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Research Article – Botany

In vitro seed germination of *Cymbidium elegans* Lindl.

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Abstract

Orchids are nature's most extravagant group of flowering plants distributed throughout the world from tropics to high alpine. They exhibit incredible range of diversity in size, shape and color of their flowers. Though orchids are grown primarily as ornamentals, many are used as herbal medicines, food, and other have cultural value by different cultures and tribes in different parts of the world. Orchids have been used in many parts of the world in traditional healing system as well as in the treatment of a number of diseases since the ancient time. Though Orchidaceae is regarded as a largest family of plant kingdom, few studies have been done regarding their medicinal properties. Linking of the indigenous knowledge of medicinal orchids to modern research activities provides a new reliable approach, for the discovery of novel drugs much more effectively than with random collection. Many of these orchids face the extreme danger of extinction due to over-exploitation and habitat loss. Plant tissue culture could be one of the most suitable alternative tools to minimize the pressure on natural population of medicinal orchids and their sustainable utilization.

Key words: Medicinal, orchids, propagation, conservation, culture

Introduction

Orchids are commercially important plant species known for their medicinal properties as well as horticultural cut flowers. There is a growing demand for orchid cut flowers in national and international markets with high commercial requirements. Orchids are well known for their highly colorful and attractive flowers whose long shelf life and varied shapes and sizes have great value in the floriculture industry as cut flower and potted plants. In nature, seed germination of both epiphytic and terrestrial orchid species is very slow and utilizes fungi as the carbon source. This fungal association provides the seed with essential growth nutrients for the effective germination and plant development of orchids (Arditti, 1968; Arditti *et al*., 1981; Kauth *et al*., 2008). Therefore *in vitro* seed germination has been utilized for the micropropagation of many orchids to meet commercial requirements. *In vitro* propagation of orchids is a powerful tool for conservation and management of orchid resources (Morel, 1964; Rao, 1977; Sharma *et al*., 1991; Lakshmanan *et al*., 1995; Ichihashi, 1997, 1998; Kanjilal *et al*., 1999; Malabadi *et al*., 2004, 2005; Teixeira da Silva *et al*., 2006; Das *et al*., 2007; Malabadi and Nataraja, 2007a, 2007b; Malabadi *et al*., 2008a, 2008b, 2008c; Malabadi *et al*., 2009a, 2009b).

Plant tissue culture techniques have become a unique tool in plant biotechnology. As a result, orchid seed germination through plant tissue culture would be a viable alternative for the production of large number of seedlings. Commercial orchid tissue culture holds valueonly if large numbers of plantlets are produced in a short time frame and with minimum input expenses. Hence, the time taken for plant regeneration becomes a crucial factor as far as the economy of production for orchid commerce is concerned. The use of expensive plant growth regulators can be cut down by replacing them with low cost natural additives such as smoke saturated water (SSW) which is a natural source of plant growth hormone. In this investigation, the effect of SSW on the seeds and shoots and formation of *Xenikophyton smeeanum* (Reichb..) plants in a simple and cost-effective manner. *X. smeeanum* is a native epiphytic orchid from the Western Ghat Forests of Karnataka State, India (Krishnaswamy *et al.*, 2004). Although this species is facing rapid destruction, no one has paid attention to its conservation to date. Since vegetative propagation methods are not available, development of *in vitro* methods are essential for conservation and commercialization of this species. Keeping these limitations in mind, the present preliminary studies were conducted to develop an efficient protocol for the rapid propagation of *X. smeeanum* (Reichb. f.) *via in vitro* seed germination. Our present study constitutes the first report of a successful and efficient *in vitro* propagation protocol for large scale production of *X. smeeanum* (Reichb. f.).

Materials and methods

Plant material

Mature capsules of *H. edgeworthii* were collected from Suwakholi, Tehri Garhwal, Uttarakhand, India (latitude 30°240, longitude 78°170; altitude 2,800 MSL) during October 2007. The botanical identity was authenticated by the Botanical Survey of India, Dehradun (Uttarakhand) and the voucher specimen deposited at the GBPIHED herbarium, Kosi-Katarmal, Almora (voucher specimen no. GBP 3101). Capsules were stored at room temperature, 25 ± 2 °C, over silica gel desiccant for 1 month.

Results and Discussion

Propagation of orchids by the development of secondary

PLBs from protocorms or PLBs has been defined for several orchids including *Phalaenopsis* (Chen and Chang, 2004; Murdad *et al*., 2006), *Dendrobium* (Saiprasad *et al*., 2004), *Aerides* (Sheelavanthmath *et al*., 2005) and *Cymbidium* (Teixeira da Silva and Tanaka, 2006). Numerous comparative analyses of plant responses to various types of plant growth regulators and the addition of organic additives to the culture medium to promote *in vitro* growth and proliferation of orchid

Fig. 1. Explant of *Cymbidium elegans*

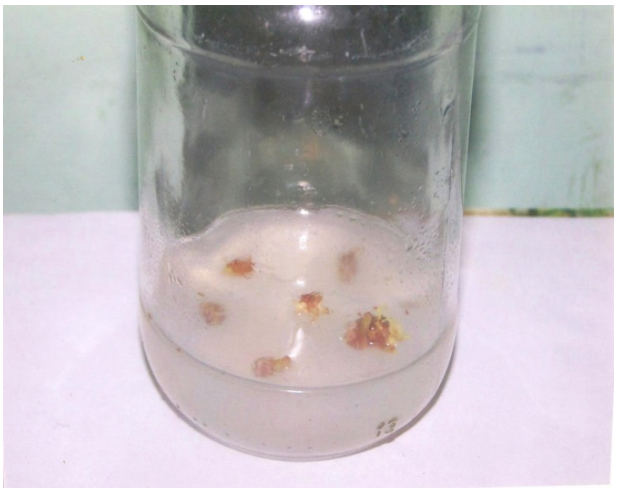

Fig. 2. Callus formation of *Cymbidium elegans*

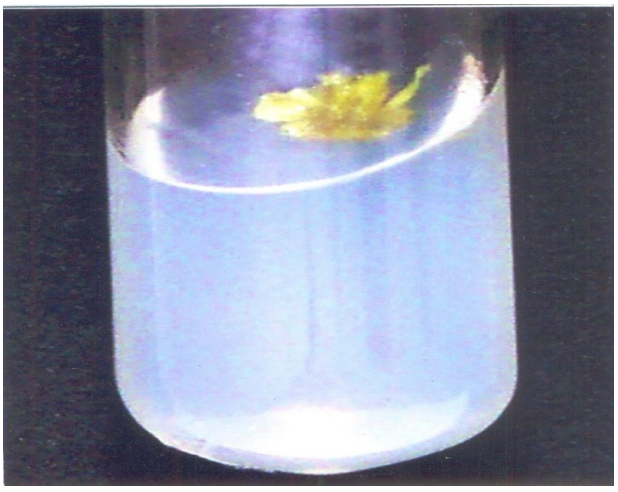

Fig. 3. Shoot and root formation of *Cymbidium elegans*

Fig. 4. Mother plant of *Cymbidium elegans*

is a common practice (Ichihashi and Islam, 1999; Chai *et al*., 2002; Islam *et al*., 2003; Rahman *et al*., 2004; Arditti, 2008, George *et al*., 2008). The use of organic additives and PGRs may add toward the development of a simple and economical plant culture (Islam *et al*., 2003). Numerous comparative analyses of plant responses to diverse types of cytokinin (BA and kinetin) have revealed that the presence of BA in the culture medium produces higher frequencies of induction and proliferation of PLB in some orchids, such as *Dendrobium nobile*, *Dendrobium Densiflorum*, and *Cymbidium aloifolium* (Nayak *et al*., 2002)

Sheelavanthmath *et al*., 2005; Luo *et al*., 2008). However, Teixeira da Silva and Tanaka (2006) specified that kinetin was more effective in promoting the formation of PLB in *Cymbidium* hybrids when supplemented together with NAA to the culture medium. In contrast, Chen *et al*. (2002) described that both BA and kinetin were equally effective for PLB proliferation in *Epidendrum radicans*. Instead, Chai *et al*. (2002) described that the addition of a suitable amount of organic additives to the culture media significantly promoted the growth of PLBs in *Phalaenopsis*. This review displayed that varying conditions are desirable for diverse types of orchids to form their optimum The histological observation showed that the protocorm-like body of *Mokara* Broga Giant orchid comprises of the shoot apical meristem (SAM) and leaf primodia of sheath leaves at the interior region and larger cells act as the storage area at the posterior region. Histological observation presented the presence of an area having dense cytoplasm located on the anterior side of the PLB, showing meristematic cells, embedded below it with parenchymatous tissue with thinwalled cells. This parenchymatic mass of cells protruded from the surface on the upper part of the protocorm and associated with vascular tissue (Figure 3).

Furthermore, dividing cells of the SAM were smaller in size and were more densely organized. Further development of these cells gave SAM a shape of a dome. The shoot primordial (SP) was also noted and is different. They comprised of small cells, undergoing anticlinal and periclinal divisions, still surrounded by large, apparently isodiametric parenchyma cells. SEM study observation using freeze drying method displayed the presence of enlarged globular PLBs shape which consists of epidermal cells with rough surface texture, which gradually tend to become rougher and wrinkled at the further developmental stages (4 weeks old).

The embryo axis also displayed the development of LP after formation of a constriction (C) at the top of the globular head. There are more than one constriction (C) can be detected on a single PLB head. The leaf primordial (LP) was also noted. Even though wide research has been carried out on *in vitro* PLB in cultivated orchids, there are limited complete structural details known on the histological observation of PLBs (Park *et al*., 2002a). In the histological analysis, we presented that the presence of parenchyma cells next to the meristematic cells. The histological and SEM studies carried out in the present work displays that the meristematic core was enveloped by a mass of parenchymatic cells. The existence of meristematic tissue at the leaf apex discloses a characteristic that is inherent to the Orchidaceae family (Churchill *et al*., 1973). This study offered the morphological development observed with naked eye is substantiated with the details based on histological as well as SEM observations. Haensch (2004) described that *Pelargonium hortorum*observed that although globular and heart-shaped embryo-like regenerants were formed, histological analysis revealed that they lacked a defined root pole. In contrast to somatic embryogenesis where the globular-stage usually comprises of small cells with dense cytoplasm and large nuclei (Haensch, 2004), globular embryo-like structures had vacuolizedparenchymatous cells (Salaj *et al*., 2005). Similar structures observed in other plant species have also been stated to as meristemoids, promeristems, meristemoid-like precursors (Hicks, 1994), protocorm-like bodies (Young *et al*., 2000; Tian *et al*., 2008), nodules (Batista *et al*., 2000; Xie and Hong, 2001; Ferreira *et al*., 2009) and nodular meristemoids (McCown *et al*., 1988). McCown *et al*. (1988) describe nodules as independent, spherical, dense cell clusters which are cohesively bound together and display

consistent internal cell or tissue differentiation and loosely resemble protocorms. In contrast, a meristemoid can be described as a cluster of cells acting together as a meristematic centre (McCown *et al*., 1988). Hence, a histological approach is vital in distinguishing true somatic embryos and nodular meristemoids. Many published reports on somatic embryogenesis have drawn conclusions on the basis of morphological appearance only and should be reviewed critically (Bassuner *et al*., 2007). Histological approaches can offer critical information to allow the application of the most suitable *in vitro* plant regeneration methods (Woo and Wetzstein, 2008). Histological and SEM observations through the presence of meristematic cells clearly presented that PLBs can develop from explants wound surface and can be converted into plantlets through sequential organogenesis or somatic embryogenesis. Since the PLBs comprised of multiple meristematic centres, it can be differentiated into shoot, leaf and then into plantlets gradually. Tian *et al*. (2008) reported that differentiation of globular cells led to formation of meristematiccenters, which developed into PLBs. The SEM observations also definite that PLBs clearly exhibit shoot primordial and a meristem done. The formation of PLBs is a unique characteristic of Orchidaceae and the term specifies a structure from induction of globular swellings until SP occur, without a root apparatus (Batygina *et al*., 2003).

Conclusion

Therefore, the presence of abundant meristematic cells within the PLBs displayed importance in orchid propagation through orchid tissue culture which makes it to be a prospective explant for cryopreservation and genetic transformation studies.

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