

**MOLECULAR CONSERVATION OF
Paphiopedilum rothschildianum AND
Phalaenopsis gigantea, TWO SPECIES OF
ENDANGERED ORCHIDS IN SABAH**

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Pemuliharaan Molekular *Paphiopedilum rothschildianum* dan *Phalaenopsis gigantea*, Dua Spesies Orkid Terancam di Sabah.

Sinopsis

Lokus-lokus mikrosatelit pada *P. rothschildianum* dan *P. gigantea* telah dikenalpasti dan penanda molekul spesifik berasaskan lokus tersebut telah dibangunkan. Penanda-penanda ini telah digunakan untuk mencirikan struktur populasi kedua-dua sepsis orkid yang terancam ini. Keptuasan kajian ini telah diaplikasikan untuk membangunkan satu strategi pemuliharaan yang berfokuskan kepada pembaikbakaan terpilih berasaskan kepada jarak genetik. Disamping itu, sebanyak sepuluh penanda molekul baru telah dibangunkan. Penanda-penanda ini mempunyai potensi untuk diaplikasikan secara jangka panjang untuk kajian genetik populasi, program pembiakbakaan dan pengurusan, dan pengesanan forensik dalam perdagangan orkid antarabangsa. Maklumat tentang umur kapsul yang sesuai untuk percambahan biji orkid secara *in vitro* telah dikenal pasti. Media-media untuk percambahan biji *P. rothschildianum* and *P. gigantea* dilaporkan. Lebih daripada satu media multiplikasi telah dibangunkan untuk kedua-dua spesies. Media-media tersebut adalah ringkas tetapi efektif; kehadiran air kelapa sahaja pada kepekatan yang tertentu berupaya untuk mengaruh pengandaan kedua-dua spesies, kehadiran arang teraktif dalam media pengandaan mempromosi penghasilan protokom *P. gigantea*. Biji benih *P. rothschildianum* berjaya disesuaikan dengan keadaan luar kultur seawal 12 bulan daripada percambahan manakala *P. gigantea* mengambil masa 15 bulan. Protokol untuk pengawetankrio berasaskan vitrifikasi menggunakan biji sebagai eksplan telah dibangunkan untuk kedua-dua spesies. Biji terselamat dan hidup setelah disimpan dalam cecair nitrogen dan plantlet *P. rothschildianum* berjaya didapati dari biji yang telah melalui pengawetankrio. Biji *P. gigantea* berjaya bercambah dan menghasilkan protokom setelah biji diawetkrio, walau bagaimanapun, kadar biji yang terselamat adalah rendah jika dibandingkan dengan *P. rothschildianum*. Protokol pengawetankrio untuk *P. gigantea* perlu dinilai dengan lebih lanjut tetapi menggunakan biji dengan viability yang tinggi. Viabiliti biji *P. rothschildianum* yang telah melalui pengawetankrio dan disimpan di dalam cecair nitrogen selama 6 bulan berjaya dikekalkan menggunakan protokol yang dibangunkan ini.



Molecular Conservation of *Paphiopedilum rothschildianum* and *Phalaenopsis gigantea*, Two Species of Endangered Orchids in Sabah.

Synopsis

Microsatellites loci in *P. rothschildianum* and *P. gigantea* have been isolated and specific molecular markers based on these microsatellite loci developed. These markers were used to characterize population structure of the two endangered orchids. The results have been applied to develop a conservation strategy focused on selective breeding on the basis of genetic distance. In addition, ten molecular markers were developed. These markers have a potential long-term application in population genetic studies, breeding and management programmes and the forensic detection in international trade. Information on the appropriate age of capsules for *in vitro* germination of seeds has been determined. The germination media for *P. rothschildianum* and *P. gigantea* are reported. More than one multiplication media were obtained for both species. These media are simple but effective; coconut water at specific concentration alone was able to induce multiplication of both species, activated charcoal in the multiplication medium was found to have promoting effect on production of *P. gigantea* protocorm. Seedlings of *P. rothschildianum* were acclimatised as early as 12 months after germination while that of *P. gigantea* took around 15 months. Cryopreservation protocols based on vitrification using seeds as explants were developed for both species. Seeds survived liquid nitrogen storage and plantlets of *P. rothschildianum* were successfully obtained from cryopreserved seeds. Protocorms were successfully recovered from *P. gigantea* cryopreserved seeds, however the recovery rate was lower as compared to *P. rothschildianum*. Cryopreservation protocol developed for *P. gigantea* require testing with high viability seeds. Seeds of *P. rothschildianum* that had undergone cryopreservation and stored in liquid nitrogen for 6 months had maintained their viability.

