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Research review paper

Frozen beauty: The cryobiotechnology of orchid diversity

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ABSTRACT

Orchids (Orchidaceae) are one of the most diverse plant groups on the planet with over 25,000 species. For over a century, scientists and horticulturalists have been fascinated by their complex floral morphology, pollinator specificity and multiple ethnobotanical uses, including as food, flavourings, medicines, ornaments, and perfumes. These important traits have stimulated world-wide collection of orchid species, often for the commercial production of hybrids and leading to frequent overexploitation. Increasing human activities and global environmental changes are also accelerating the threat of orchid extinction in their natural habitats. In order to improve gene conservation strategies for these unique species, innovative developments of cryopreservation methodologies are urgently needed based on an appreciation of low temperature (cryo) stress tolerance, the stimulation of recovery growth of plant tissues *in vitro* and on the 'omics' characterization of the targeted cell system (biotechnology). The successful development and application of such cryobiotechnology now extends to nearly 100 species and commercial hybrids of orchids, underpinning future breeding and species conservation programmes. In this contribution, we provide an overview of the progress in cryobanking of a range of orchid tissues, including seeds, pollen, protocorms, protocorm-like bodies, apices excised from *in vitro* plants, cell suspensions, rhizomes and orchid fungal symbionts. We also highlight future research needs.

Contents

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1. Introduction

1.1. Orchids and their importance in human life

With an estimated 880 genera and over 25,000 species, the Orchidaceae is the largest family of flowering plants (Givnish et al., 2015). Orchids have been known, appreciated, used, and frequently overused for centuries in different parts of the world. The significance of orchids in human life cannot be overestimated (Fig. 1). Many orchids possess high medicinal, ornamental and cultural value in their countries of origin.

Cultural significance extends to symbolism in myths the orchids have inspired works of art, literature and poetry. In ancient Japan, China and Korea the oriental *Cymbidiums* have featured in ink drawings which, when combined with elegant poetry writing, were meant to adorn the life of noble families (Paek and Murthy, 2002). The tradition of orchid ink painting persists even today (Fig. 1).

Regarding their use in medicine, all parts of orchids have been utilized, including leaves, roots, flowers, pseudobulbs, tubers, rhizomes and whole plants (Jalal et al., 2010; Singh and Duggal, 2009; Gutiérrez, 2010; Pant, 2013). The medicinal value of orchids first received recognition in ancient China (Hew et al., 1997; Pant, 2013). In traditional Chinese medicine (TCM), mainly native *Dendrobium* and *Cymbidium* orchid species serve as ingredients of therapeutic preparations for treating various ailments, including diabetes, lung cancer, stomach diseases, allergies and fatigue (Hu, 1971; Ng et al., 2012; Paek and Murthy, 2002; Liu et al., 2014). Plants of *Dendrobium* spp., *Gastrodia elata* Blume,[2] and *Bletilla striata* Rchb.f. continue to be an important part of the Chinese herbal industry (Bulpitt et al., 2007). Similarly, India has a long tradition of using orchids in the "Ayurveda" system of medicine, including four species of the genera *Malaxis* and *Habenaria* (Singh and Duggal, 2009; Jalal et al., 2010). Plants of *Cypripedium*, *Vanilla*, *Arpophyllum*, *Bletilla* and *Epidendrum* genera have also been collected for medicinal use by different ethnic groups in North America and Mexico, while Europeans were mostly aware of the medicinal value of terrestrial orchids, such as *Orchis* spp., *Dactylorhiza* spp. and *Epipactis* spp. (Bulpitt, 2005; Pant, 2013). Other medicinal properties relate to tonic, antibacterial, aphrodisiac, anti-tumour, anti-pyretic and wound-healing properties (Bulpitt et al., 2007; Singh and Duggal, 2009; Gutiérrez, 2010) as well as contributing to curing and helping reveal the symptoms of tuberculosis, indigestion, headache, fever, fractured bones, stomach diseases and, even, snake bites (Bulpitt, 2005; Pant, 2013). The medicinal value of some traditionally-used orchid species has been recently proved by clinical trials (*e.g.* Liu and Mori, 1992; Zheng et al., 1998; Shih et al., 2002; Kim et al., 2003; Morita et al., 2005; Li et al., 2011). The presence of medicinally active chemicals such as polysaccharides and secondary metabolites including alkaloids, glycosides, phenolic compounds, and many others have been also documented in orchid tissues (reviewed by Gutiérrez, 2010; Ng et al., 2012).

Beyond medicine, orchid products are widely used the food and beverage industries. Specifically, vanillin extracted from the seed pods of *Vanilla planifolia* Andrews (now mostly produced chemically) and salep ("Sahlep" in Arabic) made from dried tubers of *Orchis morio* [now *Anacamptis morio* (L.) R.M. Bateman, Pridgeon & M.W. Chase; (Fig. 1; Bulpitt, 2005)].

However, in the modern world, orchids are known and grown primarily as ornamentals, mostly for their exotic, long lasting and often fragrant flowers (Fig. 1). Orchids represent 8% of the global floriculture trade (Martin and Madassery, 2006). In China, Taiwan, Korea and Japan, oriental *Cymbidiums* are popular horticultural plants with high commercial value. For example, a single plant of *Cymbidium goeringii* (Rchb.f.) Rchb.f. can sell for US$ 10,000 (Paek and Murthy, 2002). The wholesale value of potted orchids in the United States in 2011 reached US$ 200 million, making them the second most popular potted flowering plant in the country (Teixeira da Silva et al., 2014). Orchid propagation by both large and small industries has depended on traditional as well as modern orchid breeding programmes utilizing available genetic resources (Paek and Murthy, 2002; Liu et al., 2014), and interest in sourcing wild species for unique gene combinations remains high. Hundreds of new varieties are registered annually. However, the systematic preservation of both old and these new plant genetic resources has not been seriously addressed.

1.2. Orchid biodiversity and conservation strategies

Orchids are an important part of plant biodiversity on the planet due to their high variability among species and their habitats. The highest diversity of orchid species has been found in the Andes of Colombia and Ecuador, tropical rainforests of Borneo, Sumatra, New Guinea and Madagascar (Cribb et al., 2003; Swarts and Dixon, 2009). Areas of particular species abundance are India, SW China, temperate SW Australia, South Africa and Bhutan (Cribb and Govaerts, 2005). Every year botanists discover over one hundred new orchid species (*e.g.* Carnevali et al., 2014; Vale et al., 2014; Kolanowska, 2015); for example in 2013, nearly 370 new species were described (Schuiteman, 2015). Clearly, our knowledge of orchid genetic diversity is fairly incomplete, and there is the prospect that many orchid species may be lost before their discovery.

Compared to other vascular plants, orchids are considered to be the most highly evolved of vascular plants. Having once been abundant worldwide, some species have now become extremely rare (Koopowitz and Kaye, 1983; Koopowitz, 1986; Dasgupta et al., 2004). Their high specificity for insect pollinators, minute seeds (often weighing μg) without endosperm, and a unique life cycle requiring an association with specific mycorrhizal fungi during the early stages of development has left orchids vulnerable to minor biotic and abiotic changes (Arditti and Ernst, 1984; Vinogradova and Andronova, 2002; Kandavel et al., 2004). The widespread degradation of ecosystems, for example as a result of an increased use of weed killers and artificial fertilizers, deforestation, and land clearance, has imperilled orchids in their natural habitats (Farrell and Fitzgerald, 1989; Wood, 1989; Kandavel et al., 2004; Swarts and Dixon, 2009). Moreover, global warming is predicted to produce irreversible changes in orchid communities (Seaton et al., 2010). Such effects are likely to be the most serious in mountain and tropical regions, including many orchid biodiversity hotspots in Asia and Latin America (Seaton and Pritchard, 2011).

All orchids are listed under Appendix II or I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (n.d.), http://www.cites.org). The approach to conserve these

[2] Wherever possible, authorities for species names are given at the time of first mention as recorded in The Plant List, (http://www.theplantlist.org) or the International Plant Names Index (http://www.ipni.org/)