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# REBUILDING OF ORCHID GERMPLASM CONSERVATION VIA MOLECULAR MULTI-OMICS APPROACHES

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**Abstract:** The Orchidaceae family, the world's second-largest flowering plant, dominates the International Union for Conservation of Nature (IUCN) red list. Threats to orchid survival include illegal harvesting, habitat destruction, temperature change, and unique association requirements in pollination systems and orchid mycorrhiza interaction. Some research breakthroughs in ex-situ and in-situ germplasm integration techniques for conservation application, including automated precision approaches in orchid research. Ex-situ conservation approaches, including gene bank, seed bank, cryopreservation, field gene bank, and in-vitro culture, can play indispensable roles in the success of orchid conservation and preservation, especially for endangered orchids. Recently, multi-omics technology emerged as an important tool to bridge the knowledge gaps in orchid conservation so more effective measurement could take place. These provide a good conservation framework that confluences with technology and knowledge.

Keywords: In-situ conservation, ex-situ conservation, environment exposome, climate change, diversity.

## Introduction

Orchidaceae are the second-largest angiosperm family, expanding throughout every continent except Antarctica (Fay, 2018). Every year, a large number of new species names are released. More than 30,000 orchid species spanning around 763 genera have been scientifically recognised (Kindlmann *et al.*, 2023). Orchids are commercially traded for a variety of purposes, including horticulture, medical resources, and unusual delicacies, in addition to being ornamental plants. Orchid has a long history as a source of herbal remedies in traditional medicine since the 17th century in China (Bulpitt *et al.*, 2007). Extract from the stem, leave and root organs of orchids contained many phytochemical compounds, such as flavonoids, alkaloids, bibenzyl derivatives, anthocyanins, sterols, and phenanthrenes (Ahmad *et al.*, 2022). The medicine and pharmaceutical effects have been proven effective in treating many disorders and ailments, such as inflammation, tumours, acidity, arthritis, chest pain, tuberculosis, asthma, malaria, hepatitis, and bone fractures (Mudoi *et al.,* 2023). Orchids, therefore, have strong market value and high global market demand. Orchids have been considered edible, medicinal, ritual, and aesthetic plants for millennia. Therefore, it is the most traded plant globally (Hinsley *et al.*, 2018; Ticktin *et al.*, 2020). Horticultural and floricultural trade for ornamental orchids locally and internationally involves 347 orchid species in 93 genera, of which many are listed as endangered (Phelps & Webb, 2015), significantly pushing some orchids, such as Renanthera, Cattleya, Laelia and slipper orchids like *Cypripedium* sp., *Paphiopedilum* sp., and *Phragmipedium* sp., to the brink of extinction (Fay, 2018). High commercial demand and high market values have become key drivers that lead to unsustainable harvest and illegal trade of orchids (Schuiteman *et al.*, 2008). The Convention on International Trade in Endangered Species (CITES) regulates the international trade of plants, with orchids representing 75% of all listed species.

Thailand is known as the global leader in orchid trade, having consistently ranked among the best sellers of potted plants in international commercial trade, followed by Taiwan, Myanmar, and Lao People's Democratic Republic. (Phelps & Webb, 2015; Hinsley *et al.*, 2017). Statistical data showed that 99.9% of approximately 1.1 billion orchid plants and more than 31 million kg of stems were artificially propagated for commercial horticultural trades between 1996 and 2015 (Phelps & Webb, 2015). Mexico bears 1,300 orchid species that are permitted for domestic trading (Ticktin *et al.*, 2020).

Trade in edible orchids drives a large-scale, unregulated harvest. *Salep*, the dried tubers of orchids, is a common ingredient used to produce ice cream and creamy drinks in Turkey (Veldman *et al.*, 2014). It has been reported that 40-50 million orchid plants involving 85% of 150 orchid species in Turkey were destructively harvested to produce approximately 30 tonnes of *Salep* yearly (Veldman *et al.*, 2014). Therefore, the trade of orchids for *Salep* has affected 19 species in seven genera in countries including Turkey, Iran, and Greece (Ghorbani *et al.*, 2017). *Chikanda*, also known as *Chinaka*, a traditional Zambian dish made from wild-harvested orchid tubers and peanuts, has exhausted Zambian orchid resources. To meet the high market demand for *Chikanda*, Zambia annually drives indiscriminate harvest and transport of 2-4 million orchid tubers of genera *Disa, Satyrium,* and *Habenaria* from Tanzania (Kasulo *et al.*, 2009). Reports found that the trade in *Chikanda* has put 85 Tanzanian orchid species at risk due to overharvesting (Veldman *et al.*, 2014). They are globally important edible orchids, *Vanilla* spp. Seed pods are harvested unripen for the food trade as a flavouring. Surveys reported that Madagascar produces the most Vanilla, with 3719 tonnes Vanilla produced, followed by Indonesia 2,000 tonnes in 2014 (Hinsley *et al.*, 2017).

Orchids have been medicinally used to treat many ailments and diseases for millennia. In China, 25% (350 species) of 1,388 local orchid species are used in traditional chinese medicine (Liu *et al.*, 2014). Among 78 *Dendrobium* sp. locally found, roughly 50% of them are harvested for various health purposes due to their high medicinal value like antimicrobial, anti-inflammatory, antipyretic, hepatoprotective, eyesight protective, and immunoregulatory properties (Gutierrez, 2010; Panda & Mandal, 2013; Liu *et al.,* 2014). The high demand for *Dendrobium* sp. for traditional chinese medicine affects the wild orchid population of neighbouring countries like Vietnam and Laos when they import wild orchids from these countries in recent years (Liu *et al.*, 2014). It has been reported that Laos exports an average of 100,000 kg of dried Dendrobium stem from the wild every year (Schuiteman *et al.*, 2008). Ayurvedic medicine in India also involves the collection of many wild orchids including *Habenaria edgeworthii* Hook. fex Collet, *H. intermedia*, *Malaxis acuminata D. Don, M. muscifera, Encyclia citrina, Laelia autumnal, Stanhopea hernandezii, Arpophyllum spicatum, Bletia catenulate* and *Epidendrum pastoris*, *Dendrobium* sp., *Gastrodia elata,* and *Anoetochilus* sp. (Hossain, 2011; San *et al.*, 2015). Illegal export of medicinal orchids from Nepal has affected approximately 60 orchid species from genera *Gastrodia, Vandam Coslogyne, Dendrobium,* and *Crepidium* for traditional treatments and infectious disorders (OSG *Ex-situ* Conservation Group, 2020). Our review findings show that although governments ban or restrict the harvest of wild orchids (Ticktin *et al.*, 2020), enforcement and governance without scientific backup are mainly ineffective, especially in countries with few financial resources.

Since the establishment of Orchidaceae in 1737, research studies focused on orchids have begun and the history of orchid conservation has been summarised in Figure 1. In the early 1990s, the common orchid cultivation practice mainly depended on vegetative propagation techniques through stem cutting and pot planting methods (Fay, 1994). In-vitro micropropagation was first introduced in the early 1990s, and it only involved basic sterilisation techniques in the simple growth media available at that period. Over the decades, conservation techniques developed rapidly with the advancements in multi-omics approaches, which enable the examination of orchid changes at a cellular level (Tikendra *et al.*, 2021; Wagner *et al.*, 2021; Wang *et al.*, 2021a). Due to high market demand, orchids have long been one of the world's most fragile plants, meaning they are at dire risk of extinction (Gale *et al.*, 2018; IUCN, 2021). IUCN Red List has divided orchid species into 7 main categories, of which 5 among the species are assessed as "Extinct", 270 as "Critically Endangered", 401 as "Endangered", 215 as "Vulnerable to Extinction", 95 as "Near Threatened", and 615 as "Least Concern" (IUCN, 2021). Another 232 species are currently "Data Deficient", which means appropriate information on their abundance and distribution is lacking for assessing their extinction risk (IUCN, 2021). Although they are just a small fraction of the estimated 30,000 orchid species worldwide, Red List provided a quick review of the conservation status of the threatened species suffering from anthropogenic activities (Huda & Jahan, 2019) including adverse climate change, illegal and unsustainable harvest, and habitat destruction, as well as facing challenges from the restriction of highly specific association with mycorrhizal fungi and pollinators.

## Key Factors Influence Orchid Conservation

### *Climate Change*

Global climate change has had significant effects on orchid biodiversity. Since the 1880s, the Earth's average temperature has risen more than 2 degrees Fahrenheit, with 2020 being the warmest year recorded by Nasa (Shaftel *et al.*, 2021). Since the Industrial Revolution, increasing greenhouse gases have been 50 times more intense than the Sun's radiance, thus resulting in a strong warming effect on Earth's climate (Government of Canada, 2019). Global climate change interacts with habitat loss and fragmentation, introduction and growth of invasive species and severe ecosystem modification are major threats to wild orchids (De & Medhi, 2019; Kolanowska, 2023). Based on the statistical prediction, climate change will probably drive 15-37% of all taxa to extinction by 2050 (Bellard *et al.*, 2012). This could greatly impact orchids because they are symbiosis with other organisms such as pollinators, mycorrhizal fungi, and host trees, which are also affected by the intensified climate change (Fay, 2018). Population demographic models have proven the effect of weather conditions, population dynamics and distribution of orchids (Evans *et al.*, 2020). Increasing evidence has been reported that global climate change could influence orchid distribution by causing migration and

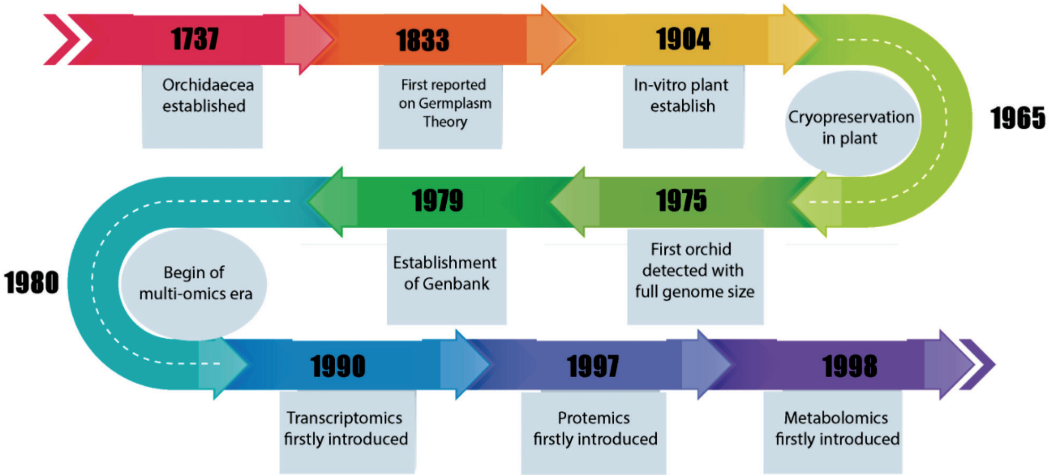

Figure 1: The history development in orchid conservation
Source: Authors

translocation of species to climate-suitable localities (Fay, 2018). As temperature rises, vegetational zones may gradually shift to cooler and higher altitudes and latitudes, resulting in both migrations of tropical and subtropical species to subtropical and temperate areas, respectively, thus eventually eliminating the species in the highest zone (De & Medhi, 2019).

Climate change is a major threat to pollination services and orchid mycorrhizal fungi (OMF) diversity, triggering phenology changes in plant-pollinator relations and flowering mechanisms (Kolanowska, 2023). Raising the temperature could affect the flowering period of the orchid and the flight period of the pollinators. The bee flight dates are advancing faster than orchid flowering, provoking the uncoupling of interactions (Willmer, 2014). Researchers found that climate change has threatened a rare spider orchid (*Ophrys sphegodes*) that tricks bees (*Andrena nigroaenea*) into mating. Warmer springs have caused spider orchids to flower earlier and simultaneously release female bees from hibernation in advance, affecting pollination (Willmer, 2014; Patrick, 2018). According to NASA (2021), more and more regions are becoming prone to drought today as global warming climbs and rising temperatures dry up the land. A previous study reported that lower humidity increases the fungal mortality rate, therefore lowering the orchid mycorrhizal fungi abundance and diversity, which is needed for orchid germination and development (Querejeta *et al.*, 2007; Izuddin *et al.*, 2019).

With the extent of climate change, global warming causes forests in lower rainfall regions to be more susceptible to fire, resulting in habitat destruction and probable extinction of local orchid species (De & Medhi, 2019). Environmental change such as disturbance of microclimate results in high turnover in species composition of orchids in communities (Solano & Hernández Pérez, 2014). The spontaneous regeneration of original vegetation in the destruction area is challenging as wild orchids have a very long life cycle and require complex conditions for propagation and cultivation (Cardoso *et al.*, 2016).

The diversity in life history, growth conditions, and morphology in orchids exert various physiological properties (Zhang *et al.*, 2018). Physiological variation under different environmental stress and growth stages can provide clues on the orchid survival and evolutional adaptation (Feng *et al.*, 2022). For instance, physiological characteristics include water storage in leaves, slow water loss during transpiration, and rapid water uptake through velamen radicum in drought conditions (Zhang *et al.*, 2018). It is, hence, critically important to examine each developmental stage of orchids that corresponds to climate change.

### *Habitat Changes*

Habitat changes greatly impact the orchid conservation efforts, including the loss of native habitat, fragmentation of habitat, habitat modification due to altered microclimate, introduction of invasive species, pollinator disruption, and land use practices (Meekers & Honnay, 2011; Newman *et al.*, 2013; Nemésio *et al.*, 2016).

Due to the narrow ranges of secondary successional habitats, orchids are extremely sensitive to environmental changes (Vogt-Schilb *et al.*, 2016). Most of the land areas in the world today have been widely utilised for human use, leading to the depletion of natural habitat and further limiting the natural habitat for orchids (Ballantyne & Pickering, 2012; Aguilar *et al.*, 2019). Many orchid species, especially the myco-heterotrophic orchids, require dense forest cover in such a way that small canopy exposure causes the depletion of genetic diversity, lowering the reproduction success rate, disturbing the mutual interaction in sexual reproduction, and reducing the seed dispersal opportunities (Balestrini *et al.*, 2014; Lander *et al.*, 2019).

A long-term field investigation assessment reported that 32 out of 187 orchid species from 22 genera are recognised as extinct from the flora of Bangladesh, and habitat fragmentation is blamed for the extinction's root cause (Huda & Jahan, 2019). Moreover, habitat fragmentation also affects the long-distance orchid bee's pollinator (*Apidae Euglossini*), leading to the bee species' extinction and severely impacting the dispersal of orchid seeds (Botsch *et al.*, 2017).

In short, understanding the intricate relationships between orchids and their habitats is crucial for effective conservation. Orchid conservation efforts must address the broader issues of habitat preservation and restoration, considering the ecological intricacies that influence orchid populations.

### *Specific Association with Pollinators and Mycorrhizal Fungi*

Orchids are well known for their canalised pollination systems. Only precise pollination mechanisms with specific pollinator species can make the pollination successful for each orchid species. Approximately 60-70% of orchids are estimated to hire only one pollinator species (Cheng *et al.*, 2009; Štípková *et al.*, 2020). This specialised pollination system makes orchids vulnerable to fluctuation in pollinator abundance, thus strongly affecting the survival and distribution of orchids (Štípková *et al.*, 2020).

In general, orchids are categorised into nectariferous and nectarless species through the specificity of pollination mechanisms, which may involve food-foraging, territorial defence, pseudo-antagonism, rendezvous attraction, brood-site and shelter imitation, sexual response, or habitat-selection behaviours of their pollinators (Štípková *et al.*, 2020). Due to low pollinator abundance and frequency of visits, pollinator constraint is the primary limiting factor on reproduction success (Chen *et al.*, 2020). *Serapias vomeraceao*, a nectarless orchid, was found to be poorer in open-pollination conditions than in hand-pollination conditions, demonstrating the need for available pollinators (Pellegrino *et al.*, 2005).

Apart from depending only on optimum temperature and pollinators, orchids rely on their specific mycorrhizal fungi at different stages of the life cycles. Mycorrhizal interaction is especially important in the early stage of seed germination. Due to the lack of endosperm in orchid seeds, mycorrhizal fungus supplies food, triggering seed germination and promoting protocorm growth (Herrera *et al.*, 2019). The embryos often rely on fungal symbionts for carbohydrates as the carbon source to develop into protocorm (Alghamdi, 2019). As the seedling grows into an autotrophic adult, the myco-heterotrophy system may continue throughout the orchid's lifetime nutrients. This integration provides 80% of the mineral supply to the orchid (Shailes, 2014; Yeh *et al.*, 2019). Therefore, ecology degradation causes changes in the mycorrhizal population, thus further stripping orchid species to extinction (Teixeira da Silva *et al.*, 2017; Waud *et al.*, 2017; McCormick *et al.*, 2018).

## Germplasm Conservation in Orchids

Germplasm conservation strategies can broadly be divided into three ways: In-situ, ex-situ conservation, and trans-situ. In-situ conservation is an effort to protect the endangered species in their natural habitat, while ex-situ conservation emphasises the protection of the threatened species via the relocation of species from their natural habitat to a protected area (Ajayi, 2019), while trans-situ conservation integrates both in-situ and ex-situ conservation techniques to maximise the effectiveness of conservation and at the same time reduce time consumption (Riordan & Nabhan, 2019). An overview of in-situ and ex-situ conservation has been compared in Figure 2.

### *In-situ Conservation*

In-situ conservation deals with the preservation of genetic resources in natural ecosystems, where its main advantage is that it contributes to the preservation of the entire ecosystem rather than just a few species. In-situ conservation underpins efforts to maintain genetic resources and genetic diversity through the adaptative process and natural selection that give rise to

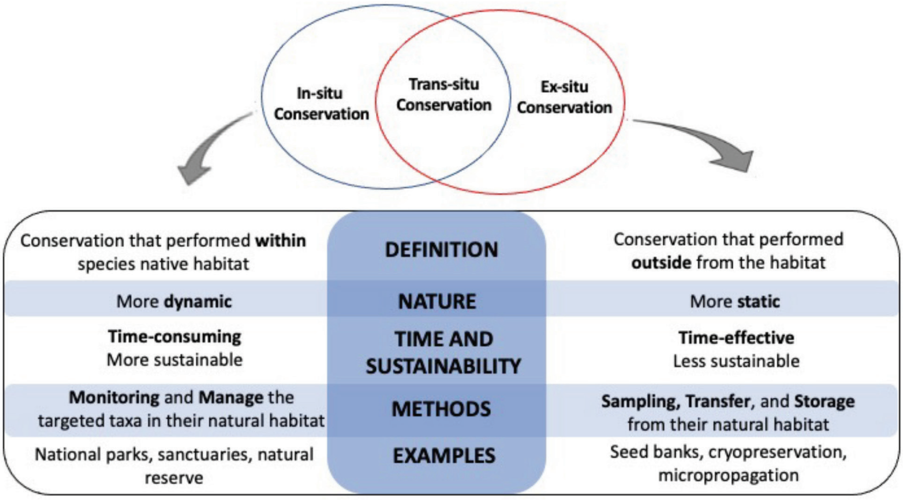

Figure 2: Overview comparison of in-situ and ex-situ conservation

new genetic traits that grow against unfavourable environmental conditions and stress (De & Pathak, 2018).

In-situ conservation efforts can be seen in many systematic protected areas, such as national parks, sanctuaries, biosphere reserves, national monuments, and cultural landscapes. These protected areas are equipped with conservation techniques including biodiversity monitoring, management, and designation of growth ecosystem (Anwar, 2020). For instance, approximately 341 orchid species are being reported from the protected Crocker Range National Park in Sabah, Malaysia (Majit *et al.*, 2014). Sessa Orchid Sanctuary in India has more than 200 orchid species including seven unique endemic saprotrophic orchids (State Forest Research Institute, 2021) and 12 rare species orchids (Wikipedia contributors, 2021). Being protected from disruption, rare orchids like Bromeliads (Newborn, 2012) and *Ponthieva brittoniae* (Sadle *et al.*, 2005) are found returning to Everglades National Park in Florida, United States. Several practices of in-situ conservation provide strong evidence for restoring threatened or rare orchid species, where the negative impact caused by anthropogenic disturbances can be minimalised, especially through the restriction of illegal and premature harvesting. A three-year monitoring program found that the population density of endangered *Dactylorhiza hatagirea* (*D. Don*) in a protected area is significantly higher than in unregulated areas, with higher reproductive fitness and tuber biomass (Chapagain *et al.*, 2021).

In the recent decade, automated technology or precision conservation techniques were established in in-situ conservation. The automated and precision conservation approach (APCA) integrates technologies and procedures to link and map variables in orchid monitoring to develop appropriate management actions. APCA provides high accuracy in identifying orchid species at risk of extinction through index-based or prediction-based methods (Zizka *et al.*, 2021). APCA has overcome the limitation of conventional assessments, which depend on manually recording resource intensity and the global species richness based on the highly traded and commercially important species of traditional conservation.

The creation of APCA by processing images from wireless WebCAMs software on the low altitude remote sensing platform, which allows for the monitoring of *Dendrobium* sp. orchid growth and access to pest information within the plantation (Samseemoung *et al.*, 2017). According to Millner *et al.* (2020), online assessment tools known as geospatial conservation assessment tools (GeoCAT) effectively provide biodiversity data for the semi-automated IUCN red list assessment. Google Earth, a 3D satellite imaging system, examines location within a population and establishes habitat loss from Google Earth detection (Simkin *et al.*, 2022). Real-time monitoring of environmental parameters such as pH, temperature, light, humidity, and carbon dioxide ($CO_2$) concentration can be accessed from the wireless sensor network (WSN) technology (Ratnapinda *et al.*, 2020). WSN technology coupled with a dynamic converge cast tree algorithm provides environmental measurements with a better spatiotemporal resolution to achieve precision cultivation management for orchids (Jiang *et al.*, 2016). In addition, WSN is equipped with Internet of Things (IoTs) technologies, which enable the automated monitoring of data storage in the cloud (Abbasi *et al.*, 2019). The integration of spatial technologies such as geographic information systems (GIS), global positioning systems (GPS), and remote sensing technology (RST) demonstrated good monitoring systems in the habitat of butterfly weeds are suggested to be used in orchid plantation management (Neigel, 2018; Bouyer *et al.*, 2021).

The formation of APCA addressed the shortcomings of traditional conservation assessments. However, APCA is a new method, and its dependability for estimating threat categories in the Red List is unknown. More extensive research must be conducted to discover the method's reliance on predicting the status of more orchids so that appropriate actions can be taken before extinction occurs.

### *Ex-situ Conservation*

Increasing anthropogenic and natural factors drastically reduce the genetic variability of orchid species (Vendrame *et al.*, 2014). Orchid conservation through natural reserves alone cannot adequately protect wild orchid species (De & Medhi, 2019). This highlighted the importance of ex-situ integration conservation as a backup method for conserving genetically important species (Irawati, 2013; De & Medhi, 2019). Germplasm ex-situ conservation includes the preservation of the whole plant, tissues and cells, preservation of genetic materials of seeds, pollen and competent mycorrhizal fungi in laboratories (Irawati, 2013; Wu et al., 2016a; OSG Ex-situ Conservation Group, 2020). The botanical garden usually accomplishes the traditional ex-situ germplasm preservation, private nurseries and orchidarium, whereas cryopreservation, field gene banks, DNA banking, in-vitro micropropagation have contributed indispensable roles in ex-situ germplasm conservation (Pal *et al.,* 2020). Ex-situ germplasm conservation rapidly conserved the threatened germplasm caused by habitat destruction, rescuing recalcitrant orchid seeds, bulking up germplasm for long-term storage, promoting habitat restoration through the reintroduction of preserved germplasm in artificial habitat (XTBG Information Group, 2010).

Gene banks are among the key players in ex-situ germplasm conservation. Germplasm of orchids has been collected from the natural habitat and stored in the plant genetics resource (PGR) gene bank (Pal *et al.*, 2020). PGR gene banks are responsible for storing, reproducing, and maintaining the active living genetic materials of orchids (Wambugu *et al.*, 2018). Gene bank offers several advantageous features, such as acting as an ideal storage platform to ensure cell viability in a good quality condition in the long term, proper monitoring of the genetic source with complete documentation and characterisation of orchid species (Pamarthi *et al.*, 2019). Hence, genetic erosion can be minimised, and a large orchid species can be preserved in optimal conditions.

Several organisations such as the Food and Agricultural Organisation of the United Nations (FAO), International Board of Plant Genetic Resources (IBPGR) and International Plant Genetic Resources Institute (IPGRI) have contributed an indispensable role in supporting germplasm conservation (Engels & Ebert, 2021).

Since then, various standard protocols such as germplasm collection, long-term storage, drying and packaging process, reintroduce and regeneration of orchid seed recommended by IPGRI provide a good guideline for orchid developers and farmers for orchids germplasm conservation (Reed *et al.*, 2004).

Germplasm is also preserved in the form of seed bank. The prevalence of seed banks in orchid conservation is high due to their simple storage procedure (Magrini *et al.*, 2019). The seed stored under specific conditions using modern plant biotechnology techniques prolongs the viability of the seed and minimises the need to regenerate the genetic source. Seed bank storage of orchids does not require high-technology equipment and is only occupied in a relatively small space. Community seed banks have been established regionally to serve as an important platform for preserving local varieties of orchids (Vernooy *et al.*, 2014). An example of a well-known seed bank is Orchid Seed Stores for Sustainable Use (OSSSU) which commenced in 2007 to connect global networks of orchid seed banks (Seaton *et al.*, 2010). Cryopreservation is ideal for seed bank conservation programs because its small size and weight enable the seeds to be stored in large quantities at a minimum volume (Kaur, 2018; Franceschi *et al.*, 2019).

Cryopreservation techniques involve the storage of orchid germplasm in ultra-low temperatures (Mweetwa *et al.*, 2006), usually in liquid nitrogen at –196°C, where metabolic processes are deactivated and the reaction of molecules are drastically slowed down (Mweetwa *et al.*, 2006). This allows the long-term storage of biological material as biological deterioration is arrested (Vendrame *et al.*, 2014). Cryopreservation could be a viable long-term strategy for preserving orchid germplasm as the method can ensure phenotypic and genotypic stability and minimise the risk of somoclonal variation (Gogoi *et al.*, 2012). Moreover, liquid nitrogen seed storage improved germination and seedling development of *Phalaenopsis* by 37%, suggesting the low-temperature treatment can trigger the breaking of dormancy (Mweetwa *et al.*, 2006). Pretreatment to remove water before cryopreservation such as desiccation, prevents the cell and tissue damage caused by ice crystal formation (Wu *et al.*, 2018). However, an optimised drying method required many orchid seeds to propose the time, temperature and humidity needed for complete dehydration (Wu *et al.*, 2016a). Another alternative is vitrification, immersing the seeds in high osmolarity vitrification solutions such as glycerol, dimethyl sulfoxide, and ethylene glycol (Mohanty *et al.*, 2012; Wu *et al.*, 2016a). Although vitrification improves the seeds' tolerance to cryopreservation, exposure of some orchid seeds, such as *Oncidium flexuosum* and *Dendrobium* hybrid, to high concentrations of the toxic cryoprotectants was found to lose viability. Therefore, further study should be conducted to minimise the toxicity exerted on orchid seeds during vitrification pretreatment (Wu *et al.*, 2016a).

A field gene bank was developed to create an artificial ecosystem for the re-establishment of endangered orchid species and allow the orchid species to breed naturally in the field, which can ensure the genetic variety of the orchid species (De & Pathak, 2018). The main conservation strategy of field gene banks is to preserve the genetic resource of recalcitrant, perennial and vegetatively propagated orchid species (Rajasekharan & Ramanatha Rao, 2019).

### *In-vitro Culture*

Orchids are evergreen, deciduous plants with a long life span (Zhang *et al.*, 2018). Some species, like *Cypripedium calceolus*, can live for 30 to 100 years. Most species undergo a long pre-flowering vegetative stage that usually lasts four to seven years or even longer (Zhang *et al.*, 2018) (Figure 3). Therefore, in-vitro culture is an indispensable key technology that shortens the life cycle and produces a mass volume of live germplasm in an artificial environment by supplementing nutrients and growth factors (Vudala *et al.*, 2019). In-vitro culture can be done by in-vitro seed germination, dual culture with symbiotic mycorrhizal fungi and organogenesis

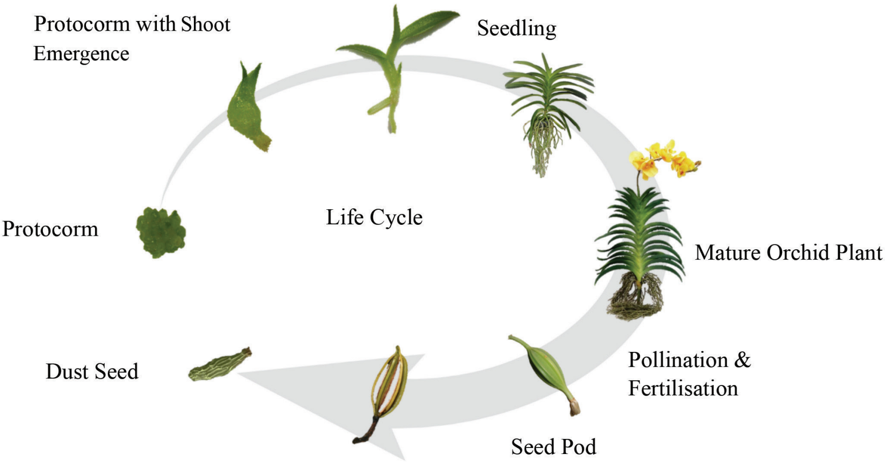

Figure 3: Life cycle of orchid. Seed pod which bears numerous dust seeds lack endosperm followed by pollination and fertilisation. As the seed pod matures, it burst, releasing dust seeds which are being dispersed to a further area. In favourable conditions, the seed germinates, forming a primary structure, known as protocorm. As the protocorm develops, shoot starts to emerge followed by roots. As both shoot and roots are established, the seedling is formed and continues to grow into an adult plant. In certain seasons, the mature plant flowers

(Dolce *et al.*, 2020). Organogenesis in in-vitro culture techniques allows the cultivation of orchids without seeds, which solves the natural limitation of low seed densities in nature (Ma *et al.*, 2020). In-vitro culture micropropagation overcame the limitation of seed that required an average of five to six years to grow only a meagre number of plants (Sarmah *et al.*, 2017). In-vitro micropropagation is economically sustainable in producing large-scale disease-free cloning materials (Sarmah *et al.*, 2017). Accumulative reports showed that some orchid species like the endangered *Beltia urbana* (Rubluo *et al.*, 1989), *Anoectochilus formosanus* (Ket *et al.*, 2004), *Geodorum densiflorum* (Bhadra & Hossain, 2003), *Dendrobium officinale* (Chen *et al.*, 2014), and *Phalaenopsis gigantea* (Murdad *et al.*, 2007) that are being cultured in-vitro can be restored into their natural environment.

To date, in vitro production of orchids through thin cell layers (TCL), which involves the culture of small-sized explant excised either longitudinally (longitudinal TCL techniques) or transversely (transverse TCL techniques) from various plant organs, has been reported (Chugh *et al.*, 2009). Protocorm-like body (PLB) and callus formation were successfully induced in TCL culture in species like *Aranda, Coelogyne cristatam Cymbidium* sp., *Dendrobium* sp., *Doritaenopsis, Paphiopedilim, Renanthera, Phynchostylis, Spathoglottis, Xenikophyton* (Teixeira da Silva, 2013), and *Brasilidium forbesi* (Gomes *et al.*, 2015). The longitudinal TCL method involves only one tissue type, while the transverse TCL technique requires several tissue types like epidermal, cortical, cambium, and parenchyma cells. TCL systems are highly efficient compared to conventional in vitro culture techniques but have yet to be fully applied commercially (Gomes *et al.*, 2015). Although in-vitro micropropagation has received critiques and scepticism for genetic variability reduction and propagated plants might be less resilient to diseases, this technique is so far from the most efficient solution for critically threatened species to be rapidly restored to their environment (Plant Cell Technology, 2021a). In addition, micropropagation has been employed to develop orchid hybrids where self-incompatibility is a major problem estimated to occur in 10% of orchid species (Niu *et al.*, 2017). Through in-vitro propagation, the germplasm of important, endangered, and rare orchids can be saved and further stored with other ex-situ conservation strategies such as gene banks with cryopreservation techniques. The combination of in-vitro propagation and in-situ conservation, also known as trans-situ conservation, could promote natural selection and genetic variation of the important orchid (Nadarajan *et al.*, 2021). Trans-situ conservation often involves ecological restoration, reintroduction, and collection, which require horticulture, education, and research unit support to maximise the conservation output (Newton & Oldfield, 2012). Riordan and Nabhan (2019) mentioned that applying the trans-situ model can lead to advances in crop improvement, especially in unprecedented climatic changes, anthropogenic issues, and political and economic uncertainties. It is hence recommended that efficient conservation of rare and endangered orchid species should involve a combination of local livelihood creation, in-situ protection, ex-situ conservation and restoration (Wang *et al*., 2021).

## Multi-omics Approaches for Orchid Conservation

Multi-omics represents an innovative approach integrating datasets from various omics disciplines during analysis. The diverse omics strategies encompassed in multi-omics include genome, proteome, transcriptome, epigenome, and microbiome. Notably, intercorrelations exist among these omics groups. Significant efforts have been made to briefly summarise the recent advancements in omics technologies over the past decade. Additionally, multi-omics technology enables scientists to assess and predict the impacts of climate change, global warming, and other environmental factors on the cellular responses of orchids. This information is crucial for understanding orchid survival and facilitating effective restoration efforts.

Over the years, omics research has centred on collecting, measuring, qualification, and characterising a wide range of biological molecules that change and translate into the functional biosystem (Fay, 2018). The science of omics provides insight into molecular mechanisms and physiological responses to cope with environmental change, therefore increasing the survival and adaptation of orchids either in-vitro or ex-situ germplasm conservation program (Balilashaki *et al.*, 2020; Carducci *et al.*, 2020). Remarkable progress in whole-genome sequencing and omics technologies has greatly enhanced our understanding of orchid biology, resulting in successful application in producing unique varieties (Tiwari *et al.,* 2023).

### *Genomics and Transcriptomics Approaches*

Many hybrid orchids have been introduced, making orchid species identification difficult. In addition, the orchid's life cycle is generally longer than that of other angiosperms, taking up to many years (2-8 years for the first blossom from seed germination) (Plant Cell Technology, 2021b). It is difficult to identify the orchid species without its flower and it has become the major challenge for orchid conservation without the intercept of genomic and transcriptomics in the past decade. Interestingly, the major risk factor pushing orchids *Caladenia huegelii* and *Phaius australis* to become endangered has been identified due to increasing in-breeding and the influence of lack of gene flow (Simmons *et al.*, 2018). Similarly, multiple generations of orchid subcultures in in-vitro micropropagation could have a similar risk to the genetic variability of orchids. Therefore, the inception of a genomics study is very important to assess the risk and solution for such a situation.

The study of genome sequences and gene expression offers valuable information about the development and role of gene families and a comprehensive collection of genome sequences, including orchids, is currently accessible in a database (Miura *et al*., 2023). Wagner *et al.* (2021) reported using genome-wide association studies (GWAS) to examine endangered orchid's conservation status and taxonomic delimitation. According to Wagner *et al.* (2021), genomic analysis techniques involving genetic structure, phylogenomic analysis, and co-ancestry provide detailed insights into the genetic distinction, which aids in maintaining a good genome and strong phylogeny. Genetic study of *Dactylorhiza* sp. in the region of the Mediterranean Basin enables scientists to understand taxonomic efforts with species number and explore the taxonomic inflation of Dactylorhiza sp. in Western Europe (Fay & Chase, 2009; Wraith *et al.*, 2020). The advancement of genomics allows for a better understanding of the orchid's evolutionary history as distinct from mutated and hybrid species (Phillips *et al.*, 2020). It is critical to track inheritable genetic information in orchids, and it is especially crucial in the mitigation of orchid species loss caused by habitat fragmentation and global climate change (Swarts & Dixon, 2009). In addition, the detection of new species through molecular phylogenetic analysis such as *Vanda funingensis* has been reported (Zou *et al.* (2016). Zhang *et al.* (2017) identified the changes in the flowering mechanism MADS-box genes and detected new gene families with a genetic mapping technique for a better understanding of the diverse suite of the developmental process during orchid evolution.

Multiple genomic approaches have been developed for the detection of gene mutation. For example, the development of Random Amplified Polymorphic DNA (RAPD) molecular marker is used for rapid gene mutation detection in protocorm-like bodies PLBs in orchids (Cardoso *et al.*, 2020). In addition, this tool is also used to explore gene expression during the developmental process in Orchidaceae. For instance, gene expression detection in flower development and inflorescence. Genomic identification of MADS-box genes (type I and type II) in *Dendrobium officinale* and *Phalaenopsis equestris* has been reported to play an important role in flowering mechanism and reproduction development in orchids (He *et al.*, 2019). Molecular markers such as DNA amplification fingerprinting (DAF), Restriction fragment length polymorphism (RFLP), and Random Amplified Polymorphic DNA (RAPD) have been used in diversity studies of Phalaenopsis (Balilashaki *et al.*, 2019). Sequence-based microsatellite markers have been applied to study molecular relationships and characterisation in orchids (Fattmah & Sukma, 2011; Chung *et al.*, 2017; Jin *et al.*, 2021).

Detailed molecular information on the regulation mechanism of orchids against unfavourable environmental conditions can be obtained through the transcriptomics approach (Miura *et al.*, 2023). Transcriptomics provide a complete set of gene expressions that reveal the changes in transcript patterns of an organism under the influence of various environmental and developmental factors such as diseases, drugs, and hormones (Balilashaki *et al.*, 2020; Valadares *et al.*, 2020; Tikendra *et al.*, 2021). For instance, the regulation mechanism of crassulacean acid metabolism (CAM) pathway against drought stress in Dendrobium catenatum had been reported (Zou *et al*., 2018). Transcriptomic analysis of *Phalaenopsis equestris* using Illumina RNA-seq has been carried out to develop drought-resistant varieties (Wan *et al*., 2018). Another literature revealed that 17 miRNAs detected in Dendrobium officinale showed growth-regulating factors and homeostatic regulation against external stress (Meng *et al*., 2016). Culturing orchids in template country could be possible since the fold transcriptome that regulate cold acclimation has been identified in orchids (Wu *et al*., 2016b). The detection of regulatory gene expression in orchids and transcriptomics data can provide valuable resources for researchers to develop resistance genes against various adverse conditions. With the development of more resistant orchid traits that resist environmental stress, orchids can exhibit higher recovery rates and are more fit to survive under evolutional changes.

The functional genes for flower development, including the ABCE functional gene, had been expressed in *Dendrobium* sp. (Xu *et al.*, 2006), B and E genes reported from *Phalaenopsis* (Pan *et al.*, 2014) and Cymbidium (Xiang *et al.*, 2018), and Oncidium genus with B (PI lineages and AP3) function gene (Hsu *et al.*, 2010). The transcriptome-wide analysis had been applied to study the MADS-box gene in the orchid *Erycina pusilla* (Lin *et al.*, 2016). In addition, transcriptomic profiling was conducted to investigate the flower scent biosynthesis pathway in the orchid *Cymbidium faberi* (Xu *et al.*, 2019). De-nova transcriptome analysis with collective datasets on flowering development and floral organ differentiation had been performed in *Phalaenopsis equestris* (Niu *et al.,* 2016) and *Cymbidium ensifolium* (Yang *et al.*, 2015). The exploration of transcriptome can improve the flowering development system through multiple pathways, including attracting more natural pollinators with the production of flower scent and flower colour, increasing orchid breeding, and hence promoting higher orchid survival (Zhang *et al.*, 2020). There are 29 MADS-box genes that were expressed during the examination of inflorescence in the tissue of Orchid *Orchis italic* (Valoroso *et al.*, 2019). Apart from flower development, MADs-box genes are crucial in controlling many other developmental aspects such as fruiting, gametophyte, embryo and seed development (Becker & Theissen, 2003; Gramzow & Theissen, 2010). The expression of DOAP1 genes showed an early flowering mechanism in the orchid *Dendrobium Chao Praya Smile*, which was used to rescue the floral organ defect from the mutant genes (Sawettalake *et al.*, 2017). Key findings on the DOAP1 gene expression are important evolutionary conservation indicators for the floral meristem specification, flowering promotion, and regulation of floral development in orchids (Sawettalake *et al.*, 2017).

Seed germination rates can be improved by unique mycorrhizal associations with orchid species (Perotto *et al.*, 2014; Fochi *et al.*, 2017). During the colonisation process of orchid seeds and *Tulasnela* sp. mychorizal fungus, a systematic gene expression study on the optimal reference genes was conducted (Dearnaley, 2007; Suetsugu *et al.*, 2017; Wang *et al.*, 2021b). The molecular signalling pathways associated with orchid mycorrhiza have also been identified (Favre-Godal *et al.*, 2020). Putative genes have been identified in the cell endocytosis process of orchid Gastrodia elata and mycorrhizal fungi Mycena (Zeng *et al.*, 2017). Cell-specific gene expression during orchid and mycorrhiza interaction has also been analysed by Laser Microdissection (LMD) technology coupled with RT-PCR analysis (Balestrini *et al.*,2018).

The association of orchid and mycorrhizal fungi and its mechanisms detected through genomic and transcriptomic approaches is shown in Figure 4. There are four processes detected during orchid and mycorrhizal fungi associations, including (1) secretion of mycorrhizal factors, (2) activation of molecular signalling, (3) Evasion of pathogen-associate molecular patterns (PAMPs), and (4) secretion of phytohormones (Favre-Godal *et al.*, 2020). Secretion of mycorrhizal factors activates the common symbiosis genes involved in biological activities such as root colonisation and nutrient uptake or exchange upon the orchid mycorrhizal (OM) interaction (Delaux *et al.*, 2014). OM fungi can recognise symbiosis signal molecules such as strigolactones and further trigger the production of lipochito-oligosaccharides in response to the symbiosis relationship (Zanetti *et al.*, 2017; Gough *et al.*, 2018). Secretion of different phytohormones such as auxin, gibberellins, cytokinins and ethylenes from both orchids or fungi promoting seed germination, increasing the number of leaves and biomass, modulating the concentration of phytohormone to response in particular biological response (Faria *et al.*, 2013). In turn, fungi can enhance plant defence mechanisms by producing effectors such as small inhibitory proteins and the evasion of PAMPs (Cord-Landwehr *et al.*, 2016).

### ***Proteomics Approaches***

Proteomics research investigates dynamic protein products, their activities, and relationships in all expressed proteins (Balilashaki *et al.*, 2020). The contribution of proteomics studies in orchid conservation can be seen in the detection of various functional proteins in the development process of orchid species. For instance, proteomic analysis using high-definition Mass Spectrometry coupled with nano ultrapure liquid chromatography (UPLC) has been used for LFY-like protein and OrcLFY

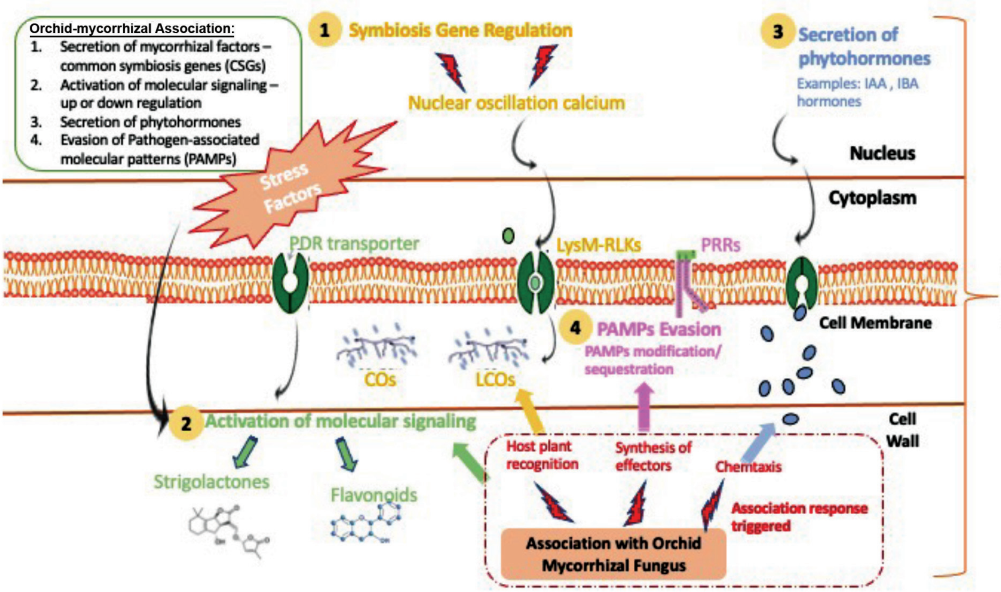

Figure 4: Overview of the association between orchid and mycorrhizal fungi with its possibly occurring events and molecular pathways detected through genomic and transcriptomic approaches

protein expressions study in *Vanilla bahiana*, which play significant roles in the production of fruit, flower, and also vanilla flavours in *Vanilla bahiana* (Lopes *et al.*, 2019).

The use of proteomics in pollination detects self-compatibility and self-incompatibility proteins, which are important for species survival and genetic diversity (Abdallah *et al.*, 2019). About 54 differentially expressed proteins (DEP) had been identified through 2D electrophoresis with tandem mass spectrometry technique in both self-pollination and cross-pollination processes (Wang *et al.*, 2017). The identification of highly functional DEP enables the identification of the mechanism involved in pollination responses and identifies specific genes responsible for pollination (Wang *et al.*, 2017).

The study and identification of drought-resistance proteins in *Cymbidium tracyanum* and *Cybidium sinense* could improve the orchid survival rate during prolonged drought seasons (Li *et al.*, 2018). Surviving in a water-stress environment is key to a successful conservation program, as the water supply in natural conditions is unpredictable (Zhang *et al.*, 2016). The regulation mechanism of defence-related proteins in the orchid *Oncidium sphacelatum* has been reported and these defence-related proteins play critical roles in the orchid-mycorhiza interaction (Valadares *et al.*, 2014). Late embryogenesis-abundant proteins (LEAP) in *Dendrobium officinale* involved in the regulation mechanism against abiotic stress and stress-related hormones have also been reported (He *et al.*, 2021).

López-Chávez *et al.* (2016) reported the interaction between orchid *Oncidium sphacelatum Lindl*. identified the DEP and mycorrhiza fungus RG26 of *Thanatephorus* sp. control various developmental processes such as energy metabolism, ribosome biogenesis, purine recycling, and cell cycle process. A similar proteomics approach had been reported in orchid *Gastrodia elata* and mycorrhiza *Mycena* sp. to identify the proteins involved in the symbiotic germination process. Proteomics findings may aid in creating molecular biomarkers that will provide information on the symbiosis study and investigate mechanism alterations in different stages of symbiotic germination (Zeng *et al.*, 2018).

### *Metabolomics Approaches*

Metabolomics is a useful analytical profiling tool for understanding an organism's metabolism by measuring many metabolites presented within cells, tissues or organisms' interaction with its environmental condition (Lankadurai *et al.*, 2013; Balilashaki *et al.*, 2020). Non-targeted metabolomics helps to provide more intrinsic information on the survival and growth of a plant. For instance, identifying 391 out of 856 metabolites detected from *Dendrobium sinense* using metabolomics techniques showed significant differences during drought stress compared to control samples (Zhang *et al.*, 2021a). Metabolomics analysis conducted on the leaves part of *Dendrobium officinale* revealed that there is a higher accumulation of secondary metabolites produced such as flavonoids, jasmonate, alkaloids and sugar in adaptation to salt stress (Zhang *et al.*, 2021b).

Nutrients are important for orchid growth and survival as a critical environmental stressor. Orchids gained up to 80% of their mineral nutrient supply from interacting with mycorrhiza fungi (Suetsugu *et al.*, 2021). Metabolomics study reveals evolution towards myco-heterophyte and the orchid plant metabolism changes in orchid species (Lallemand *et al.*, 2019). Ghirardo *et al.* (2020) investigated the metabolic changes occurring in orchid mycorrhiza to explore the role of fungal lipids and their mechanism within the orchid mycorrhizal association.

There are several advanced analytical techniques developed to support the metabolomic studies including liquid chromatography-mass spectroscopy (LCMS), imaging by mass spectrometry (IMS) and Matrix-assisted laser desorption ionisation (MALDI-IMS) (Favre-Godal *et al.*, 2020). LCMS has been widely used to detect metabolite changes at different environmental stresses, and the data spectra obtained can be analysed using principal component analysis (PCA). Identifying the unique structural treatment groups or detecting similar components can be obtained from a scatter distribution plot generated in PCA (Piasecka *et al.*, 2019). Both IMS and MALDI-IMS approaches visualise and detect the distinct distribution of metabolites and discover the association between the organism (Covington *et al.*, 2017; Barbosa *et al.*, 2018).

Researchers often adopt the integration of metabolomics and transcriptomic analysis for in-depth detection of the metabolite's biosynthesis pathway and identifying the induced metabolite that responds to specific stress environments. Lallemand *et al.* (2019) applied the in-situ transcriptomic and metabolomics approaches to understand the loss of photosynthesis in the leaves of myco-heterotrophic plants. Herrera *et al.* (2018) adopted the proteomic and metabolomics approaches to explore the adaptation and tolerance mechanism developed by the symbiosis relationship between the orchid *Bipinnula fimbriate* and its specific mycorrhiza in the heavy metal-polluted ecosystem. Various molecular studies conducted through the combination of multi-omics approaches under different biological events have been listed in Table 1.

In summary, the combination of multi-omics approaches such as genomics, proteomics, metabolomics, and transcriptomics are often used simultaneously to discover intrinsic development or mechanism adaptation at a cellular level (Wang *et al.*, 2021a). Multi-omics approaches with high throughput techniques provide unprecedented opportunities for monitoring orchids' growth development and senescence. Multi-omics approaches enable scientists and orchid growers to visualise and elucidate all the complex mechanisms involved in orchids' developmental, physiological, and environmental adaptive and defensive events (Balilashaki *et al.*, 2020; Yang *et al.*, 2021b). Therefore, the application of multi-omics approaches in deciphering the underlying molecular mechanism of orchid serves as an important data resource to maximise the orchid survival during the restoration program (Figure 5). Altogether, this information provides useful insight for local government or environmental agencies to focus on priority areas based on the unique requirement of each orchid for effective conservation programs.

Table 1: Overview of the use of multi-omics analysis on various biological events in orchid culture

| Events | Omics Approaches | Orchid sp. | Study Aim | Molecular Techniques | Application to Orchid Conservation | References |
|---|---|---|---|---|---|---|
| **Seed Germination** | Genomic and transcriptomics | *Streptophyte lineages* | Identification of MADS-box gene | GWI | Identification of growth or genetic factors in seed germination for seed germination, further improves ex-situ conservation technique | (Chunmei He *et al.*, 2019) |
| | Genomic and transcriptomics | *Calanthe tsoongiana* | Study the potential regulatory genes and mechanism in protocorm development and seed germination | NGS | | (Jiang *et al.*, 2021) |
| | Genomic, proteomics and transcriptomics | *Dendrobium officinale* | Study the regulation mechanism of symbiotic germination | ITRAQ and RNA sequencing | | (Chen *et al.*, 2017) |
| **Orchid Mycorrhizal Association** | Metabolomics, proteomics and transcriptomics | *Oeceoclades maculate* | Study of defence response and metabolic changes in mycorrhizal roots | ITRAQ MS | With the identification of known associated mycorrhizal species, a high seed germination rate can be achieved by providing specific mycorrhizal during the seed germination process | (Valadares *et al.*, 2020) |
| | Transcriptomics and metabolomics | *Anacamptis laxiflora* | Study of metabolomic adjustment during symbiosis with orchid mycorrhizal | UPLC | | (Ghirardo *et al.*, 2020) |
| | Genomics and transcriptomics | *Oncidium Gower Ramsey* | Study the role of RNA between mycorrhizal fungus and orchid root | RNA Solexa sequencing | | (Ye *et al.*, 2014) |
| | Genomics and transcriptomics | *Dendrophylax lindenii* | Study on the molecular identification of mycorrhizal fungus | ITS, Sanger EST | | (Hoang *et al.*, 2016) |
| | Genomics | *Vanda coerulea grill. Ex Lindl.* | Study of the molecular associated fungi with symbiotic seed germination | DNAseq qiagen | | (Aggarwal *et al.*, 2012) |

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| **Flower and Pollination Mechanism** | Genomics and transcriptomics | *Apostasia shenzhenica, Neuwiedia malipoensis, Vanilla shenzhenica, Galeola faberi, Paphiopedilum armeniacum* | Study evolutional trends and floral development | NGS | Floral development to increase pollination and fertilisation, and adopt the omics knowledge to maintain the good gene traits or used in the hybrid study | (Tsai *et al.*, 2013) |
| | Transcriptomics | *Vanilla planifolia* | Study of the biosynthetic pathway of flavouring components | NGS | | (Rao *et al.*, 2014) |
| | Transcriptomics and metabolomics | *Phalaenopsis amabilis* | Study of flavonoid biosynthesis regulation in different flower colour | LC-MS/MS | | (Meng *et al.*, 2020) |
| | Genomics, transcriptomics, and proteomics | *Ophrys exaltata*<br>*Ophrys sphegodes*<br>*Ophrys garganica* | Identification of genes responding to pollinator attraction | LCMS/MS, EST | | (Sedeek *et al.*, 2013) |
| **Self-incompatibility** | Proteomics | *Dendrobium Chrysanthum* | Study of self-incompatibility mechanism and differentially expressed proteins between the self- and cross-pollination | MALDI-TOF/TOF-MS | Self-incompatibility problem | (Wang *et al.*, 2017) |
| **Evolutional Changes** | Genomics and transcriptomics | *Cymbidium sinense* | Identification of whole genome and evolutionary traits | Generation sequencing, NextDenovo | Species identification and evolution for conservation purposes | (Yang *et al.*, 2021a) |
| | Genomics and transcriptomics | *Dendrobium chrysotoxum* | Identify of sweet gene expression and whole genome sequencing and detection of gene loss within the same species | PacBIo Sequel System | | (Zhang *et al.*, 2021c) |

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|---|---|---|---|---|---|---|
| **Environmental Stress** | Transcriptomics and metabolomics | *Dendrobium sinense* | Phenylpropanoid and Purine Metabolism response to drought stress | LC-ESI-MS/MS | Conservation strategies to minimise environmental stress can be developed by better understanding orchid stress response | (Zhang *et al*., 2021a)<br>(Zhao *et al*., 2019) |
| | Transcriptomics and metabolomics | *Dendrobium wangliangii* | Ribonucleoside or ribonucleotide metabolic process against drought stress | NGS, IS | | (Ahmed *et al*., 2021) |
| | Transcriptomics and metabolomics | *Acampe ochracea* | Upregulations of antioxidant genes against paracetamol-induced hepatotoxicity | UPLC-TOF-MS | | (Zhang *et al*., 2021b) |
| | Transcriptomics and metabolomics | *Dendrobium officinale* | Study of metabolome and transcriptome under salt stress | IS, LCMS | | (Balao *et al*., 2017) |
| | Genomics, proteomics, and transcriptomics | *Dactylorhiza incamata, Dactylorhiza uchsia* | Genetic diversity and evolutional changes under biotic stress such as pathogen infection and herbivores | RNAseq de- novo transcriptome assemblies | | (Herrera *et al*., 2018) |
| | Genomics and proteomics | *Bipinnula fimbriata* | Study of tolerance and adaptation mechanism in heavy metal polluted environment | UPLC | | (Chao *et al*., 2018) |
| **Orchid Breeding** | Genomic and transcriptomics | *Phalaenopsis aphrodite* | Study of genetic mapping and chromosome level assembly during orchid breeding | PacBIo Sequel System | Successful breeding is critically important for germplasm conservation, especially in endangered or rare orchid species | (Meng *et al*., 2016) |

NGS = next generation sequencing, GWI = genomic-wide identification, LCMS = liquid chromatography-mass spectroscopy, UPLC = ultra-performance liquid chromatography, EST = expressed sequence tags, IS = Illumina sequencing, MALDI = matrix-assisted laser desorption/ionisation source, TOF = TANDEM time-of-flight

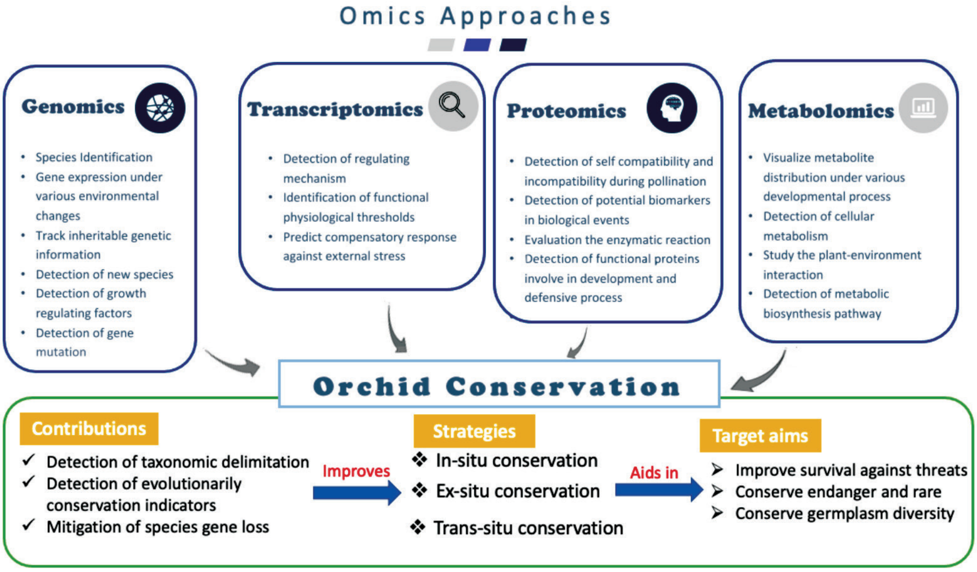

Figure 5: The summary of the contribution of multi-omics approaches in orchid conservation
Source: Authors

## Conclusions

In the past decades, orchid conservation research has focused more on the taxonomy, orchid mycorrhizal symbiosis, in-situ, and ex-situ conversation techniques, which all play vital roles in successful orchid conservation. However, climate change and global warming are expected to be the next major causes of species extinction in the near future. As a result, effective orchid conservation in the future must consider orchid responses and survival in climate change scenarios, which will rely on advances in omics technologies. Also, the integrated conservation strategies should involve unified joints among global conservation organisations, local government, researchers, orchid societies, botanies, and a single individual to achieve successful orchid conservation. All in all, the key to successful conservation is human behaviour, in which proper awareness and love for nature can greatly increase the success rate of conservation.

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## Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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