



**UNIVERSITI PUTRA MALAYSIA**

**IN VITRO CULTURE AND SYMBIOTIC RELATIONSHIPS  
OF PAPHIOPEDILUM NIVEUM, TAENIOPHYLLUM OBTUSUM AND  
VANDA HOOKERIANA WITH THEIR ASSOCIATED FUNGI**

**IRAWATI**

**FP 1987 8**



IN VITRO CULTURE AND SYMBIOTIC RELATIONSHIPS  
OF PAPHIOPEDILUM NIVEUM, TAENIOPHYLLUM OBTUSUM AND  
VANDA HOOKERIANA WITH THEIR ASSOCIATED FUNGI

by

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A thesis submitted in partial fulfilment of the  
requirements for the degree of Doctor of Philosophy  
in the Faculty of Agriculture,  
Universiti Pertanian Malaysia

September 1987



## ACKNOWLEDGEMENT

To individuals and institutions I would like to express my appreciation and sincere gratitude for their contributions in completion of this study.

- \* My supervisors, Dr. Mohammad Md. Ali (Associate Professor) and Dr. Hasan Mad for their guidance and advice throughout the course of this study and in the preparation of this manuscript.
- \* Mr. Saleh Kadzimin, for all his help since the beginning of this study.
- \* Mr. Mark A. Clements, for the valuable discussions, his helpful comments and suggestions and also for his help in fungal determination.
- \* Dr. Zaliha Christine Alang, for reading this thesis.
- \* The Faculty of Agriculture, and the Computer Centre of the Universiti Pertanian Malaysia, for the facilities in conducting this research and in preparing this manuscript.
- \* Commonwealth Mycological Institute for fungal identification.



- \* Mr Ho Oi Kuan, Mr Yusof Suki, Mr Mimbar Haryadi, Mrs. Aminah, Mr How Yee Peng, Dr. Lai Choo May and Prof. Yap Thoo Chai for their assistance and also to Ms. Fadzlon Mohd. Jusof, Mrs. Chairun Nisa Haris and Mrs. Kamariah Hamid for their kind help.
  
- \* Public Services Department of Malaysia for the award of a fellowship under the Malaysian Technical Assistance Programme.



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## LIST OF ABBREVIATIONS

BA	6-benzyladenine
2,4-D	2,4-dichlorophenoxyacetic acid
EDTA	ethylenediamine tetraacetic acid
FAA	formol acetic alcohol
IAA	indole-3-acetic acid
NAA	naphthaleneacetic acid
ppm	parts per million
TBA	tertiary butyl alcohol



An abstract of the thesis presented to the Senate of Universiti Pertanian Malaysia in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

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September, 1987

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Co-supervisor : Dr. Hasan Mad  
Faculty : Agriculture

In vitro cultures of three orchid species, namely, Paphiopedilum niveum, Taeniophyllum obtusum and Vanda hookeriana, were initiated from different parts of the plants. Proliferous protocorm-like bodies were obtained from shoot tips and axillary buds of V. hookeriana and shoot tips and inflorescence of T. obtusum. Shoot tips and axillary buds of P. niveum grew into single or multiple plantlets. Inflorescence culture of T. obtusum developed into multiple branches of inflorescences, calli or produced roots. Young leaf tip or leaf base of P. niveum and root of T. obtusum were unable to differentiate into callus. However, culture of young flower bud of P. niveum produced leaf after seven months.



High pH of the medium was suitable for T. obtusum cultures. Low concentration of modified Knudson's C medium was preferable to the standard concentration for the culture of P. niveum.

Efforts to raise seedlings of the three different orchids showed that seeds from 140 to 160-day old fruits of P. niveum and 60 to 115-day old fruits of T. obtusum germinated better than dry seeds, while for V. hookeriana dry seeds were able to germinate freely.

Mycorrhizal fungi isolated from P. niveum, T. obtusum and V. hookeriana roots were able to induce the germination of their own seeds. Fungal mycorrhiza from T. obtusum showed a greater ability to initiate germination and promote growth of V. hookeriana seeds compared to its own fungal mycorrhizas. Other fungi associated with the three orchids species were deleterious to the seeds.

Most fungi associated with these three orchids did not influence the development of protocorm-like bodies of V. hookeriana and were not compatible to T. obtusum. Their influence in the establishment of V. hookeriana and T. obtusum seedlings in the nursery was also not significantly shown in this study.



Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia sebagai memenuhi sebahagian daripada keperluan untuk ijazah Doktor Falsafah.

KULTUR IN VITRO DAN PERHUBUNGAN SIMBIOSIS  
ANTARA PAPHIOPEDILUM NIVEUM, TAENIOPHYLLUM OBTUSUM DAN  
VANDA HOOKERIANA DENGAN KULAT-KULAT SEKUTUANNYA

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Kultur in vitro bagi tiga spesies orkid, iaitu, Paphiopedilum niveum, Taeniophyllum obtusum dan Vanda hookeriana, telah dibuat dari beberapa bahagian tanaman. Struktur-struktur seperti protokom yang mampu membiak dengan cepat diperolehi dari pucuk dan tunas samping V. hookeriana dan dari pucuk dan jambak bunga T. obtusum. Pucuk dan tunas samping dari P. niveum tumbuh menjadi anak benih tunggal atau ganda. Kultur jambak bunga dari T. obtusum tumbuh menjadi jambak bunga ganda, kalus atau menghasilkan akar. Hujung dan pangkal daun yang muda P. niveum dan akar T. obtusum tidak dapat menghasilkan kalus. Akan tetapi kultur kuncup bunga muda dari P. niveum dapat menumbuhkan daun setelah tujuh bulan.



Medium yang mempunyai pH tinggi adalah sesuai untuk kultur T. obtusum. Kepekatan yang rendah dari medium Knudson's C yang diubahsuaikan adalah lebih baik bagi membiakkan P. niveum daripada yang berkepekatan biasa.

Usaha untuk menumbuhkan anak benih dari ketiga-tiga jenis orkid yang berbeza didapati biji benih P. niveum yang berumur 140 hingga 160 hari dan biji benih T. obtusum yang berumur 60 hingga 115 hari bercambah lebih baik daripada biji keringnya, manakala biji kering V. hookeriana pula bercambah dengan bebas.

Kulat-kulat mikoriza yang diasingkan dari akar-akar P. niveum, T. obtusum dan V. hookeriana mampu merangsangkan percambahan biji benih orkidnya. Kulat mikoriza dari T. obtusum menunjukkan keupayaan memulakan percambahan dan menggalakkan pertumbuhan biji-biji V. hookeriana yang lebih baik dibandingkan dengan kulat-kulat mikorizanya sendiri. Kulat-kulat lain yang bersekutu dengan ketiga jenis orkid merosakkan biji-biji benihnya.

Kebanyakan kulat-kulat yang bersekutu dengan ketiga-tiga jenis orkid ini tidak mempengaruhi pertumbuhan struktur-struktur seperti protokom dari V. hookeriana dan tidak sesuai bagi T. obtusum. Pengaruhnya terhadap penyesuaian tumbuh dari anak benih V. hookeriana dan T. obtusum pada semaian anak benih juga tidak tampak berkesan dalam penyelidikan ini.



## CHAPTER I

### I N T R O D U C T I O N

Among ornamental plants, orchids are one of the most economically important in this region. Therefore efforts are made to support the development of orchid industry through propagation techniques, breeding methods, cultivation practices and marketing studies to fulfil the demand of seedlings, plants or cut flowers. However, recently people are aware of the possible loss of many orchid species due to over-collection of wild orchids or land clearing in many countries in the world (Hagsater & Steward, 1986). Conservation measures have to be taken to prevent these valuable germplasms from extinction. Suitable propagation techniques are needed to multiply them. This effort would also encourage further breeding and utilization of the orchid species and also has a significant role on their conservation.

There are many important Malaysian orchids, and among them, three species had been chosen for this research and they are Paphiopedilum niveum, Taeniophyllum obtusum and Vanda hookeriana. Paphiopedilum niveum is restrictedly found in nature. This species is not only an attractive wild orchid but is also a potential species for breeding. Its commercial

