Advances in Bioresearch
Adv. Biores., Vol 17 (4) April 2026: 323-333

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.17.4.323333

Advances in Bioresearch

REVIEW ARTICLE

Plant-based Materials as a substitute of agar for *in vitro* Multiplication of therapeutically and Floriculturally important Orchid species- a review

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ABSTRACT
The ever-increasing exigence for orchid species, with therapeutic importance, calls for the development of applicable and sustainable in vitro cultivation techniques. Agar, which has long been extensively employed in plant tissue culture as a gelling agent, is obtained from marine algae, whose populations are being exploited to a larger extent to extract agar. Other replacement of agar as solidifier must be discovered to save the populations of marine plant species (Rhodophyta), thus, to keep marine ecosystem healthy. Materials such as coconut coir, Pinus bark, filter paper, cotton, rice husk, different types of gums (Guar gum, Tragacanth, Gum Arabic, Isubgol) are investigated so far to support in vitro regeneration and multiplication of the germplasm of orchid species. The main objective of this review paper is to explore the possibilities of replacing agar in plant tissue culture for gelling of the culture medium. Since the agar is obtained from marine algae whose populations, currently, are being exploited. This practice could figure the marine algae as rare and endangered species. Biodiversity conservation is of prime importance for the existence of every species on this globe. Current review paper presents a report on the usage of different gelling agents that have been tested so far and also reports some more materials that could be used as a substitute to agar. Literature studies reveal that no suitable alternative of agar has been discovered yet which could support the regeneration and multiplication of diverse orchid species in vitro. Our future studies are directed to find out a suitable alternative of agar.
Keywords*: Orchids, therapeutic, protocorm like bodies, culture medium, agar.*

Received 29.11.2025 Revised 23.02.2026 Accepted 28.03.2026

How to cite this article:
Shaina T and Saranjeet K. Plant-based Materials as a substitute of agar for *in vitro* Multiplication of therapeutically and Floriculturally important Orchid species- a review. Adv. Biores. Vol 17 [4] April 2026. 323-333

INTRODUCTION

The orchidaceae is one of the most diverse and species enriched family of flowering plants, encasing 850 genera and 33,000 species adapted to all continents except Antarctica [1]. It is one of the largest monocotyledonous angiosperm families, that hold significant medicinal, ornamental, ecological and evolutionary importance. Throughout their evolution they have developed unique features that contribute to their vast diversity [2]. Due to their varied growth patterns, life cycles, and habitats; the orchids display a wide range of physiological traits. Epiphytic orchids typically have thick, fleshy leaves with robust cell wall, protective cuticles, and sunken stomata, while terrestrial orchids are distinguished by the presence of rhizomes, corms, or tubers [3].

Some of the botanically interesting features of orchids are dust-like non-endospermic seeds, zygomorphic flowers, reliance on specific pollinator, requirement of mycobiont, long-lived pollen tubes, and post-pollination development of ovules. Despite their diversity, the specificity of their pollinator and fungal associations marks them highly sensitive to environmental changes [4]. Orchids, known for their extraordinary floral diversity, showcase an immense variety of unique and often mesmerising floral architecture, representing a highly advanced stage of floral evolution within monocotyledons. Along with their ecological adaptations, orchids exhibit distinct reproductive strategies, such as packaging of mature

pollen into pollinia, ovary and ovule development regulated by pollination, precise synchronization of gametogenesis for efficient fertilization, and the release of vast number of immature embryos from immature and mature pods [5].

Orchids are found in diverse regions, including the tropics, subtropics, and temperate zones, with their noticeable presence in tropical vegetation as epiphytes. In India, terrestrial orchid species flourish in the Western Himalayas, whilst epiphytic orchids are mostly found in the Eastern Himalayas and the Western Ghats [6]. The distribution of orchids is uneven, with a concentration in riparian zones and moist forest pockets where the habitat supports their growth, development, and regeneration [6]. In the North-west Indian Himalayas, terrestrial orchids grow in humus-rich, damp soil beneath tree shadows, whereas small-flowered orchids are found in the Western Ghats [7]. Geographical distribution of orchids in India has been indicated (Table 1).

Table1: Geographical distribution of Orchids in India [8].

Region	Indian state	Genera	Species
North-Western Himalayas	Jammu and Kahmir, Uttarakhand, Himachal Pradesh.	75	288
Eastern Himalayas and North Eastern India	Assam, Arunachal Pradesh, Meghalaya, Manipur, Mizoram, Nagaland, Tripura and Sikkim.	157	870
Peninsular region	Central India, M.P., Gujarat, Orissa, Andhra Pradesh, Western Ghats and East India.	89	379
Andaman and Nicobar Islands.	Islets in the Bay of Bengal and 319 Islands.	53	115

Orchids are also known as excellent ecological indicators [9]. Many species are cultivated for their economic importance, particularly for floriculture business, where they are prized as cut-flowers for their exotic beauty and long- lasting blooms [10]. Although grown for ornamental purposes, orchids are also used in traditional herbal medicine, as food, in perfumery and jewellery industry, and for cultural practices by various communities worldwide [11].

Based upon geographical area, the orchids grow as epiphytes (those that grow on other plants) usually have thick, fleshy leaves with tough outer layers and tiny sunken pores [12] and terrestrials (those that grow on the ground soil) often have perennating organs such as underground stems or storage structure such as rhizomes, corms, or tubers [13]. Orchids grow slowly, their minute seeds take long time to mature, and complete their life cycle. Their growth requirements (for instance, photoperiod; availability of soil nutrients) vary according to their habitats in which they dwell in nature. Orchids can adjust to different light conditions over time, but sudden change in light can put them under physiological stress [3].

The threatened status of many orchid species is driven by factors such as small population sizes, restricted geographic distribution, and their dependence on specific pollinators and mycorrhizal partners in their wild habitats. This delicate ecological balance is increasingly disrupted by climate change, habitat destruction, and shifting land use practices. In addition, introduction of invasive species and illegal collection of their populations further exacerbate threat to their wild populations [14].

Orchids have been used for thousands of years in traditional medicine to treat a wide range of diseases and ailments, stomach disorders, chest pain, rheumatism, cholera, arthritis, paralysis, syphilis, jaundice, including tuberculosis, gastrointestinal problems, sexually transmitted diseases, hepatitis, acidity, muscle pain, asthma, malaria, earache, leucorrhoea, cancer, piles, wounds, sores and anticancerous [15,16,17,18,19,20]. Orchids have been utilized in traditional medicine including Ayurveda, Siddha, Homeopathy and Yunani since ancient times [21].

Orchids are globally traded as both cut-flowers and potted plants, with market demand rising alongside increased trade volumes. Rich in alkaloids, polysaccharides and other beneficial compounds, orchids are also utilized in the feed and beverage in industries [22]. Beyond their significant economic and ornamental value, orchids hold special cultural, meaning in classical Chinese literature, orchids are celebrated as one of the “Four Gentlemen among the Flowers,” alongside Chinese plum, chrysanthemum, and bamboo [23]. These flowers are also used in food- flavouring products, making them economically significant in the global, horticultural and food industries [24]. However, in the Southern African region, orchids hold particular importance for their tubers, which are used as food source [25]. Orchids are also being applied in cosmetology and cosmeceuticals. Some orchids, such as *Dendrobium chrysotoxum, Rhynchostylis retusa,* and *Vanda coerulea*, are recognized for their anti-ageing properties [26]. Their plant extract helps in combating skin ageing, promote cell and tissue longevity, and restore skin hydration and

radiance by acting directly on the skin cells or stimulating the enzymes involved in the skin's ageing process [26].

In nature, the populations of medicinal orchids are declining day-by-day, primarily due to human intervention, deforestation for agricultural purpose, industry setup and for promoting housing. As a consequence, their habitats are getting destroyed which is directly impacting their species diversity. Infect, whole family orchidaceae appears in CITES appendix I and II [27].

So, there emerges strong urgency to save their populations, from getting extinct, in their natural habitats. Since conventional methods of orchid propagation face significant limitations, including slow growth, low germination rates, vulnerability to pests, and nutritional deficiencies the use of biotechnological tools and techniques have proven to be the most effective means for improving the propagation process and saving their germplasm *in vitro*, thus assisting with their conservation [28].

The initial stages of orchid reproduction and development, particularly seed germination, are strongly affected by the orchid's relationship with suitable mycorrhizal fungi and environmental conditions. Orchid seeds, which are minuscule and resemble dust particles, which are produced in large number, weigh between 0.3 and 14 micrograms, with each seed capsule containing between 1300 and 4 million seeds that are suited for wind dispersal [29]. These traits, such as their small embryos and lack of endosperm, make germination difficult [29]. The lignified pectin layers of seed coat can impede water absorption, restrict embryo expansion, and, under laboratory conditions, delay germination in terrestrial orchids [30].

In plant tissue and cell culture research, the composition of the medium plays a crucial role in determining the efficiency of the micropropagation process [31]. A large number of culture media are used to propagate orchids *in vitro* that are solidified with frequently used material such as agar. By creating a continuous three-dimensional molecular network, several colloidal polysaccharides and specific proteins of microbial and plant origin often function as solidifiers or stabilisers in the medium. Gelling agents affect the diffusion properties of medium and give it rigidity. The medium's viscosity, which in turn depends on the agent's concentration and physicochemical properties, determines the diffusion rate [32].

Agar in plant tissue culture:

"*In vitro* tissue culture" is influenced by various factors such as sterility, growth, and development [33]. While preparing any culture medium, the agar is most widely used gelling agent for over a century due to its stability, clarity, non-toxic nature, and resistance to degradation [34]. Agar is primarily made up of two polysaccharides: agarose, which makes up about 70% of the mixture and is mainly responsible for its gelling properties, and agaropectin. Both polysaccharides share a backbone composed of alternating galactopyranose sugars, forming repeating agarobiose units.

These units link together to create long chains with an average molecular weight of 120,000 daltons, equivalent to around 400 agarobiose units [35]. Over the past century, plant culture methods have advanced significantly. Progress in cell theory knowledge and research has led to substantial innovations that have transformed traditional cultivation practices [36].

A substance made up of replicated molecules of repeating agarobiose units, is termed agar. However, agarose is a least substituted agar molecule with the best gelling capability [37]. It is a hydrocolloid derived from seaweed, rich in polysaccharides and well-known for its strong gelling properties [38]. Since then, it has been used as solidifier and emulsifier in plant tissue culture, food, cosmetic, and pharmaceutical industry due to being of inert nature [39]. It acts as a solidifying agent and assists in the hardening of the culture medium that influences the growth of the cultured tissues [40]. The structure of Agar [41] is shown in (Fig. 1.).

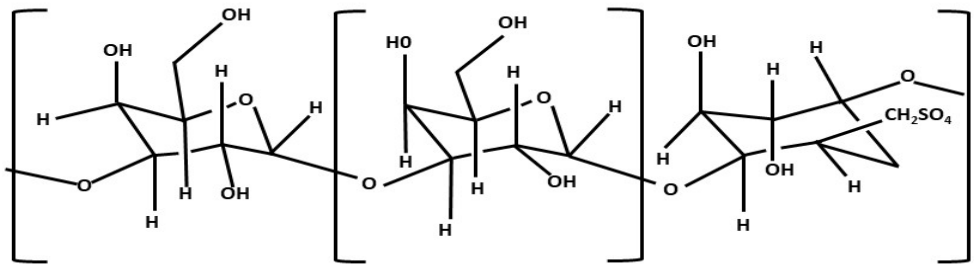

Figure 1. The structure of Agar [41].

Properties of agar:

Compared to other gelling agents, agar has a number of advantages [40]. It is found as a structural carbohydrate in cell walls and likely also plays a role in ion exchange and dialysis functions [42]. When combined with water, it melts readily at temperatures between 60 and 100°C, solidifies at about 45°C, and creates a gel that is stable at all practical incubation temperatures. Plant enzymes cannot break down agar gels, nor do they react with the components of the medium. It is frequently utilized in culture medium at 0.8–1.0% concentrations, particularly in studies involving tissue metabolism, pure agar preparations are crucial [40]. Agar, derived from the red algae, is a blend of agarose and agaropectin, with agarose being favoured for its superior properties [43]. Agar gels are essential in food preparations that require a high content of soluble dietary fiber, as agar is the food additive with the highest concentration of this type of fibre exceeding 94% [44]. Its key applications are due to its rheological characteristics, making it valuable in the food, biotechnology, and pharmaceutical sectors. Here, it serves as a thermoreversible gelling agent, stabilizer, texture enhancer, and thickener [43].

Risk of agar resource depletion:

Agar is primarily derived from *Gracilaria* (53%), *Gelidium* (44%), and certain agarophytes (3%) like *Gelidiella* and *Pterocladia*. Although agar, extracted from *Gelidium*, has high gel strength, its high cost and the depletion of natural water sources restrict its usage, highlighting the need to identify alternate natural resources of these hydrocolloids [45].

Industry Demand:

Agar-agar serves multiple roles across industries. In the food sector, it plays a role as a gelling thickening and stabilizer in products such as jellies and desserts. It is also essential in microbiology, where it is widely used as a culture medium for growing bacteria and fungi [46].

Conservation Strategies:

To maintain a steady and sustainable supply of agar-agar, effective conservation measures are crucial. These include encouraging responsible harvesting techniques, monitoring natural seaweed habitats, and investigating alternatives or synthetic sources of agar-agar [47].

Ecological Significance:

Seaweed ecosystems play a critical role in supporting marine biodiversity and maintaining oceanic health. Overharvesting seaweed can lead to ecological imbalances, harming marine habitats and diminishing ocean resilience [48].

Contribution to Food Security:

Due to its versatility in food applications, agar-agar is considered as a valuable ingredient and a sustainable alternative to animal-derived gelatin, contributing positively to global food security [49].

Additional Uses:

Beyond food and science, agar-agar finds applications in the paper and textile industries, as well as in conservation practices where it is used as a solvent gel for delicate restoration work [50].

Literature studies reveal that another solidifier such as agarose is characterized by a unique structure that allows it to form gels naturally. Beads made from agarose are highly porous, mechanically strong, chemically and physically stable, and distinctly hydrophilic [51]. These properties, which can be further enhanced by covalent cross-linking, make agarose beads especially well-suited for enzyme immobilization.

Over 20% of assessed plant species face extinction, posing significant risks to food security, particularly in developing nations. Traditional propagation method, like seeds and cuttings, cannot keep up with the rate of species loss, while tissue culture offers a rapid alternative to this conventional technique. However, tissue culture has often seen as too costly for developing countries struggling with food insecurity, with the gelling agent being the most expensive component in tissue culture medium.

Low-cost alternatives of agar:

A variety of derivation techniques can be used to modify the polymer's hydroxyl groups, enhancing its applicability [52]. However, in recent years, efforts have been made to find a suitable alternative of agar, due to concerns related to the risk of the depletion of agar plant resources, its extremely high cost, limited supply, and multi-dimensional utility, all have ignited the search for cheaper alternatives. Seaweed hydrocolloids have gained significant interest in the food industry due to their unique functional properties [53]. Agar, known for its hydrophilic gel characteristics, was the first phyco-colloid to be used in various fields, pharmaceuticals, including food, biotechnology and cosmetics due to its favourable physical and chemical properties, such as cryoprotective qualities, gelling, high biodegradability, stabilizing, and excellent water-holding capacity [54].

Since 2005, researchers have been working on the use of several cheaper substrata as a complete agar replacement for orchid multiplication *in vitro* as part of their efforts to develop cost-effective methods

[55]. Agar is obtained from marine algae and they are exploited to a larger extent. As a result, marine algas populations are also declining [56]. From one perspective, measures have been taken to save many plant species along with orchids that are at the verge of extinction, but at the same time, the source of agar (Red algae) is being depleted therefore to fulfil the purpose of Biodiversity Conservation such measures must be taken so that both group of plants remain conserved in nature. In order to save the populations of marine algae as well as ecosystem, some alternative source of agar is to be identified as soon as possible.

Till today, a variety of other materials are being tried to replace agar such as gum tragacanth, isabgol, sago palm, guar gum, gum arabic etc. Satisfactory results are still to be achieved. Literature studies demonstrate that a variety of gelling agents have been examined as a low-cost alternative of agar [57]. Various materials, such as polyurethane foam discs, chopped coconut coir, betel nut coir, and leaf litter, were processed and tested as substitutes for agar, and a comparative analysis was conducted [55]. Some other materials include Guar gum, Isabgol, Gum Arabic, Gum tragacanth and Sago-palm. Guar gum was identified as one of the most viable options for replacing agar. Due to its texture, colour and gelling nature. In term of cost, it is cheaper than that of agar. Other sources like Guar gum [58], Isabgol [59], Gum tragacanth [61], Xanthan gum [62] and Sago palm [63] have also shown good results.

Presently we have tested the efficacy of rice husk as a replacement (or a support matrix) of agar in MS medium (liquid) for multiplication of PLBs of *Cymbidium finlaysonianum.*

Low-cost Alternatives:

A Survey of literature indicates that a number of substitutes are tested such as guar gum [64], gum tragacanth [64], gum Arabic [65], Isabgol [66], isubgol and sago palm [59, 67], coconut coir [68] etc.

The husk (mesocarp) of the coconut fruit is used to make coir, which offers a sustainable substitute in liquid culture medium [68, 69]. Made up of coir fibre and pith, it is one of the toughest and most versatile natural plant fibres, readily accessible in supply. Similar to agar, coir fibres are hygroscopic, retaining about 10-12% moisture at 65% humidity and 22-25% at 95% humidity [70]. The structure and characteristics of coconuts and their by-products can vary widely based on the species, origin, and processing methods. One primary by-product derived from processing coconuts is coir, which is extracted from the outer layers, or husks [70].

Polysaccharide-based materials are among the most promising biopolymers due to their advantages, including availability, non-toxicity, biodegradability, low cost, and versatility. Guar gum, a hydrophilic polysaccharide, stands out for its exceptional thickening, stabilizing, gelling, film-forming, and emulsifying properties, positioning it as a highly promising material for various industrial applications [71]. Guar gum, a galactomannan extracted from the endosperm of *Cyamopsis tetragonoloba*, has proven effective as a standalone gelling agent for plant tissue culture media. Its effectiveness was demonstrated through various applications, including in vitro seed germination of *Linum usitatissimum* and *Brassica juncea*, axillary shoot proliferation from nodal explants of *Crataeva nurvala*, rooting of regenerated shoots from the same species, *in vitro* androgenesis in anther cultures of *Nicotiana tabacum*, and somatic embryogenesis in callus cultures of *Calliandra tweedii* [58].

Natural polymers serve a variety of purposes, and Tragacanth gum is one example. Many people are drawn to natural gums due to their appealing traits, including being environmentally friendly, derived from renewable biological sources, readily available, cost-effective, and structurally varied. A group of naturally occurring polysaccharides is referred to as gums because they typically form gels or thick solutions [72]. Gum Tragacanth is an ancient, heterogeneous exudate gum composed of a highly branched polysaccharide. This anionic polymer is widely recognized for its biodegradable, noncarcinogenic, nontoxic, and nonallergenic properties [73]. Natural tragacanth gum, derived from the sap of *Astragalus gummifer*, has been successfully utilized as a gelling agent in tissue culture media for the *in vitro* shoot development and proliferation of miniature rose and carnation [74]. The diffusion capacity and rheological characteristics of MS medium for the germination and growth of tobacco seedlings (cv. Samsun Canik) were evaluated under varying pH levels and concentrations of tragacanth gum. Findings indicated that a concentration of 11 g/L tragacanth gum at a pH of 5.6 to 5.8, which maintained low osmotic pressure, provided optimal conditions for morphological development, thus, resulting in longer shoots and roots, as well as wider leaves, compared to similar growth on agar-solidified MS medium, which exhibited higher osmotic pressure [60].

Plantago ovata Forsck., commonly known as *Psyllium* or Isabgol, is a medicinal herb that is traditionally used in folk medicine. The plant is primarily cultivated for its mucilaginous seed husks, which are known for their laxative properties. The seeds contain bioactive compounds including phenolics, flavonoids, terpenoids, and iridoid glycosides. Additionally, isabgol composition includes an excess of hemicellulose, primarily structured with a xylan backbone and linked with sugars such as

rhamnose, arabinose, and arabinoxylans [75]. Isabgol, when used alone as an alternative of agar showed slow growth of the cultures, shoots demonstrated prolific rooting in the isabgol-gelled medium, which also maintained satisfactory clarity in its gelled state [76, 59].

Study aimed to evaluate the effectiveness of combining various amounts of commercial starch and Gum Arabic as alternative gelling agents for supporting the healthy growth of banana plantlets, offering a cost-effective substitute for traditional, expensive gelling materials such as agar, agarose, and gelrite. Nine different blends, including some that incorporated conventional agar, were tested. The results showed that mixing 20–40 grams of commercial starch with 0–5 grams of Gum Arabic per litter of prepared medium produced adequate gel formation, maintained acceptable pH levels, and supported satisfactory explant development [77]. Isabgol, Gum Arabic and Sago palm are one of the cheapest and most suitable substitutes of agar, among which isabgol showed best results and the use of gum Arabic as a gelling agent resulted in significantly reduced explant regeneration, as it exhibited a loose consistency, causing the explants to partially submerge below the surface (Table 2.) [59]. Presently, efforts are being made to test the efficacy of different materials that could replace agar with a view to save plant species from where agar is being derived.

Table 2. Proliferation potential of *Vanda testacea* protocorm explants on medium solidified with agar, isubgol, gum Arabic, and sago [59]

Gelling Agents	Average number of PLBs	Average number of shoots	Complete Plantlets (week)
Agar	8.0 ± 0.40^{c}	7.25 ± 0.25^{c}	11.25 ± 0.49^{cd}
Isubgol	7.75 ± 0.50^{c}	7.00 ± 0.25^{c}	11.0 ± 0.40^{cd}
Gum Arabic	2.75 ± 0.40^{abd}	1.75 ± 0.25^{abd}	17.75 ± 0.47^{abd}
Sago	7.25 ± 0.47^{c}	8.75 ± 0.30^{c}	15.0 ± 0.40^{abc}

MATERIAL AND METHODS

Cymbidium finlaysonianum (Lindl.) protocorms (2 mm in length) were taken (36 weeks old) from *in vitro* cultures. PLBs i.e. Protocorm like bodies were used for sub-culturing from the *in vitro* cultures. Rice husk was used to replace agar in the medium. It is the thin hard outer covering which is removed from the rice grain during the milling of rice. It was in the husk form; the material was washed gently to remove any kind of dirt and other unwanted debris matter over its surface. Next the material was soaked into water @ 20g / 500 ml in individual beakers for the removal of any kind of secondary metabolite present, if any. The material remained immersed for 3-4 days. After that the water was discarded. Again, beakers were filled with fresh water and autoclaved. After autoclaving the water was again discarded. Again, fresh water was filled in flasks with the same material and kept for three days. Again, water was discarded. This process was repeated after every three days for a period of 3 months. The process was repeated till clear water was achieved in beaker with material. Murashige and Skoog (1962) liquid medium (Hi, media. Mumbai, India) + rice husk was used to initiate *in vitro* cultures. The pH of medium was adjusted to 5.7. The medium was autoclaved at a temperature of 121ºC(250ºF) and pressure 15 psi (1.03 bar) for 15-12 minutes

The inoculations were performed and the cultures were incubated at 25± 2ºC temperature and 12/12 hours of Light / Dark photoperiod. Subculturing was done as and when required. The observations were taken weekly and data was recorded consistently. The cultures were observed under microscope and the photographs were captured.

Table. 3 *In vitro* multiplication of protocorms in MS medium (liquid) / and with agar replacement

Medium and agar replacement	Regeneration response (%)	Initiation of PLBs (week)	Number of PLBs	Initiation of shoot/s (week)	Initiation of roots (week)	Complete plantlets formation (week)
MS (control)	50.00	2.4	3.0	6.3	8.5	13.5
MS + Rice husk	75.00	3	9	7.5	12	16

RESULTS

In the basal agarised MS medium only 50% explants regenerated after 2.4 weeks of culture. The explant multiplied through budding at their surface. As many as 3 PLBs could be obtained PLBs converted into shoots after 6.3 weeks. These shoots took 8.5 weeks to initiate rooting in the cultures. Plantlets were formed after 13.5 weeks. In MS medium (liquid) + Rice husk the protocorms regenerated with 75%

frequency after 3 weeks and formed plantlets after 16 weeks. The combination generously favoured protocorm multiplication through budding as many as 9 PLBs could be harvested.

Figure 2. A. Regeneration and multiplication in rice husk. B. Rooting of plant in rice husk. C. Shooting of buds in rice husk.

Protocorm-based propagation turned to be an effective method for mass multiplication, ensuring high survival rates and healthy plantlet development under controlled and sterilized culture conditions. The origin and development of secondary PLBs were efficaciously traced, offering important insights into the regeneration pathway, contributing to the enhancement of future micropropagation techniques.

COMPARISON OF COSTS AMONG DIFFERENT ALTERNATIVE FOR AGAR

Presently comparison has been made by calculating the costs of different alternatives of agar (Fig. 3.). The substitutes with lower cost are guar gum, Isabgol, gum Arabic, gum tragacanth and sago-palm. Other than these gelling agents some plant waste alternatives are also tested like coconut coir, *Pinus* needles, Wood chips, *Pinus* bark, Chia seeds, *Cycas* needles which have negligible cost as compare to above-mentioned low-cost materials. The cost of agar is approx. double as compared to the other alternatives such as Guar gum, Gum Arabic, Sago palm, Coconut coir, Chia seeds etc. Through the literature exploration it is revealed that till today there is no suitable discovered which could support the regeneration and multiplication of diverse orchid species *in-vitro*. Our future studies are directed to find out a suitable alternative to agar.

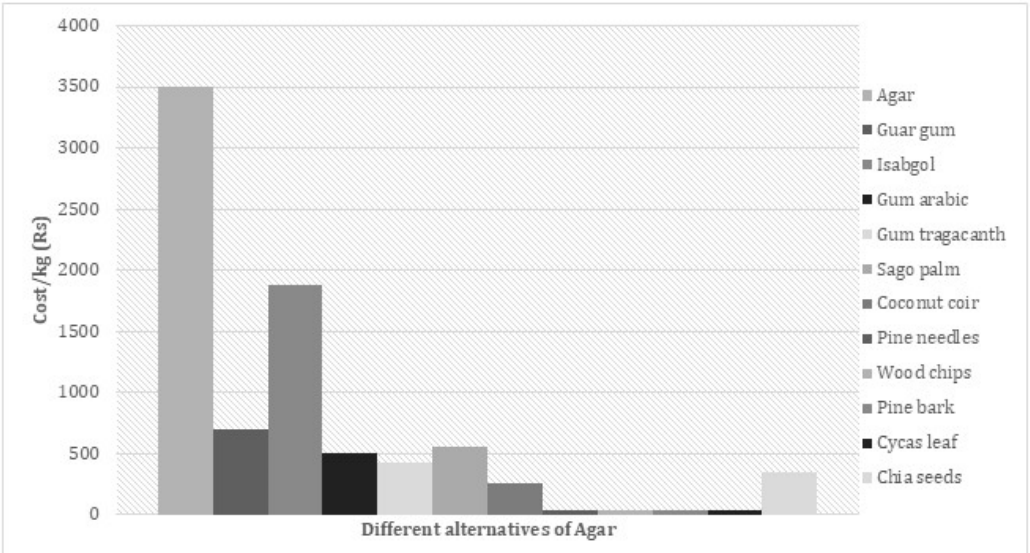

Fig. 3: Comparative cost analysis of different alternatives of agar (in rupees for 1kg). Shows the cost of different alternatives of agar is calculates in Rupee for 1kg.

CONCLUSION

Current paper presents a review on the usage of different gelling agents that have been tested so far and also reports some other materials that could be used as a substitute to agar such as rice husk. The study

investigated low-cost, ecofriendly gelling agents as alternatives to agar for in vitro multiplication of *Cymbidium finlaysonianum* (Lindl.) protocorms. Results showed that rice husk effectively promoted protocorm-like body (PLB) multiplication, shoot regeneration, and rooting almost similar to that of agar. The study concludes rice husk can be utilized in Liquid MS medium for *in vitro* regeneration and multiplication of PLBs of *C. finlaysonianum*, as a cost-effective substitutes for agar. Studies are being conducted to find a replacement of agar with a suitable alternative agent. This would help in conserving the population of orchids in their natural habitats and the sources of marine algae which are exploited ruthlessly to extract the agar.

ACKNOWLEDGMENTS

During the course of writing and compiling this review paper, the timely help provided by the lab instructors is acknowledge with deep gratitude. I offer my humble thanks and shall always be obliged to them.

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