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Conservation of Orchid Species through Leaf Culture Segment: A Review

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Abstract

The present paper reviews the possibility of using leaf segments as explants of orchid species to regenerate and multiply the germplasm in vitro.Presently, the regeneration of leaf explants was accessed in MS (1962) medium by varying salt concentration i.e. full strength and half strength versions.Although the explants regenerated in all the tested versions of MS medium, however, cent per cent explants responded in full strength version of MS medium and developed into plantlets within 12 weeks of culture. The results demonstratethat besides micropropagating Acampe praemorsa in vitro through the deviced protocol this protocol could be used to micropropagate other orchid species through leaf segment culture in vitro.

This report showcases the possibility of using leaf segments as an effective alternative, to seed and shoot apical meristems, to multiply the germplasm of rare and endangered orchid species.

Keywords: In vitro, micropropagation, leaf explant,Plb,Acampe praemorsa.

Introduction

Orchids, known for their diverse array and captivating beauty, have fascinated researchers and enthusiasts for generations, owing to their intricate growth patterns and substantial ecological importance[1]. Orchids are mostly grown for their ornamental qualities and are prized for their extended vase life in addition to their exotic beauty. They possess unique reproductive structures, specialized pollination mechanisms like pseudocopulation, mimicry, resupination[2].One of the most remarkable botanical features of orchids is their distinct flowers, exhibiting a wide spectrum of shapes, sizes, colors, fragrances,and their long vase life[1].

Beyond their aesthetic appeal and botanical significance, orchids hold substantial scientific value[3].Orchids are also known for their culinary and pharmaceutical properties, and mesmerising fragrance in addition to their economic use. It is also known that certain orchid species possess antibacterial qualities[4].Orchids contain a wide range of phytochemical substances, such as terpenoids, alkaloids, flavonoids, and derivatives of phenanthren, they are

excellent "nutraceuticals". Their bioactive compounds display immune-modulatory, hepatoprotective, anti-carcinogenic, antioxidant and neuroprotective activities. Some orchid species are utilized as food as well[5]. A popular dish in Bhutan called Olatshe is made from the blossom buds of the Cymbidium hookerianum plant[6]. Most places in the world, but particularly Bhutan, devour the inflorescence, flowers, and pseudobulbs of some Cymbidium species[5]. The most significant commercial product of orchids is the essence known as "vanillin," which is extracted from unripe Vanilla planifolia pods. Since the orchids are ruthlessly taken from the wild; as a result of this careless harvesting, many have already been classified as endangered[7].Orchids are perhaps among the most seriously threatened plant species, and belong to one of the largest families of flowering plants (CITES, 2024)[8]. The preservation of orchids in biosphere reserves, orchid sanctuaries, orchidaria, botanical gardens, and other rescue facilities is essential to successfully preventing the extinction of orchids. Plant tissue culture is a viable option for mass multiplication and conservation of rare, imperilled, and endangered orchids[8]. The invention of in vitro propagation has protected many naturally grown orchids and reduced their harvest in the wild[9].

The significance of tissue culture techniques extends beyond mass propagation; it holds promise for conservation efforts, enabling the preservation of rare and endangered orchid species which are moving towards extinction due to their habitat loss and over exploitation[10].Success in this endeavor, to conserve the orchid species not only promises advancements in orchid tissue culture techniques but, also holds implications for conservation, horticulture, and broader botanical research[11].Since most of the orchid species have become rare, endangered and threatened and Acampe praemorsa is not an exception.

Acampe praemorsa is an epiphytic wild orchid with a 16 cm long stem that is covered in sheathing leaves, a long, thick aerial root system amidst the leaves, and a persistent old inflorescence axis[12,13]. Acampe praemorsa contributes to local ecosystems as a component of forest undergrowth, providing habitats for various organisms and potentially playing a role in pollination dynamics[13].It is a therapeutically important orchid, is suffering widespread collecting and habitat loss concerns. As a result, its populations have declined, and the species is now restricted to extremely tiny areas of its native environment.Therefore,Orchid micropropagation has emerged as a successful tool to conserve ever shrinking germ plasm of orchid species such as Acampe praemorsa byselecting suitable explants such as leaf segments from in vitro grown cultures in suitable culture medium and conditions[14].

In vitro leaf segment culture allows for rapid multiplication of desired genotypes without compromising the mother plant's survival[15].The current study focuses

on the use of in vitro tissue culture techniques as a vital strategy for preserving the medicinal and floriculturally significant Acampe praemorsa orchid, one of the threatened species[16,17].

Materials and Methods

Acampe praemorsaleaves (0.5 cm long) sourced from 30 weeks old in vitro raised cultures were used as explants. Murashige and Skoog's (MS), 1962 medium (Hi media, Mumbai, India)with two different versions i.e full and half strength medium was used a carbon source. The medium was gelled with 0.9% Agar powder. The pH of medium wasadjusted to 5.7 and autoclaved at 121°C at a pressure of 1.06 kg/cm2 for 15 minutes[18]. Inoculations were done under aseptic conditions in a laminar air flow cabinet. Cultures were incubated at 25±2°C under12 h photoperiod at 3,500 lux light intensity (40W Fluorescent tubes, Philips, India). The observations were made regularly and the data was recorded accordingly. The results were recorded on the basis of time taken by the explant to initiate the cultures, pathway of regeneration, and formation of complete plantlets in full and half strength MS medium. To check the reproducibility the experiment was repeated twice.

Results

In the present investigation, 95 per cent explants responded to regeneration in MS (1962) full strength medium, demonstrating the medium's excellent effectiveness in encouraging regeneration. On the other hand, the regeneration percentage was much lower in half strength MS medium i.e. 15 per cent. The study emphasizes how important it is for Acampe praemorsain vitro tissue culture techniques to have access to nutrients. It is evident that full-strength MS medium works better to support PLb regeneration,multiplication, and plantlet development than half-strength MS medium. The tissue culture procedure devised currently could be utilized for the multiplication of germplasm of other orchid species as well.

In our cultures, the explants followed direct PLb-mediated regeneration, regardless of the strength of the nutrient medium i.e. full and half strength medium. However, full strength MS medium favoured profuse multiplication of PLbs, whereas, in half strength MS medium poor PLb multiplication was observed. This consistency indicates that while the nutrient strength does not alter the regeneration pathway, it significantly affects the efficiency and speed of regeneration. The higher success rate in full-strength MS medium can be attributed to the adequate nutrient provision essential for PLb formation and growth. The results of the experiment are summarised (Table 1; Figure1 a,b).

Discussion

For the successful regeneration of leaf explants, various factors including physiological age, source, size, responsive region and orientation of the explants, role of nutrient medium, plant growth regulators, various additives and effect of

light contributes significantly[19-24].Unlike inflorescence explants, which are only available during certain seasons, foliar explants are readily available year-round and do not necessitate the death of the mother plant[25]. Wimber (1965) pioneered leaf tissue culture and published the first well-documented report on PLB production from Cymbidium leaves[26].In monopodial orchids, leaf culture is advantageous because no mother plant is sacrificed and the explants are available all season long[21].Cells of the leaf or leaf explants differentiate into callus, somatic embryos, or shoot buds directly during the regeneration process. Many researchers have examined the process of differentiation and subsequent development of these structures[27,28,24,29,30].In vitro leaf segment culture has been practiced in a large number of orchid species of wild habitats (Table 2).
According to observations made in Vanda Kasem's Delight Tom Boykin, the meristematic cells are very active at this stage and have greater proliferative capacity over mature leaves[30]. In Saccolabium papillosum young leaves showed better regeneration than mature ones[17]. Earlier reports on in vitroAcampe praemorsa leaf segments indicate direct shoot organogenesis and in our cultures the leaf segments followed PLb mediated pathway of regeneration[41]. Literature studies reveals that the alteration in pathway of regeneration seems to be influenced by the type of nutrients present in the culture medium.Our results are in accord with similar earlier findings in Vanda testacea where leaf explants also followed PLb-mediated plantlets development[36].Explants from in vitro cultures outperformed those from naturally grown plants in terms of regeneration potential of Saccolabium papillosum, which also stands in support to our experiment i.e. the explants procured from in vitro grown (30 weeks old) cultures respond readily to regeneration[17].

In vitro propagation of orchids has forever been an alluring prospect but a test given to their nutritional desires have not been fully understood. Furthermore, the assorted vitamins and minerals, vital for their sustenance have been arduous to work out[31-33]. One of the most used plant tissue culture media is Murashige and Skoog, (1962) medium that can be found in numerous strengths[1].In that way, half-strength and full-strength MS media can have various applications and benefits depending on the plant species and its developmental stage. It is renowned for its efficacy in promoting the growth and development of plant cells, tissues, and organs invitro[1,3]. These contain macronutrients like nitrogen (ammonium nitrate and potassium nitrate), phosphorus (potassium phosphate), potassium, calcium, magnesium, and sulphur. These components are essential for cell proliferation and division[3]. Micronutrients, which are required in lesser levels, include iron, manganese, zinc, copper, boron, molybdenum, and cobalt[34]. They perform important functions in the plant's physiological and metabolic activities. Because full-strength MS medium has a high nutritional content, it is frequently used to stimulate callus development, protocorm

development and shoot bud formation from explants[34].Numerous orchid species are propagated clonally with its help, enhancing the growth of roots and shoots[9]. For the induction of somatic embryos, which can grow into whole plants, full-strength medium has been formed to be efficacious[9,35].Plant species with low nutritional needs or those that are sensitive to high salt concentrations should thrive in half-strength medium. It can be used to maintain cultures over extended periods of time without encouraging rapid development, which could lead to nutrient depletion or suboptimal circumstances[18].But in contradiction to the research done by us the best results were seen in the full strength MS medium using leaf as explant in Acampe praemorsa.

According to Kaur and Bhutani, increasing nutrition delivery in the leaf base leads to a distinct regeneration response than in other areas of the plant[36]. Comparing entire and leaf segments allowed researchers to assess Cleisostomaracimeferum Lindl's regeneration response. As previously noted in Tolumnia Louise Elmore 'Elsa', a better reaction was shown in the case of the entire leaf, and PLbs generated on the entire surface as opposed to the cut ends alone in the case of the leaf segments[19,30]. Various research noted that the size of the leaf explantshas an impact on their capacity to regenerate. Within 4-6 weeks of inoculation, quite big (>2.5 cm) leaf explants in Vanda Kasem's Delight 'Tom Boykin' became brown, produced phenolics, and eventually perished. Conversely, little (<2.5 cm) leaves responded well without releasing phenolic chemicals and produced PLBs with success[37].The regenerative potential of meristematic cells was maximal at certain leaf explant sizes, i.e., > 2.5 cm in Vanda and 1.0 cm in Phalaenopsis, and therefore, displayed optimal response. For Cymbidiumiridioides and Cymbidium aloifolium, 4-week-old in vitro-raised plants were utilized in either whole or in leaf segments, withthelengthsvaryingfrom0.5to1.5cm.

Explants with a 0.5 cm diameter had the highest level of regrowth.Choosing a particular medium during leaf culture has a big impact on the initiation of regeneration response since it contains additions, macro and micro components, and plant growth regulators (PGRs). The majority of research found that the most effective medium for leaf regeneration is (MS) medium, which was followed by Mitra medium, Knudson C (KC) medium, and Heller's medium (HM)[32,33,38,39]. Full strength MS medium allows for significant multiplication of PLbs, resulting in better regeneration efficiency and quicker plantlet growth. In contrast, poor PLb multiplication was seen in half strength MS medium, which corresponded to a lower regeneration percentage and longer time required for plantlet development.

Histological research on various orchid species have revealed that inoculating leaf explants with in an appropriate medium produces meristematic and highly cytoplasmic dense cells[42,19]. These meristematic cells are smaller than the other cells in the leaf. These cells are classified as meristemoid or embryogenic cells or somatic embryos[28,24].Meristemoids/embryogenic cells were found on

the abaxial and adaxial surfaces of Phalaenopsis amabilisvar.formosa leaves, measuring 20-30 μm in diameter[25].During regeneration, leaf/leaf explant cells differentiate into callus or somatic embryos, or directly into shoot buds. Several studies have explored the differentiation and development of these structures[27,28,24].Callus cells are spherical and divide repeatedly to form somatic embryos or PLbs during development. PLbs/somatic embryos are transferred to fresh medium with varying PGR concentrations to develop into plantlets. Several studies have found that reducing the strength of nutrient medium to half or one-fourth can induce root growth. Orchids may require organic additives to promote root differentiation and development. The time required for plantlet development varies by species[38].Plant tissue culture has been used for bulk multiplication of several species using many explants such as root, stem, leaf, stem node, inflorescence and shoot meristem[40].Biotechnological approach is required to propagate, multiply, and to carry out breeding and genetic refinement in plants.

Conclusion

It has been concluded that orchid leaves have inherent regeneration potential under in vitro conditions. Realizing the potential of donor tissues depends on several factors, including their source, juvenility, genetic composition, and nutritional requirements. Regenerative cells originate from stable epidermal/subepidermal cells, ensuring genetic fidelity[28,43].Further insights may also be obtained by doing trials with other explant kinds, such as root or stem segments. Gaining insight into the molecular mechanisms behind nutrient-mediated growth and regeneration may help improve our comprehension of the processes involved in plant tissue culture.The proposed strategy can be utilized to conserve this endangered and medicinally essential orchid species, as well as other orchid species. The regenerating ability of protocorm-like bodies (PLbs) of Acampe praemorsa was evaluated in MS medium without growth supplements because growth regulators cause somaclonal variations in the in vitro raised cultures.

The concentration of salt strength MS medium affected the initiation of regeneration response.

The regeneration response in the protocorm-like bodies (PLbs) in this experiment has shown to be dependent solely on full strength MS medium.It may be inferred from the current experiment that leaf explants can be successfully used to initiate and grow cultures in vitro. Full strength concentration of Murashige and Skoog's (1962) medium is optimum that has more potential in initiating the culture in comparison to half strength medium.

Abbreviations

PLb: Protocorm like bodies; **MS medium**: Murashige and Skoog Medium; **PGR:**Plant growth Regulator.

Author's Contribution

Ankita Gogoi: Data curation, Formal analysis, Investigation, Writing – original draft.

Dr. Saranjeet Kaur: Editing, Conceptualization, Supervision, Validation, Writing – review & editing.

Table 1:-In vitro leaf segment culture of Acampe praemorsa in different concentrations of MS medium.

Medium	Initiation of regeneration response (wks)	Percentage of regeneration	Pathway of regeneration	Development of plantlets (weeks)	Remarks
½ MS	6.00	15.00	PLb mediated	25.00	Low PLb multiplication
MS	5.00	95.00	PLb mediated	12.00	Multiplication of PLbs is high

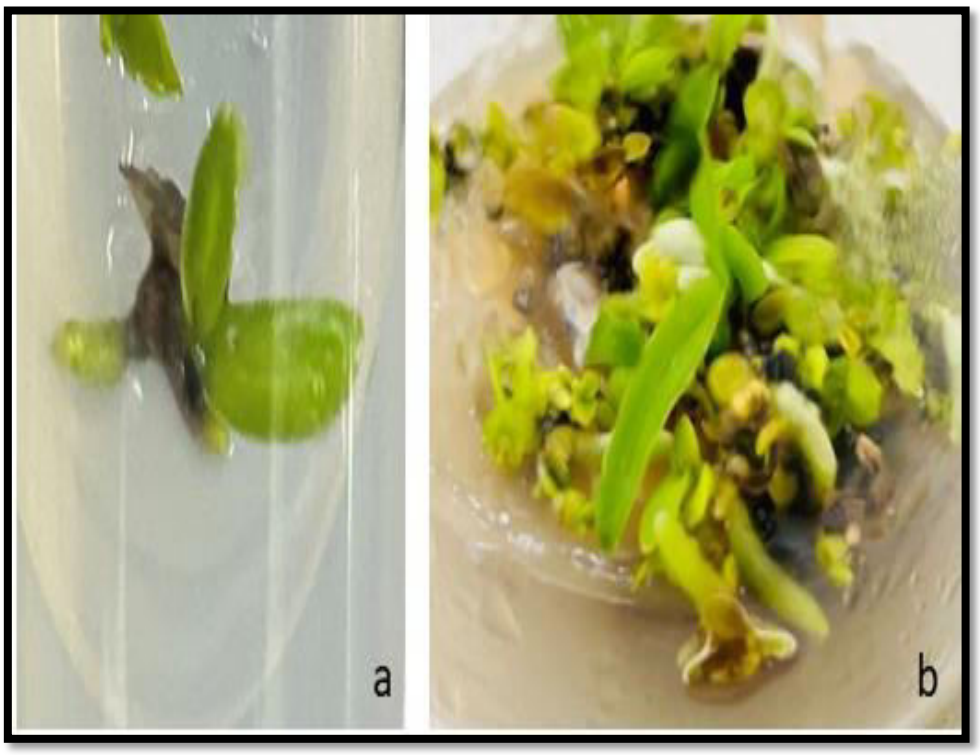

Figure1:-1.In vitroleaf segment culture in full strength MS medium a. PLb-plantlet formation in full strength ; b. Development of plantlets in full strength MS medium after 12 weeks

Table2:-Summarizing various studies on in vitro cultures of orchids using leaf explants with half and full strength of MS medium		
Name of Orchids	**Medium strength**	**Author**
Aerides crispum L	Full strength	Sheelavanthmath et al., 2005
Aerides maculosum Lindl	Full strength	Murthy and Pyati, 2001
Cattleya walkeriana	Full Strength	Prado et al., 2018
Dendrobium cheingmai	Half strength	Chung et al., 2005
Dendrobium transparens	Full-strength	Joshi et al., 2023
Epidendrum radicans	Half-strength	Devi et al., 2017
Mokara 'Chark Kuan'	Full strength	Ghani and Harris, 1992
Micropera pallidaLindl.	Half strength	Bhadra and Hossain, 2004
Oncidium(Gower Ramsey)	Half Strength	Chang et al., 1999
Paphiopedilum insigne	Full Strength and Half Strength	Zhao et al., 2021
Paphiopedilum philippinense	Full Strength and Half Strength	Chen et al., 2004
Phalaenopsis Little Steve	Half Strength	Kuo et al., 2005
Saccolabium papillosum	Full strength	Kaur and Vij, 2000
Sedirea japonica	Half-strength	Nguyen et al., 2020
Tolumnia(Snow Fairy)	Half-strength	Chookoh et al., 2019
Tolumnia louise Elmore 'Elsa'	Half-strength	Shen et al., 2018
Vanilla planifolia Andr.	Full strength	Janarthanam and Seshadri, 2008

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