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Review Article

Organic Compounds: Contents and Their Role in Improving Seed Germination and Protocorm Development in Orchids

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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 rate. The nonsymbiotic 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 and growth and development of various orchids. They also stimulate seedling development, 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

Orchids, member of the Orchidaceae, are one of the largest and most diverse families of flowering plants, consisting 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 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 for stimulation 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, 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 seeds 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 mysorensense*, 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 with 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, Figure 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. [64, 65], 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 [66, 67]. It also consists of various organic ions such as phosphorus, magnesium, potassium, calcium, iron, and manganese [68, 69], all of which facilitate germination [70]. Furthermore, CW contains indoleacetic acid (IAA), abscisic acid (ABA), gibberellic acid (GA), and zeatin [71], 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 [23] reported 93% germination of *Rhynchosstylis retusa* seeds after the addition of 150 $\text{mL}\cdot\text{L}^{-1}$ of CW to a half-strength Murashige and Skoog medium ($\frac{1}{2}$ MS; Murashige and Skoog [72]). Similar conclusions were reached by Piri et al. [24], who stated that the addition of CW 150 $\text{mL}\cdot\text{L}^{-1}$ to Mitra medium (M; Mitra et al. [73]) was most suitable for seed germination of *Acampe papillosa*.

According to Huh et al. [25], 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 $\text{mL}\cdot\text{L}^{-1}$ CW resulted in the highest germination rate (70.8%) and protocorm formation rate (74.2%). Zeng et al. [26] also established that $\frac{1}{2}$ MS medium containing 0.5 $\text{mg}\cdot\text{L}^{-1}$ α -naphthaleneacetic acid (NAA) and 1.0 $\text{g}\cdot\text{L}^{-1}$ activated charcoal (AC) supplemented with CW, enhanced seed germination and seedling formation of *Paphiopedilum wardii*. Among the different organic supplements, including CW, PH, BH, tryptone (T), and P with various concentrations, 100 $\text{mL}\cdot\text{L}^{-1}$ CW increased seed germination (65.33%) and had the highest seedling formation (35.67%) compared to other treatments, while Wu et al. [27] concluded that $\frac{1}{4}$ MS medium containing 0.5 $\text{mg}\cdot\text{L}^{-1}$ NAA and 1 $\text{g}\cdot\text{L}^{-1}$ AC, supplemented with 200 $\text{mL}\cdot\text{L}^{-1}$ CW and 1 $\text{g}\cdot\text{L}^{-1}$ 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. [28], the Hyponex medium, Kano [74], supplemented with CW effectively enhanced plantlet growth of ornamental orchid *Calanthe* hybrids. Of the various CW concentrations tested (0, 10, 30, 50, and 100 $\text{mL}\cdot\text{L}^{-1}$), a concentration of 50 $\text{mL}\cdot\text{L}^{-1}$ significantly increased plantlet dry weight. Maharjan et al. [29] assayed the effect of different concentrations of CW (0, 50, and 100 $\text{mL}\cdot\text{L}^{-1}$) on shoot formation of medicinal orchid *Vanda pumila* protocorms and found that the number and length of shoot increased when cultured on $\frac{1}{2}$ MS medium supplemented with CW. Similar conclusions were made by Punjansing et al. [30] who determined that the addition of CW (50 $\text{mL}\cdot\text{L}^{-1}$) to $\frac{1}{2}$ MS medium containing 50 $\text{g}\cdot\text{L}^{-1}$ PH was the best for promoting shoot multiplication of *Phaius tankervilleae* var. *alba*. This result was supported earlier by Jainol and Jualang [31] who 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 $\text{mL}\cdot\text{L}^{-1}$ resulted in the highest number of shoots. Kaur et al. [32] investigated the effects of the addition of various organic supplements (BH, CW, and control without organic additives) at various concentrations on the *in vitro* multiplication of protocorms in a medicinal orchid (*Dendrobium nobile*). In their study, M medium supplemented with 200 $\text{mL}\cdot\text{L}^{-1}$ CW was the most suitable for the enhancement of protocorms multiplication.

TABLE 1: Effects of organic supplements on orchid seed germination and protocorm development.

| Media | Species | Organic supplements (OS) | Key results obtained | References |
|---------|--|--|---|------------|
| ½ MS | <i>Rhynchostylis retusa</i> | CW: 0, 50, 100, 150, 200 mL·L ⁻¹ | The fastest germination (14 days) and highest germination rate (93%) were found in ½ MS medium supplemented with CW (150 mL·L ⁻¹). Of the forty-five plantlets transplanted to soil, forty survived. | [23] |
| M | <i>Acampe papillosa</i> | CW: 0, 150 mL·L ⁻¹ YE: 0, 2 g·L ⁻¹ AC: 0, 2 g·L ⁻¹ | Addition of CW at 150 mL·L ⁻¹ to M medium accelerated germination and seedling formation, with a germination rate of 70.75%. Complete seedlings were then transferred to clay pots with a media mixture of brick pieces, sphagnum moss, pine bark, and charcoal pieces (1:1:1:1), where 70% of seedlings survived. | [24] |
| MS | <i>Cypripedium macranthos</i> | CW: 0, 50, 100, 200 mL·L ⁻¹ BS: 0, 50, 100, 200 mL·L ⁻¹ MPS: 0, 50, 100, 200 mL·L ⁻¹ BH: 0, 15, 30, 60 g·L ⁻¹ P: 0, 1, 2, 4 g·L ⁻¹ | An optimum seed germination rate of 70.8% and protocorm formation rate of 74.2% were obtained on MS medium with 100 mL·L ⁻¹ CW. | [25] |
| ½ MS | <i>Paphiopedilum wardii</i> | CW: 0, 50, 75, 100, 150 mL·L ⁻¹ PH: 25, 50, 75, 100 g·L ⁻¹ BH: 25, 50, 75, 100 g·L ⁻¹ T: 0.5, 1, 1.5 g·L ⁻¹ P: 0.5, 1, 1.5 g·L ⁻¹ | The fastest germination (65 days) and an optimum seed germination rate (65.33%) and seedling formation rate (35.67%) were achieved on ½ MS medium containing NAA 0.5 mg·L ⁻¹ and AC 1 g·L ⁻¹ supplemented with 100 mL·L ⁻¹ CW. Zeng et al. [26] also reported that plantlets, 5 cm in height, were then transplanted into pots with a media mixture (1:2:1; v/v/v) of shattered fir bark, stone for orchid, and sieved peat. After 180 days in a greenhouse, 92.33% of plantlets survived. | [26] |
| ¼ MS | <i>Renanthera imschootiana</i> | CW: 0, 100, 150, 200, 250 mL·L ⁻¹ T: 0, 0.5, 1, 1.5 g·L ⁻¹ P: 0, 0.5, 1, 1.5 g·L ⁻¹ PH: 0, 0.5, 1.5, 2.0 g·L ⁻¹ BH: 0, 0.5, 1.5, 2.0 g·L ⁻¹ CW: 200 mL/L + P 0.5 g·L ⁻¹ CW: 200 mL/L + P 1 g·L ⁻¹ CW: 200 mL/L + P 1.5 g·L ⁻¹ | ¼ MS medium containing 0.5 mg·L ⁻¹ NAA, 1 g·L ⁻¹ AC, supplemented with 200 mL·L ⁻¹ CW, and 1 g·L ⁻¹ P was most suitable for seed germination (93.10%) and protocorm development (41.10%) at stage 5 (seedling). Survival of <i>Renanthera imschootiana</i> plantlets (95%) was found 60 days after transplanting into pots with sphagnum moss in the greenhouse. | [27] |
| Hyponex | <i>Calanthe hybrids</i> | CW: 0, 10, 30, 50, 100 mL·L ⁻¹ | A CW concentration of 50 mL·L ⁻¹ significantly increased plantlet dry weight and fresh weight. | [28] |
| ½ MS | <i>Vanda pumila</i> | CW: 0, 50, 100 mL·L ⁻¹ Kin: 0, 1, 2 mg·L ⁻¹ BAP: 0, 1, 2 mg·L ⁻¹ | The highest number of shoots (9.5 shoots/culture) was obtained when the protocorm was cultured on ½ MS medium containing 1 mg·L ⁻¹ Kin added to 100 mL·L ⁻¹ CW. The longest shoots (0.7 cm/culture) were obtained when protocorm was cultured on ½ MS medium containing 2 mg·L ⁻¹ BAP added to 100 mL·L ⁻¹ CW. | [29] |
| ½ MS | <i>Phaius tankervilleae</i> var. <i>alba</i> | CW: 0, 50, 100, 150, 200 mL·L ⁻¹ PH: 0, 25, 50 g·L ⁻¹ | The highest number of shoots (3.0 shoots/explant) was found when protocorm was cultured on ½ MS medium containing 50 g·L ⁻¹ PH and supplemented with 50 mL·L ⁻¹ CW. | [30] |
| KC | <i>Dimorphorchis lowii</i> | CW: 100, 150, 200 mL·L ⁻¹ TJ: 100, 150, 200 mL·L ⁻¹ BH: 25, 75, 125 mL·L ⁻¹ P: 1, 2, 5 g·L ⁻¹ YE: 0, 2 g·L ⁻¹ | A CW concentration of 150 mL·L ⁻¹ yielded the highest percentage of explant forming shoots (100%), number of shoots per explant (13.83), and shoot length (38.86 mm). | [31] |

TABLE 1: Continued.

| Media | Species | Organic supplements (OS) | Key results obtained | References |
|-----------|--|---|--|------------|
| M | <i>Dendrobium nobile</i> | BH: 0, 10, 20, 30 g·L ⁻¹ CW: 100, 200, 300 mL·L ⁻¹ | A CW concentration of 200 mL·L ⁻¹ in M medium resulted in the highest percentage of regeneration (80.25%) and the highest number of PLBs/protocorm (10). | [32] |
| VW | <i>Dendrobium lasianthera</i> | P: 0, 1, 2, 3 g·L ⁻¹ | After 12 weeks of culture, a seed germination rate of 100% was observed in all treatments. Plantlets, 2-3 cm in height, were transplanted into plastic pots with a media mixture of coconut fiber and sphagnum moss (3:1; v/v) and acclimated in greenhouse. More than 90% of plantlets survived. | [33] |
| PM, MS, M | <i>Epidendrum ibaguense</i> Kunth | P: 0, 2 g·L ⁻¹ | Supplementation with 2 g·L ⁻¹ P in M and PM media increased germination and improved protocorm growth (90%) over the control (without P). The healthy <i>in vitro</i> plantlets with 2 to 3 leaves were individually grown in pots with a mixture of peat moss, brick pieces, and charcoal pieces (0.25:1:1), and 90% plantlets survived. | [34] |
| PM, MS | <i>Spathogottis plicata</i> Blume | P: 0, 2 g·L ⁻¹ | The highest percentage of seed germination (95%) was obtained on PM medium supplemented with 2 g·L ⁻¹ P. The well-rooted plantlets were transferred to pots containing a mixture composed of saw dust, coconut coir, humus, and coal pieces at (1:1:1:2; w/w/w/w) and had an 80% survival rate in an outside environment. | [35] |
| KC | <i>Dendrobium parishii</i> | P: 0, 2 g·L ⁻¹ | The highest percentage of seed germination (100%) was obtained when KC medium was supplemented with 2 g·L ⁻¹ P | [36] |
| KC, MS | <i>Aerides ringens</i> (Lindl.) Fischer | P: 0, 0.25, 0.5, 0.75 g·L ⁻¹ | The highest seed germination rate (89.28%) was recorded in KC medium containing 8.9 μM BAP supplemented with 0.5 g·L ⁻¹ P. Seeds cultivated on this medium also had the fastest growth (55-65 days) and the largest protocorm size (1.89 mm). Plantlets regenerated via <i>in vitro</i> seed germination processes were transferred into pots with a mixture of charcoal, broken brick pieces, and/or tree fern roots and had an 80% survival rate. | [37] |
| ½ MS | <i>Taprobanea spathulata</i> | P: 1.25, 2.5, 5 g·L ⁻¹ TJ: 5, 10, 15% (w/v) CW: 5, 10, 15 mL·L ⁻¹ | A P concentration of 5 g·L ⁻¹ resulted in the most significant induction of multiple protocorm (14.64). | [38] |
| BM-1 | <i>Paphiopedilum venustum</i> | BH: 0, 10, 20, 30 g·L ⁻¹ P: 0, 1, 2 g·L ⁻¹ | A P concentration of 1 g·L ⁻¹ facilitated the highest regeneration of adventitious shoots per explant (3.05) and the highest total number of plantlets per shoot (4.05). | [39] |
| Hyponex | <i>Vanda roxburgii</i> | PH: 0, 2.5, 5, 10, 20% (w/v) | A PH concentration of 20% significantly increased germination from 17.2% (control) to 78.24%. | [40] |
| VW | <i>Dimorphorchis lowii</i> | CW: 0, 10% (v/v) PH: 0, 10% (w/v) TJ: 0, 10% (w/v) | Seeds from 150-day-old capsules, grown on VW medium supplemented with 10% PH, showed the highest germination rate (98.02%), followed by 10% CW (97.23%), 10% TJ (93.71%), and the control (91.79%). | [41] |
| ½ MS | <i>Dendrobium tosaense</i> | BH: 0, 8% (w/v) PH: 0, 8% (w/v) CW: 0, 8% (v/v) | At a PH concentration of 8%, the highest seedling fresh weight (0.39 g) and longest root length (7.7 cm) were obtained. | [42] |

TABLE 1: Continued.

| Media | Species | Organic supplements (OS) | Key results obtained | References |
|------------------|--|---|--|------------|
| ½ MS | <i>Dendrobium officinale</i> | PH: 0, 25, 50, 100% (w/v) | The optimal shoot multiplication and shoot growth medium for <i>Dendrobium officinale</i> was ½ MS containing 0.1 mg·L ⁻¹ NAA and 2 mg·L ⁻¹ BA and supplemented with 100% PH. This medium yielded a multiplication rate of 6.2 and a shoot length of 2.5 cm. | [43] |
| VW | <i>Bulbophyllum nipondhii</i> | PH: 0, 25, 50, 75 g·L ⁻¹ | VW medium containing 100 mL·L ⁻¹ CW and supplemented with 75 g·L ⁻¹ PH, showed the highest number of new pseudobulbs/explant (3.5), number of roots/explant (8.1), mean root length (13.6 mm), and mean leaf length (19.2 mm). | [44] |
| MM | <i>Chloraea gavilu</i> | BH: 0, 100 g·L ⁻¹ TJ: 0, 100% (w/v) | BH successfully improved germination, reaching a rate of over 90%. | [45] |
| ½ MS | <i>Dendrobium</i> sp. | BH: 0, 2.5, 5, 10, 20% (w/v) | At a BH concentration of 10%, PLB regeneration was highest with 554 PLBs/culture. | [46] |
| HO ₂₆ | <i>Paphiopedilum hangianum</i> | BH: 0, 50, 100 g·L ⁻¹ | HO ₂₆ medium containing 1 g·L ⁻¹ P and 1 mg·L ⁻¹ NAA and supplemented with 100 g·L ⁻¹ BH, was suitable for plantlet formation (95.33%). Plants regenerated by <i>in vitro</i> germination processes were successfully grown in greenhouse conditions for 180 days with a survival rate of 88.5%. | [47] |
| Harvais | <i>Cypripedium macranthos</i> | CW: 0, 50, 100 mL·L ⁻¹ PH: 0, 25, 50 g·L ⁻¹ BH: 0, 25, 50 g·L ⁻¹ | Among the investigated organic supplements, the addition of BH (25 and 50 g·L ⁻¹) improved shoot number, root number, shoot length, and root length. | [48] |
| KC | <i>Hadrolaelia purpurata</i> | BH: 0, 90 g·L ⁻¹ P: 0, 1 g·L ⁻¹ CW: 0, 150 mL·L ⁻¹ | Highest seedling number (90.08) and leaf number (3.19) were obtained in KC medium supplemented with 90 g·L ⁻¹ BH. | [49] |
| VW | <i>Phalaenopsis amboinensis</i> | CW: 150 mL·L ⁻¹ CW: 150 mL·L ⁻¹ + BH 5 g·L ⁻¹ CW: 150 mL·L ⁻¹ + BH 10 g·L ⁻¹ CW: 150 mL·L ⁻¹ + BH 15 g·L ⁻¹ | Among the surveyed organic supplements, 150 mL·L ⁻¹ CW together with 10 g·L ⁻¹ BH in VW medium increased plantlets growth to the maximum height (62.1 mm) and dry weight (15.5 g). Plants regenerated via asymbiotic seed germination processes were successfully acclimatized in greenhouse conditions, and the survival rate was greater than 85%. | [50] |
| VW | <i>Dendrobium bigibbum</i> and <i>Dendrobium formosum</i> | CHT polymers: P ₇₀ , P ₈₀ , P ₉₀ with five concentrations: 0, 10, 20, 40, 80 mg·L ⁻¹ CHT oligomers: O ₇₀ , O ₈₀ , O ₉₀ with five concentrations: 0, 10, 20, 40, 80 mg·L ⁻¹ | The highest rate of seed germination (91.2%) was obtained with CHT type O ₈₀ at 10 mg·L ⁻¹ using <i>Dendrobium formosum</i> . | [51] |
| VW | <i>Dendrobium</i> sp. | CHT: 0, 5, 10, 15, 20, 25 mg·L ⁻¹ | The highest fresh weight of PLBs (0.83 g), number of PLBs (12.33), and number of plantlets (6.66) were obtained using VW medium supplemented with 15 mg·L ⁻¹ CHT. | [52] |
| VW | <i>Dendrobium phalaenopsis</i> | Shrimp CHT: 0, 5, 10, 15, 20, 25 mg·L ⁻¹ | At a CHT concentration of 15 mg·L ⁻¹ , the highest PLB growth from meristematic buds was observed, with a fresh weight of 0.15 g. | [53] |
| ½ MS | <i>Grammatophyllum speciosum</i> | CHT: 0, 5, 10, 15, 20, 25, 50, 100 mg·L ⁻¹ | The use of CHT at 15 mg·L ⁻¹ increased the PLB diameter (69 mm) when compared to the control (54 mm). Similarly this treatment yielded the highest number of new PLBs/explant (11.2), shoots/explant (3.1), and leaves/explant (1.8). | [54] |

TABLE 1: Continued.

| Media | Species | Organic supplements (OS) | Key results obtained | References |
|------------------|---------------------------------|---|---|------------|
| VW | <i>Phalaenopsis gigantea</i> | CHT: 0, 5, 10, 15, 20, 25 mg·L ⁻¹ | After 20 weeks of cultivation, 10 mg·L ⁻¹ CHT resulted in the highest increase in PLB fresh weight (3.4 g) compared with other treatments: control (1.5 g), 5 mg·L ⁻¹ (1.2 g), 15 mg·L ⁻¹ (0.8 g), 20 mg·L ⁻¹ (1.3 g), and 25 mg·L ⁻¹ (1.2 g), respectively. | [55] |
| Green house | <i>Grammatophyllum scriptum</i> | CHT: 0, 0.5, 0.75, 1 g·L ⁻¹ | The effective addition of CHT at concentrations of 0.5–0.75 g·L ⁻¹ resulted in a plant height of 0.8 cm and a leaf length of 0.5 cm. | [56] |
| Green house | <i>Dendrobium cv. Sonia 17</i> | CHT: 2.5, 5, 7.5, 10 mg·L ⁻¹ | The addition of 7.5 mg L ⁻¹ CHT resulted in the highest number of spikes/plant (9.93), number of florets/spike (8.45), spike length (32.87 cm), and flower diameter (7.92 cm). | [57] |
| Green house | <i>Dendrobium Suree Peach</i> | CHT: 0, 10, 30, 50, 100 mg·L ⁻¹ | At 10 weeks after transplanting in the greenhouse, the application of 100 mg·L ⁻¹ CHT, together with 5 g·L ⁻¹ lotus extract, resulted in the highest number of leaves (8.5). | [58] |
| KC | <i>Vanda helvola</i> | TJ: 0, 10, 15, 20, 40% (v/v) CW: 0, 10, 15, 20% (v/v) P: 0, 0.1, 0.2, 0.3% (w/v) YE: 0, 0.1, 0.2, 0.3% (w/v) | After 90 days of cultivation, KC medium supplemented with 15% TJ was the most suitable for seed germination, and 40% TJ supported the highest root number and length, as well as the widest leaf width. David et al. [59] also reported that the KC medium supplemented with TJ promoted rapid germination of seed (23 days) when compared to the control without TJ (28 days). Plantlets regenerated via the <i>in vitro</i> seed germination process were successfully acclimatized in greenhouse conditions. | [59] |
| ½ MS MS KC | <i>Geodorum densiflorum</i> | TJ: 5, 10, 15% (v/v) CW: 5, 10, 15% (v/v) PH: 5, 10, 15% (v/v) | The ½ MS medium supplemented with 5% TJ resulted in the highest germination rate and protocorm growth. | [60] |
| VW | <i>Vanda Kasem's Delight</i> | PH: 0, 5, 10, 20, 30% (w/v) PE: 0, 5, 10, 20, 30% (w/v) TJ: 0, 5, 10, 20, 30% (v/v) | VW medium supplemented with 20 and 30% TJ promoted rapid PLB development. | [61] |
| MS | <i>Dimorphorchis rossii</i> | YE: 0, 0.1, 0.2, 0.3% (w/v) | Protocorm proliferation was highest on 0.2% YE (41.67%), followed by the control (33.33%), 0.3% YE (31.25%), and 0.1% YE (12.50%) after 130 days of culture. | [62] |
| KC | <i>Vanda dearei</i> | YE: 0, 0.15, 0.2, 0.25, 0.5% (w/v) P: 0, 0.15, 0.2, 0.25, 0.5% (w/v) BH: 0, 2.5, 7.5, 12.5% (w/v) CW: 0, 10, 15, 20% (v/v) TJ: 0, 10, 15, 20% (v/v) | The addition of 0.5% YE significantly enhanced seed germination (85.9%) and shortened germination time to 23 days. Seedlings, approximately 6.5 cm in height, were adapted to the greenhouse environment and continued to develop into healthy plantlets. | [63] |

5. Peptone

P is occasionally used as a medium 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. [33] evaluated the effect of different P concentrations (0, 1, 2, and 3 g·L⁻¹) on seed germination and shoot formation of *Dendrobium lasianthera*, observed at 4, 8, and 12 weeks after culture. 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 the highest (84.0%) in the *Vacin and Went* medium (VW; *Vacin and Went* [75]) 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™, Sigma Chemical Co., USA) supplemented with 2 g·L⁻¹ P than on treatments that did not contain P [34, 35]. Similarly, Buyun et al. [36] found a seed germination rate of 100% for *Dendrobium parishii* with Knudson medium (KC; Knudson [13]) supplemented with 2 g·L⁻¹ P. According to Srivastava et al. [37], the seed germination rate of *Aerides ringens* increased significantly when cultured on KC

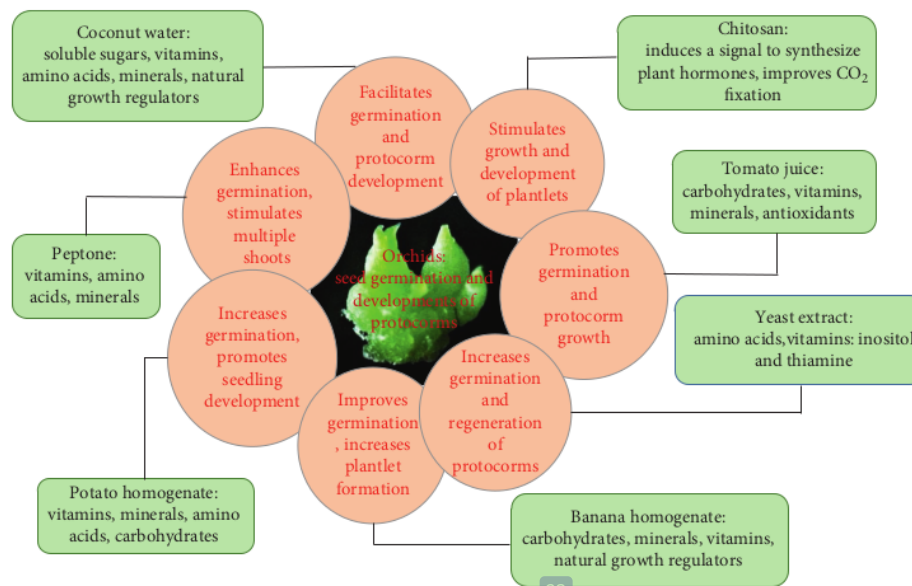


FIGURE 1: Overview on the contents and roles of some organic supplements in seed germination and further development of the protocorm in orchids.

medium containing $8.9\mu\text{M}$ benzylamino purine (BAP) and supplemented with P. Among the different concentrations of P tested (0.25 , 0.5 , and $0.75\text{ g}\cdot\text{L}^{-1}$), the highest germination rate (89.28%) was obtained in $0.5\text{ g}\cdot\text{L}^{-1}$ P as an additive after 30 days of incubation.

P is also known to stimulate the multiple shoot and protocorms formation. Devi et al. [38] reported that P improved the *in vitro* multiplication of protocorms of medicinal epiphytic orchid *Taprobanea spathulata*. Of the different concentrations tested, $5\text{ g}\cdot\text{L}^{-1}$ P was a suitable concentration for the induction of multiple protocorms. Kaur and Bhutani [39] 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; Waes and Debergh [76]) supplemented with P ($1\text{ g}\cdot\text{L}^{-1}$) 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 [77] because it contains high levels of amino acids [78] and vitamins, including thiamin, biotin, pyridoxine, and nitrogen [79].

6. Potato Homogenate

Orchid seed germination was significantly affected by the presence of PH in the medium (Table 1). Islam et al. [40] 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. [41] evaluated the effect of different organic supplements (PH, CW, and TJ) at various concentrations on seed germination of

Dimorphorchis lowii and found that 10% (w/v) of PH resulted in the highest seed germination rates. Additionally, PH enhanced seedling growth of *Dendrobium tosaense* [42] and stimulated the multiplication and growth of *Dendrobium officinale* shoots [43]. Besides, $75\text{ g}\cdot\text{L}^{-1}$ PH in VW medium containing $100\text{ mL}\cdot\text{L}^{-1}$ CW was most suitable for *in vitro* proliferation in *Bulbophyllum nipondhii* pseudobulbs [44].

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 [80, 81]. Miransari and Smith [82] 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. [45] 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 [83]) supplemented with BH successfully improved seed germination.

Islam et al. [46] 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 $\frac{1}{2}$ MS medium supplemented with 10% BH was the most suitable for PLBs regeneration. According to Zeng et al. [47], plantlet formation of ornamental lithophytic orchid *Paphiopedilum hangianum* increased significantly when PLBs were subcultured on HO₂₆ medium (Zeng et al. [84])

11 containing $1 \text{ g}\cdot\text{L}^{-1}$ P and $1 \text{ mg}\cdot\text{L}^{-1}$ NAA and supplemented with BH. The same study concluded that Harvais medium (Harvais [85]) 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 $50 \text{ g}\cdot\text{L}^{-1}$ improved shoot and root number and shoot and root length [48]. Gonçalves et al. [49] found that the number of *Hadrolaelia purpurata* seedlings produced was the highest on KC medium containing $90 \text{ g}\cdot\text{L}^{-1}$ of BH. Furthermore, Utami and Hariyanto [50] observed the effect of supplementation with $150 \text{ mL}\cdot\text{L}^{-1}$ CW with different BH concentrations (0, 5, 10, $15 \text{ g}\cdot\text{L}^{-1}$) 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, zinc, thiamin, riboflavin, niacin, pyridoxine, pantothenic acid, ascorbic acid, folic acid [86], and natural growth regulators such as zeatin, gibberellin, and IAA [87, 88]. It is also rich in carbohydrates, supplying energy to heterotrophic plants during the early stages of *in vitro* cultivation [89].

8. Chitosan

24 CHT, a biopolymer derivate of chitin, is mostly found in the exoskeleton of arthropods and crustaceans [90] and has been applied in various fields, including agriculture [91].

Numerous studies have shown that orchid seed germination is affected by the addition of CHT to the media (Table 1). Kananont et al. [51] 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: P₇₀, P₈₀, P₉₀; CHT oligomers: O₇₀, O₈₀, O₉₀) at five concentrations (0, 10, 20, 40, and $80 \text{ mg}\cdot\text{L}^{-1}$) 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 O₈₀ at $10 \text{ mg}\cdot\text{L}^{-1}$. Restanto et al. [52] 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 $25 \text{ mg}\cdot\text{L}^{-1}$), the PLB number, PLB fresh weight, and number of plantlets were the highest using $15 \text{ mg}\cdot\text{L}^{-1}$ of CHT. Nge et al. [53] also studied the effect of shrimp CHT at different concentrations (0, 5, 10, 15, 20, and $25 \text{ mg}\cdot\text{L}^{-1}$) on the meristematic bud growth of *Dendrobium phalaenopsis* and showed that VW medium supplemented with $15 \text{ mg}\cdot\text{L}^{-1}$ CHT resulted in the highest fresh weight of PLBs. Additionally, the same study concluded that $\frac{1}{2}$ 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, and $100 \text{ mg}\cdot\text{L}^{-1}$), a concentration of $15 \text{ mg}\cdot\text{L}^{-1}$ resulted in the highest growth rate (4-fold increase) [54]. Samarfard and Kadir [55]

investigated the effects of CHT on PLB proliferation in *Phalaenopsis gigantea*, and among the six concentrations (0, 5, 10, 15, 20, and $25 \text{ mg}\cdot\text{L}^{-1}$) tested, $10 \text{ mg}\cdot\text{L}^{-1}$ 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. [56] studied the effects of CHT on the growth of *Grammatophyllum scriptum* plantlets and suggested that CHT had significant effects on some parameters, including plantlet height and leaf length. Kumari et al. [57] investigated the effects of different CHT concentrations (2.5, 5, 7.5, and $10 \text{ mg}\cdot\text{L}^{-1}$) on the growth and development of *Dendrobium* cv. Sonia 17. Of the four concentrations tested, $7.5 \text{ mg}\cdot\text{L}^{-1}$ 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 [58] 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 $100 \text{ mg}\cdot\text{L}^{-1}$ within 10 weeks of transplanting. The increase in plantlet growth was allegedly under the influence of photosynthesis. According to Barka et al. [92], the addition of CHT derivative, i.e., Chitogel, improved CO₂ fixation by 1.5-fold and O₂ production by 2-fold, indicating that CHT derivatives have the potential to increase photosynthesis, thereby increasing plant growth. According to Uthairatanakij et al. [93], the beneficial effect of CHT may induce a signal to synthesize plant hormones, auxin and gibberellin.

9. Tomato Juice

TJ significantly influences the germination of orchid seeds and subsequent protocorm development (Table 1). David et al. [59] investigated the effect of different complex additives (TJ, CW, P, and YE) at various concentrations on seed germination in *Vanda helvola in vitro*. In their study, KC medium supplemented with 15% (v/v) TJ was the most suitable for promoting germination. Muthukrishnan et al. [60] also examined the effects of various organic supplements (TJ, CW, and PH) at different concentrations on seed germination in *Geodorum densiflorum* and showed that $\frac{1}{2}$ MS medium supplemented with 5% (v/v) TJ resulted in the highest rate of seed germination and protocorm growth. Similarly, Gnasekaran et al. [61] 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 [94], glucose, and fructose [95], 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 [96]. Tomatoes also contain other antioxidants, such as α -carotene, β -carotene, and ascorbic acid [97–100]. According to Gnasekaran et al. [61], 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 [101]. Numerous studies have shown that orchid seed germination and protocorm regeneration are affected by YE in the cultivation media (Table 1). Gansau et al. [62] investigated the effect of different YE concentrations (0, 0.1, 0.2, and 0.3%; w/v) on protocorm proliferation and growth of *Dimorphorchis rossii*. In their study, MS medium supplemented with 0.2% YE increased protocorm regeneration. Earlier, Jualang et al. [63] 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 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.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Both authors contributed to literature search, participated in manuscript writing, and read and approved the final manuscript.

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