especially inositol and thiamin, and has been effectively used to increase seed germination and regeneration in many orchid species [100]. Numerous studies have shown that orchid seeds germination and protocorm regeneration is affected by YE in the cultivation media (Table 1). Gansau et al. [101] investigated the effect of different YE concentrations (0, 0.1, 0.2, 0.3%; w/v) on protocorm proliferation and growth of *Dimorphorchis rossii*. In their study, MS medium supplemented with 0.2% YE increased of protocorm regeneration. Earlier, Jualang et al. [102] evaluated the effect of different organic supplements (YE, P, BH, CW, and TJ) at various concentrations on seed germination of *Vanda dearei* and found that 0.5% (w/v) YE enhancement seed germination.

11. Conclusion

Based on the literature survey, we conclude that the addition of the organic supplements, including CW, P, BH, PH, CHT, TJ, and YE, to orchid seed germination media supports seed germination, precipitates seedling formation, and yields vigorous plantlets. They also effectively increase the number of PLBs, induce multiple shoot formation, and stimulate the growth and development of plantlets under greenhouse conditions. These organic supplements represent natural sources of amino acids, vitamins, minerals, organic acids, sugars, proteins, and natural growth regulators, assisting in orchid propagation by stimulating the development and morphogenesis in asymbiotic seed culture. Many orchid species are threatened by land conversion and habitat mismanagement. Asymbiotic seed germination with the addition of organic supplements is an excellent technique for the mass propagation and efficient acclimatization of orchids for reintroduction to natural habitats, which will facilitate the conservation of endangered orchid species.

Author Contributions

Both authors contributed to the literature search as well as to the writing of the manuscript. Both authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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