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REVIEW ARTICLE

A Review on Undefined Organic growth Supplement stimulants for *in vitro* multiplication of medicinally and floriculturally important orchid species: *Cymbidium finalaysonianum* Lindl.

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ABSTRACT
Plant growth regulators at higher concentrations are known to induce somaclonal variations in the in vitro raised plantlets, which are undesirable in micropropagation. Occurrence of variations beats the primary purpose of clonal propagation, where clones are to be raised. To save the germplasm of rare and endangered orchid species, use of organic growth supplements can be employed. When compared to plant growth regulators, organic growth supplements are also effective at promoting the growth and development of in vitro cultures. In addition, they ensure production of true-to-type plantlets by maintaining the genetic homogeneity of the in vitro raised cultures. The aim of this study is to report a thorough review of the efficacy of various undefined organic growth supplements such as banana homogenate, apple juice, tomato juice, pineapple juice, potato extract in invoking the neo-formations in the regenerants, in order to exclude plant growth regulators.

Keywords: Orchid, undefined organic supplements, multiplication, *in vitro*, germplasm.

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INTRODUCTION

The family Orchidaceae is one of the largest family among angiosperms all over the world with 25,000 and 33,000 species belonging to 704-850 genera [1]. Orchids are cosmopolitan in distribution. They are distributed in almost all phytogeographical regions, including temperate, tropical, and subtropical regions with different microclimates and habitats ranging from tropical to temperate regions. There are 1484 orchid species, approximately, known to exist in India, out of which, 856 species inhabit the North - Eastern region (NER) [2]. Several horticulturally important indian orchids such as *Aerides multiflorum, Aerides odoratum, Arundina graminifolia, Arachnis, Bulbophyllum, Calanthe masuca, Coelogyne elata, C. flavida*, and *C. corymbose* have been utilized in various hybridization programs to raise hybrids of international repute. Numerous other orchid genera and species such as *Dendrobium aphyllum*, *Paphiopedilum venustum, D. nobile, D. chrysanthum, D. farmeri, D. chrysanthum, D. densiflorum, D. moschatum, D. fimbriatum, D. jenkinsii, Phaius wallichii, Pleione praecox, Renanthera imschootiana, Rhyncostylis retusa, Thunia alba, Vanda cristata, Vanda coerulea, Vanda coerulescens, P. spicerianum, P. hirsutissimum*, and *P. insigne* are also utilized in raising innumerable admirable hybrids of world-wide prominence [3]

Majority of orchid species have adopted epiphytic mode of habit. This adaptive strategy is particularly advantageous in rainforests, where orchids use the upper canopy's light and moisture for growth. Terrestrial orchids, on the other hand, grow in soil and are often found in temperate climates, where they contribute to forest floor biodiversity [4]. Orchids play several vital ecological roles, particularly as epiphytes in rainforest ecosystems. Epiphytic orchids grow on the branches and trunks of trees, utilizing them as structural support to access sunlight and moisture from rain and air. This positioning allows orchids to occupy vertical space within dense forests, contributing to the stratification of rainforest ecosystems [4]. By occupying these unique niches, epiphytic orchids create microhabitats for insects and small organisms, adding to the biodiversity and ecological complexity of the forest. Terrestrial orchids, such as those found in temperate forests, often act as indicators of environmental health due to their sensitivity to soil conditions and the presence of specific mycorrhizal fungi. Since orchids require specific fungal symbionts to germinate, their presence can signify a healthy and stable ecosystem.

Orchids play unique roles in various ecosystems, particularly in rainforest canopies, where many epiphytic orchids grow. They contribute to the vertical layering of forests, creating microhabitats for insects and small organisms, thus, increasing habitat complexity and supporting biodiversity [4]. Pollinator relationships of orchids are known for their complex and specific relationships with pollinators. Many species have co-evolved with certain pollinators, such as bees, birds, and moths. This intricate mutualism not only ensures orchid pollination but also helps sustain pollinator populations, demonstrating orchids' role as keystone species in their ecosystems [5]. Terrestrial orchids are excellent soil health indicators. They are often sensitive to environmental changes, and also act as valuable bioindicators of soil and ecosystem health. The presence and diversity of orchid populations in a given area can indicate the health of the soil and local ecosystems, as orchids require specific soil fungi (mycorrhizae) to grow and reproduce [6]. Throughout their evolution, orchid have developed unique specialized organs and structures that contribute to their ecological diversity. They exhibit diversity in morphology within their genera and as well as at species level.

Morphologically, the orchid flowers have developed certain unique features such as magnificent blooms with wide range of shape and size, dust-like, non-endospermic seeds, obligatory associations with mycorrhizal fungi, crassulacean acid metabolism (CAM), and exceptional reproductive structures such as pollinia, rostellum, and gynostemium that enhance their adaptability [7]. The architecture of orchid flowers is extremely unique in its complexity, largely due to the evolutionary needs for efficient pollination accomplishment. Orchid, flowers possess perianth; they generally consist of three sepals and three petals, with one petal gets modified into a labellum or "lip," which serves to attract and guide pollinators. This distinctive floral structure is a prime example of co-evolution, as orchids have adapted to attract specific pollinators, ranging from bees to birds, depending on their geographic location and ecological niche on this globe. Orchids employ various strategies to ensure successful pollination. For instance, certain orchids mimic the appearance or scent of female insects to lure males for the process known as "pseudocopulation and, thus, facilitates pollination [8]. Other orchids emit strong fragrances to attract specific pollinators, such as nocturnal moths or butterflies, during the precise times of day or night [5].

Another crucial element in the life cycle of orchid species is their symbiotic relationship with mycorrhizal fungi. Orchid seeds are tiny and microscopic in structure and also lack endosperm. As a consequence, these reduced seeds rely on specific fungi for nutrient uptake during germination from soil. This relationship is essential for the survival of orchid species, particularly those in nutrient-poor environments like tropical rainforests, where the fungi facilitate nutrient uptake from the environment [9]. Noteworthy genera include *Phalaenopsis, Cymbidium, Odontoglossum, Cattleya, Paphiopedilum, Cypripedium, Oncidium, Zygopetalum, Mokara* etc. known for their large, resilient blooms that are widely popular as houseplants; *Cattleya*, often recognized for their vibrant colours and commonly used in corsages; and *Paphiopedilum* known as lady's slipper orchids, possess distinctive pouch-shaped posterior petal called labellum or lip to trap pollinators. These abovesaid genera exemplifies the structural diversity within the family orchidaceae, and showcases the wide range of adaptations that orchids have developed to ensure their survival and reproduction in nature.

ORCHIDS OF MEDICINAL AND FLORICULTURAL POTENTIAL

A large number of orchid species, holds huge medicinal potential, have played a long-standing role in traditional medicine across various cultures. They pose high therapeutic properties, such as anti-inflammatory, antioxidant, immunomodulatory properties [10]. *Dendrobium* species are used for their antipyretic and restorative properties, helping with immune health and vitality. These herbal species contain alkaloids, phenols, and polysaccharides with potential health benefits [10]. Literature explorations reveal, bioactive compounds in *Gastrodia* that are known for their antioxidant and anti-inflammatory activities, which have been associated with potential therapeutic applications in neurological conditions such as Alzheimer's and Parkinson's disease [11]. *Vanilla planifolia*, while primarily valued for its culinary applications, also have medicinal properties; *Vanilla* extract contains vanillin, a compound with antioxidant and anti-inflammatory properties, often used for calming, anti-stress purposes, and digestive support [12]. Since the majority of the drug businesses rely on plants to produce pharmaceutical chemicals, medicinal plants are of tremendous interest to biotechnology researchers. *Malaxis acuminata*, a terrestrial species, also known as "Rishbhak," is well-known for its significant therapeutic value since its dried pseudobulbs are a key component of "Ashtavarga" medications, which are used to make the ayurvedic remedy "Chyavanprash." The species is a powerful aphrodisiac and is used to treat tuberculosis [13]. Numerous orchids are beneficial herbs that are used to treat a range of issues connected to virility and fertility along with treatment of respiratory, digestive, rheumatic, cardiac, neurological, and dermatological ailments [14]. Orchids have also carved a niche for themselves in the world of horticulture. Some of the notable species are:

***Phalaenopsis* (Moth Orchid):**

This widely cultivated orchid is known for its aesthetic appeal, *Phalaenopsis* is one of the most popular species used in horticulture. Its resilience and ease of propagation make it a most sought-after orchid flower species in both commercial and personal collections [15].

***Paphiopedilum insigne* (Lady's Slipper Orchid):**

This species is notable for its unique pouch-like structure, which aids in trapping pollinators. *Paphiopedilum* orchids are appreciated for their intricate beauty and are often featured in conservation efforts due to their vulnerability in the wild [16].

***Vanda coerulea* (Blue *Vanda*):**

Known for its striking blue flowers, *Vanda coerulea* is both culturally significant and endangered. It has become a symbol of conservation efforts within the orchid family, and its unique pigmentation has made it highly desirable in horticulture [17].

***Orchis mascula* (Early Purple Orchid):**

This European terrestrial orchid is valued for its role in traditional folk medicine and is often used as a symbol of spring in local cultures. *Orchis mascula* was historically utilized as an herbal remedy for various ailments and is now primarily protected due to habitat loss [18]. In the horticulture and floriculture commerce, the most popular species are those with beautiful flowers, but there are other species with tiny sizes, nice scents, or leaf patterns which are also preferred as ornamental plants [19].

THREAT TO ORCHID POPULATION IN THEIR WILD HABITATS

The beauty and resilience, in terms of long vase life, of these orchids have made their mainstay in floral arrangements in weddings, and ornamental displays, symbolizing luxury, and sophistication. Orchids play a very significant role in the global floricultural market because of its long-lasting large magnificent blooms. Owing to their extended shelf-life, variety of colours, and other desirable qualities that buyers seek depending upon the occasion and culture, orchids are currently quite popular on the global market [20]. Cut-flowers and artificially propagated plants cultivated in commercial greenhouses make up the great majority of the orchid commerce today. Up to 10% of the world's cut-flower commerce is made up of orchid blooms.

Because of their beauty, cultural appeal, and therapeutic potential, orchids face numerous threats in their natural habitats. They face survival challenges due to habitat fragmentation and overexploitation [21]. Habitat destruction due to logging, agriculture, and urban development is among the most pressing issues, particularly for orchids in tropical rainforests, where biodiversity is highest [6]. Some rare orchids, particularly those with unique colours or patterns, are highly sought after by collectors, leading to overharvesting and the depletion of natural populations. As forests are cleared, not only the orchids are directly removed, but their host trees, fungi necessary for their germination, other co-existing microorganisms, flora and fauna also get wiped away. This disrupts the delicate balance of the ecosystem in which they flourish. Above all, the illegal trade of wild orchids also creates another significant threat to their populations.

Another prominent factor such as climate change also represents a more insidious threat to orchid populations in their natural environment particularly in regions where temperature and rainfall patterns tend to fluctuate rapidly. Changes in climate can affect orchid habitats, fungal partners, and their pollinators, all of which are critical to the orchid reproduction and survival. It is also found that orchids in alpine and temperate regions are especially vulnerable to climate change, as rising temperatures force species to migrate to higher altitudes or face extinction [22]. Environmental changes and overexploitation of orchid species to meet global demands have put many orchid species at the verge of extinction.

CONSERVATION OF ORCHIDS

Numerous orchids have become endangered due to unsustainable (sometimes unlawful and/or unrecorded) harvest for horticulture, food, or medicine, in addition to threats like habitat destruction and climate change. Therefore, in order to successfully save orchids for future, there is a strong need to develop workable strategy that could enable us to address the threats on a broader scale [23]. Due to decline in orchid populations, conservation efforts for orchids are increasingly focused on both *in situ* (within natural habitats) and *ex situ* (outside of natural habitats). *In situ* conservation aims to protect orchids by preserving their native habitats, promoting sustainable land use practices, and restoring degraded ecosystems. This method is vital for preserving the complex ecological relationships between orchids, their fungal partners, and their pollinators [6]. *Ex situ* conservation includes the cultivation of orchids in botanical gardens, seed banks, and research laboratories. These initiatives are crucial for endangered orchids, allowing for the propagation of species that may otherwise disappear from the wild. Recent advances in symbiotic propagation, where orchid seeds are germinated with their specific mycorrhizal fungi, have allowed researchers to grow orchids from seed more effectively, thus, aiding in conservation and restoration projects [9].

Keeping an eye on orchid populations and habitats, by managing species in *ex situ* conservation, creating artificial propagation and gene banks, protecting land and habitat and using communication, would help to raise awareness and educate people that could help in saving the orchid species in their natural habitats [28]. Seeds must come into contact with a certain mycorrhizal fungus in order to germinate in their native habitats, and they may need to rely on another fungus at a later stage of development. The orchid flowers frequently require a particular pollinator, to complete their life cycle. Orchid flowers and their insect pollinators frequently have complex connections.

Another method that is used to save orchid species is micropropagation. It is a productive technique for producing homogenous plant material in large quantities [24]. The most frequently discussed topics in micropropagation is to optimize the best combinations of macro elements, microelements, vitamins, amino acids, growth regulators, and occasionally antibiotics and chemicals that bind phenolic compounds, is crucial for rare plant species that have not been previously regenerated *in vitro*.

ROLE OF PGRS AND ORGANIC GROWTH SUPPLEMENTS

Phytohormones are a class of chemical substances that are found naturally and have a small effect on physiological processes. Although other processes like stomatal movement may also be impacted, the processes that are impacted primarily include growth, differentiation, and development [25]. The substance, known as a plant hormone, is synthesized inside the plant. Present review is aimed to report the replacement of plant growth regulators by undefined organic growth supplements in the culture medium. Literature studies reveal that, the use of plant growth regulators at high concentration leads to the occurrence of the somaclonal variations [26]. Plants originating from any type of cell culture were referred to as "clones," and the genetic variations among these plants was referred to as "somaclonal variations." Only mitotic cell division is required for the asexual reproduction *in vitro* and regeneration into whole plants [27]. Somaclonal variations are genetic and epigenetic abnormalities result from the instability of *in vitro* cultures. These modifications can occasionally have positive outcomes; for instance, they can be employed in breeding initiatives to create new cultivars with desired traits [28]. Fruit and vegetable-based organic growth supplements are nutrient-dense and jam-packed with bioactive chemicals, which might promote orchid development and increase the plant's resistance to environmental stresses. Phenolics, flavonoids, and organic acids are among these substances, and they have shown to promote plant development in a variety of plant species and orchid species. To illustrate the point, fruit extracts' phenolic components may operate as natural insecticides and fungicides, protecting plants against harmful invaders [29].

IMPACT OF PGRS CONCENTRATION AND SOMACLONAL VARIATIONS

The ability of organic supplements to promote plant tissue growth *in vitro*, especially in orchid cultivation, is widely known. These supplements, which include different fruit homogenates and plant extracts, are incorporated into culture medium for their vitamins, fibres, proteins, and hormones as well as natural carbon sources [30]. Organic growth supplements that can increase multiplication rate and promote plantlet growth include potato extract, apple juice, banana homogenate, coconut water, and other organic growth additions in tissue culture medium. Many sources are investigated for their ability to incorporate into tissue culture as a replacement for synthetic nutrient media, given in Table 1 Adding these organic growth additives to the culture medium, helps to enhance the number of shoots, roots, and leaves and also promote shoot regeneration [31].

Application of organic nutrients in the medium can enhance the growth and morphogenesis in cultured plant cell and tissues. The organic growth supplements are the rich source of vitamins, minerals, carbohydrates, amino acids, and other unidentified supplements (including some compounds that are not technically animal vitamins). The requirement of different component of medium depends on the genotype of the species [32]. Micronutrients like potassium and calcium are vital for plant metabolism, enzyme activation, and cellular processes; they are found in organic supplements [33].

Table. 1 List of organic growth supplement sources.					
S. No.	Organic Supplement	Source Plant / Material	Active Constituents	Reported Effects on Orchids	References
1	Coconut water	*Cocos nucifera*	Cytokinins, auxins, sugars	Enhances shoot proliferation	[34]
2	Banana pulp	*Musa paradisiaca*	Sugars, auxins, potassium	Improves protocorm development	[35]
3	Potato extract	*Solanum tuberosum*	Starch, vitamins B and C	Promotes shoot and root growth	[35]
4	Pineapple juice	*Ananas comosus*	Sugars, bromelain	Induces somatic embryogenesis	[36]
5	Sugarcane juice	*Saccharum officinarum*	Sucrose, minerals	Supports shoot growth and chlorophyll synthesis	[37]
6	Tomato juice	*Solanum lycopersicum*	Vitamins A, C, minerals	Stimulates callus and protocorm formation	[38]
7	Orange juice	*Citrus sinensis*	Vitamin C, sugars	Enhances pigmentation and cell division	[39]
8	Corn extract	*Zea mays*	Sugars, minerals	Improves rooting and plantlet elongation	[40]
9	Alfalfa extract	*Medicago sativa*	Saponins, phytohormones	Enhances shoot differentiation	[41]
10	Spinach juice	*Spinacia oleracea*	Iron, chlorophyll, vitamins	Enhances greening and chlorophyll content	[42][35]
11	Papaya extract	*Carica papaya*	Enzymes, sugars	Improves callus and embryo formation	[35]
12	Aloe vera gel	*Aloe barbadensis*	Polysaccharides, salicylic acid	Enhances rooting and disease resistance	[43]
13	Carrot juice	*Daucus carota*	Carotenes, vitamins	Promotes morphogenesis	[35]
14	Chickpea extract	*Cicer arietinum*	Protein, isoflavones	Enhances shoot development	[44]
15	Mung bean extract	*Vigna radiata*	Proteins, phytohormones	Enhances protocorm formation	[45]

In-vitro propagation is quintessential methodology for the conservation of endangered orchid species. Rather than using costly synthetic additives, cost-effective organic ingredients can be added to the tissue culture mix to change it. Fruit juice and coconut water are two examples of organic sources that are great additions for *in vitro* production because they include high levels of vitamins, amino acids, and organic compounds that can act as growth regulators [31]. Numerous organic substances, such as Coconut water (CW), Banana homogenate (BH), Potato homogenate (PH), Tomato juice (TJ) can encourage the germination of seeds as well as the growth and development of different orchid species. Additionally, they promote the development of numerous shoots, plantlets, protocorm-like bodies (PLBs), and seedlings [46]. Presently, efforts were made to test the efficacy of tomato juice in MS medium on the multiplication of PLBs of *Cymbidium finlaysonianum* Lindl.

MATERIALS AND METHODS

Protocorms (2mm in length) were taken 34 weeks old from *in vitro* cultures. Tomatoes were obtained from market and used in the form of juice extract in the medium. After adding tomato juice, pH of the medium was adjusted to 5.8 with 1N HCl / 1N NaOH before autoclaving. The inoculation was conducted in the aseptic condition in laminar fair flow cabinet and cultures were incubated at 25 ± 2°C for 12 hours either in the dark or under a light photoperiod of 40 μ mol^{-2} s^{-1} in the tissue culture lab. Weekly observations of the cultures were made, and the data was documented appropriately. The results are represented as mean ± standard deviation.

RESULTS

Presently, the protocorm segments regenerated readily in MS medium supplemented with tomato juice (Fig.1a). Organic supplement proved advantageous as its addition supported elevated regeneration response i.e. 87.5 ± 0.25 % (Fig. 2); as many as 6.50 ± 0.70 PLBs, after 3 weeks of culture, were obtained (Fig. 3). The PLB multiplied profusely in MS medium supplemented with tomato juice and also formed shoots earlier than those present in MS medium devoid of tomato juice (Fig.1b).

Fig. 1: *In vitro* multiplication of PLBs in MS medium and its supplementation with organic growth supplement a. Initiation of PLB in control b. Profuse multiplication of PLBs and shoot formation in MS + tomato juice

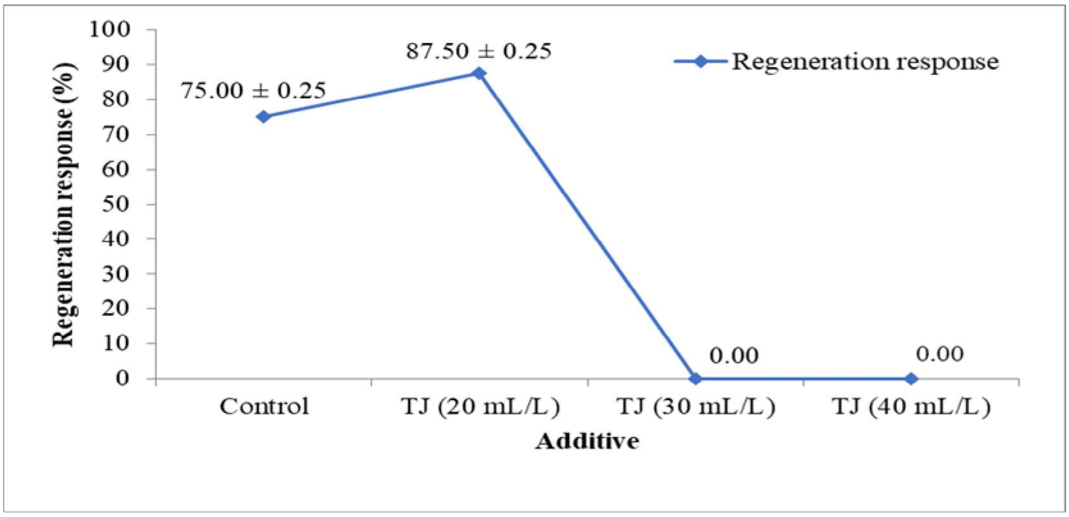

Fig. 2: Represents comparative analysis of regeneration response (%) of PLBs treated with different concentrations of Tomato juice (TJ) in MS (1962) medium and as control; Data represented as Mean ± Standard Deviation (SD)

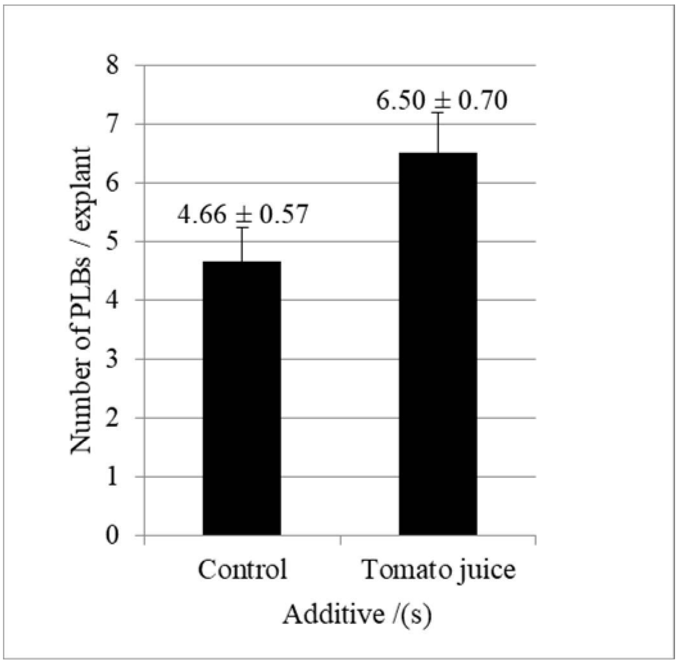

Fig. 3: Represents the number of PLBs formed MS medium (control) and MS + TM; Data represented as Mean ± Standard Deviation (SD)

Earlier, according to [53], in *Physalis angulata*, tomato juice (TJ) encouraged shoot regeneration and multiplication and also supported maximum shoot number (12.5) in MS supplemented with TJ @5% and maximum shoot length (10.7 cm) in MS supplemented with TJ@7.5%.

Literature studies reveal that, adding banana homogenate (50 g/l) to basal medium can significantly alter the percentage of responding explants [34]. In *Cymbidium pendulum*, up to 80.5% of explants regenerated, and the resulting shoot was robust in size when medium was supplemented with banana homogenate [34]. According to [47] BH can improve *in vitro* regeneration of *Dendrobium* orchid PLBs. BH (10%) can lengthen the shoots in *Vanda* species. [48] reported that BH (10%) has positive impacts on *Spathoglottis kimballianai* leaf size. In cultures of *Dendrobium nobile*, banana homogenate dramatically boosted the number of leaves [49]. Orchid roots might benefit from the potassium-rich banana juice which increase their robustness and adaptability to a wider range of growth conditions [34]. Orchid roots might benefit from the potassium-rich banana juice, which is essential for osmoregulation and nutrient transport in plants. Bananas also have the plant hormones auxins and cytokinin, which promote growth by increasing cell division and elongation [50]. Literature exploration also points about the effects of banana homogenate and potato extract on the growth of several ornamental plant species. Due to having nutritional qualities, banana homogenate is favoured among these natural substances and is primarily utilized in orchid crops. The addition of BH to the medium has been shown to improve the rates of protocorm-like body formation, seed germination, and shoot and root growth in tropical orchid cultures [51]. Another organic growth supplement such as coconut water is heavily loaded with minerals, vitamins, thiamin, pyridoxine, and ascorbic acid, as well as soluble carbohydrates, which are a natural source of carbon. Additional organic ions that have been demonstrated to promote germination include calcium, magnesium, potassium, phosphorus, iron, and manganese [52]. The high bioactivity of coconut water is due to its rich composition of minerals, amino acids, sugars, cytokinin's, and auxins, among other plant bio-stimulants. Coconut water contains cytokinins, which is able to trigger cell division and enhance root elongation; this may explain why it speeds up the germination process in certain orchid species [54]. Orchids get magnesium from coconut water, which helps with the photosynthetic efficiency and leaf colouration since magnesium is a key ingredient in chlorophyll production. the *in vitro* propagation of *Laelia rubescens* using Phytamax medium supplemented with 100 ml/L pineapple juice produced the highest seedling height (1.31 cm), number of leaves per seedling (3.33), and roots (2.33). In contrast, pineapple juice proved best for *in vitro* development of *Laeliocattleya* on MS medium at 200 ml/L [55].

Potato extract has also been proven advantageous in enhancing growth of the *in vitro* raised plantlets. The high starch content of potato extract ensures that orchids have a consistent supply of carbohydrates, which they use as an energy source and to support their metabolic processes. In addition, vitamins and amino acids included in potatoes have a role in energy generation and chlorophyll synthesis [56].

CONCLUSION

Based on literature studies, we can conclude that the incorporation of a variety of organic supplements such as banana homogenate, coconut water, tomato juice, pine apple juice, potato extract to the culture medium remains promotive in most of orchid species at optimum concentrations. As organic growth supplements are the source of combination of growth factors and organic nutrients, which assist in high rate of seed germination, growth and development of the plantlets / seedlings, PLB multiplication and high regeneration response of the explants. Further research is required in this field to find the complexities of organic growth supplements and their abilities for other plant species as well, which enhance positive growth of the seedlings and plantlets, thus, minimizing or even excluding the use of PGRs in the nutrient pool.

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