Orchid Science and Biotechnology

Abbreviation: Orchid Sci. Biotech.
Print: ISSN 1752-3443

Scope and target readership: *Orchid Science and Biotechnology* is dedicated to significant advances in orchid science and biotechnology.

Orchid Science and Biotechnology aims to examine the following aspects of terrestrial or epiphytic orchids (symbiotic or asymbiotic):

1) Breeding science (genetic modification, hybridisation and embryo rescue);
2) *In vitro* propagation (micropropagation, somatic embryogenesis, tissue culture, including *in vitro* culture of pseudobulbs and keiki-derived tissue);
3) Mycorrhizal symbioses (and effects on orchid productivity, reproduction and disease resistance *in vitro* or *ex vitro*);
4) Physiology, molecular biology, structural botany (integrated, pure and applied);
5) Orchid diseases, their management and control;
6) Post-harvest technology as applies to cut orchid flowers and foliage (deterioration, preservation, shipping, and marketing);
7) Production of secondary metabolites, organic and inorganic biochemistry, and phytochemistry from any orchid organ;
8) Storage of valuable genetic material (cold-storage or cryopreservation);
9) Velamen development and physiology;
10) Orchid flower scent, volatile and colour analysis;
11) International legislation, including CITES, anti-trafficking measures, stimulation and maintenance of biological diversity, and programmes that stimulate biodiversity at any system scale are strongly encouraged.

For publication in *Orchid Science and Biotechnology* the research must provide a highly significant new contribution to our understanding of orchids and must generally be supported by a combination of either: physiological, biochemical, genetic or molecular analyses. All areas of study are welcome and the experimental approaches used can be wide-ranging.

Global Science Books, Ltd.
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TW7 5NQ, United Kingdom

Editorial Office: Takamatsu, Japan
Accounting: Lagos, Portugal

GSB homepage: www.globalsciencebooks.com
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Cover photos: Left-hand side plate: *In vitro* growth and development of epiphytic hybrid *Cymbidium* Twilight Moon 'Day Light'. More details in Teixeira da Silva *et al*., pp 15-23. *In vitro* plant regeneration of *Dendrobium nobile* via shoot buds and PLBs. More details in Song *et al*., pp 51-55. Symbiotic germination of *Anoectochilus formosanus* Hayata seeds on Hyponex medium for non-mycorrhizal control and oat meal agar for mycorrhiza plant (R04) for 2 months. More details in Chang *et al*., pp 56-60. Regenerated *Phalaenopsis* protocorms (RPs) formed on a seed-derived protocorm (SDP). More details in Chen *et al*., pp 40-43.

Printed in Japan on acid-free paper.

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Number 1

Apiradee Uthairatanakij (Thailand), Jaime A. Teixeira da Silva (Japan), Kullanart Obsuwan (Thailand) Chitosan for Improving Orchid Production and Quality (pp 1-5)

ABSTRACT

Invited Mini-Review: Chitosan is a deacetylated derivative of chitin that is derived from the cell walls of fungi, crustacean exoskeletons, cuticles of insects and some algae. It is considered environmental friendly for agricultural uses as it is easily degradable and non toxic to humans. Chitosan and its derivatives have been reported to elicit natural defence responses in plants and it has been used as a natural compound to control pre- and postharvest pathogenic diseases. Chitosan application has also been shown to increase yields of some agricultural crops. Chitosan has recently been reported to act as a plant growth promotor in some species including orchids. The degree of deacetylation and concentrations of chitosan have varying effects on the growth and development of orchid cultured *in vitro*. Spraying with chitosan has been shown to significantly reduce the severity of leaf spot disease in orchids. Also, it has been shown that application of chitosan to *Dendrobium* orchid plants tended to increase the size of open florets and length of the inflorescences, but did not affect the display-life of cut orchids.

Upatham Meesawat, Kamnoon Kanchanapoom (Thailand) Understanding the Flowering Behavior of Pigeon Orchid (*Dendrobium crumenatum* Swartz) (pp 6-14)

ABSTRACT

Invited Mini-Review: Inflorescences of pigeon orchid (*Dendrobium crumenatum* Sw.) are comprised of 3 floral buds, each at a different developmental stage. Only one floral bud at the responsive stage could be stimulated to flower at exactly 9 days after the rainfall event (days to anthesis, DTA=9). Floral buds at this stage were characterized as perfect miniature flowers in which microsporocytes were in a pre-meiotic stage. The binucleate pollen grains appeared 3 days prior to anthesis. The conditions on the day of induction, required a decrease of 10°C in temperature from the peak temperature at noon within 1-2 h to trigger events leading to anthesis. Both cooling and a pre-warming treatment before cooling could stimulate anthesis at 19 (DTA=19) and 21 (DTA=21) days after treatment, respectively. Concentrations of 10^{-2} M gibberellic acid (GA_3) and 10^{-1}-10^{-2} M N^6-benzyladinine (BA) applied separately to the inflorescences of grown plants could stimulate anthesis but simultaneous application from all permutations of concentrations of both chemicals failed to do so. *In vitro* clonal propagation from axillary bud culture through callus-derived protocorm like-bodies (PLBs) and plant regeneration was developed through both embryogenesis and organogenesis. *In vitro* floral organs were induced from the 4-week-old PLB-derived plantlets while subsequent floral organogenesis and anthesis were dependent on the conditions of the rainfall event only. The *in vitro* induced plants flowered within 8-12 months as compared to 5-7 years for natural plants. This system shortened the juvenile period of this orchid. This knowledge is very useful and critical for many aspects of future flowering research.

Jaime A. Teixeira da Silva (Japan), Dam Thi Thanh Giang (Vietnam), M-T. Chan, Sanjaya (Taiwan), Atsushi Norikane (Japan), M-L. Chai (Japan), Julio Chico-Ruíz (Peru), Suprasanna Penna (India), Tom Granström (Finland), Michio Tanaka (Japan) The Influence of Different Carbon Sources, Photohetero-, Photoauto- and Photomixotrophic Conditions on Protocorm-Like Body Organogenesis and Callus Formation in Thin Cell Layer Culture of Hybrid *Cymbidium* (Orchidaceae) (pp 15-23)

ABSTRACT

Original Research Paper: It is common to utilize sucrose as a carbon source in the induction of *Cymbidium* PLB and callus, and as an energy source in the tissue culture of other orchids. This study found carbon sources to be clustered into 4 groups according to their suitability for PLB and/or callus induction: cluster A (high PLB and callus inducers): fructose, sucrose; cluster B (intermediate PLB and callus inducers): galactose, glucose, turanose (a rare sugar); cluster C (intermediate PLB and low callus inducers): lactose, maltose, starch, mannitol, sorbitol; cluster D (poor PLB and callus inducers): mannose, xylose, raffinose, cellulose. Plants regenerated from control PLB-derived shoots were cytologically (using flow cytometry) and genetically (using 10mer RAPD) identical, despite varying photosynthetic capacities. Callus exhibited high levels of polysomaty.

Plants could root under photoauto-, photohetero- and photomixotrophic conditions, with the latter showing the highest photosynthetic capacity at almost all photosynthetic photon fluxes (0-300 μmol $CO_2.m^{-2}.s^{-1}$). Plants were acclimatized from all treatments with a 100% survival rate, although those derived from photomixo- and photoautotrophic conditions appeared (visually) to be much stronger, sturdier and more robust.

Pinaki Sinha, Mohammad Lokman Hakim, Mohammad Firoz Alam (Bangladesh) Clonal Propagation of *Cattleya labiata* cv. 'Pearl Harbor' through *in Vitro* Culture of Thin Cell Layers From Young Shoots of Mature Plants (pp 24-28)

ABSTRACT

Original Research Paper: A protocol for the clonal propagation of *Cattleya* was developed from thin cell layers (TCLs) derived from protocorms through *in vitro* culture. The TCLs from young shoots of a mature *Cattleya* hybrid was cultured for one week on half strength Murashige and Skoog (MS) liquid medium supplemented with N^6-benzyladenine (1.5 mg l^{-1}), α-naphthaleneacetic acid (0.5 mg l^{-1}), 2% (w/v) sucrose, 10% (v/v) coconut water, 2 g l^{-l} peptone and 1 g l^{-l} activated charcoal, followed by culture on agar-gelled full strength MS medium with same supplementing components, which produced an average of 8 protocorm-like bodies (PLBs) within 12 weeks. Clumps of PLBs were subcultured for 8 weeks on fresh medium with the same nutrient composition and cut into eight pieces and subcultured on Gelrite-gelled MS medium with 2% (w/v) sucrose + 10% (v/v) coconut water + 2 g l^{-l} peptone + 150 mg l^{-l} L-glutamine + 1 g l^{-l} activated charcoal, where each clump fragment of the PLBs produced an average of 220 PLBs within 8 weeks. After a further four weeks of subculture the PLBs were found to have enlarged with leafy shoots and new PLBs induced from the base of the old leafy bodies. Plantlet development from leafy shoots was achieved on ½MS medium supplemented with 2 g l^{-l} peptone, 2% (w/v) sucrose, 10% (v/v) CW and 1 g l^{-l} activated charcoal, where 100% of explants developed into plantlets with roots within 8 weeks. The addition of 50 g l^{-1} banana pulp in the medium enhanced the number and length of roots. Within the first 41 weeks after initiation of culture 1260 plantlets as well as a huge amount of PLBs were induced from a single explant (TCL) section. Repeating the subculture of PLBs on proliferation medium and culturing leafy shoots on plantlet regeneration medium could produce 500,000 plantlets every 32 weeks.

R.V. Sreedhar, L. Venkatachalam, K. Roohie, N. Bhagyalakshmi (India) Molecular Analyses of *Vanilla planifolia* Cultivated in India using RAPD and ISSR Markers (pp 29-33)

ABSTRACT

Original Research Paper: The natural "vanilla flavour" obtained from the pods of *Vanilla planifolia* is the largest flavouring ingredient in food industry. In order to evaluate the usefulness of genetic markers such as RAPD and ISSR for assessing the diversity among clones of *V. planifolia* and to create a database for the available germplasm, we investigated the existing population cultivated in India. The genetic diversity among 25 accessions collected from 13 major locations was studied. Forty random amplified polymorphic DNA (RAPD) and 11 inter-simple sequence repeats (ISSR) primers resulted in 326 scorable bands ranging in size from 200 bp to 2800 bp and 83 scorable bands from 200 bp to 2500 bp, respectively. Banding pattern among the different samples collected within accessions was similar indicating that the morphological difference observed within accession had no genetic background. On the other hand, molecular analysis among different accessions from different locations also yielded identical PCR band profiles in both RAPD and ISSR analysis. These results clearly indicate that *V. planifolia* cultivated in India is likely to share the same genetic background and therefore, the genetic diversity is extremely low. Therefore we propose other biotechnological approaches to induce genetic variations to improve the agronomical traits.

Number 2

Davina Chai, Hao Yu (Singapore) Recent Advances in Transgenic Orchid Production (pp 34-39)

ABSTRACT

Invited Mini-Review: With their attractive flowers and wildly diverse forms, orchids are a mainstay of the global floricultural trade. New varieties with improved floral characters and extended vase-life are continuously being generated by classical breeding techniques, though the selection process is necessarily time-consuming and remains a major obstacle to the rapid production of commercially-valuable orchids. Manipulation of specific floral traits and other desirable characteristics such as flowering time and vase-life by conventional sexual hybridization methods is also practically impossible. In the past decade, researchers have used molecular genetic techniques to revolutionize orchid biotechnology, which typically employs gene transformation systems coupled with rapid selection and regeneration methods for the production of new orchid varieties with

the desired traits. Here, we summarize recent findings in transgenic orchid production, particularly with regards to improved transformation methods and the use of novel selectable markers.

Jen-Tsung Chen, Wee-Peng Gow, Wei-Chin Chang (Republic of China) Zygotic and Somatic Embryogenesis of *Phalaenopsis* (pp 40-43)

ABSTRACT

Invited Mini-Review: Tissue culture protocols for zygotic and somatic embryo induction of a popular economical important orchid, *Phalaenopsis*, are reviewed in this report. In *P. amabilis*, repetitive embryogenesis was achieved using seed-derived protocorm as explants. In *P. amabilis*, *P.* 'Little Steve' and *P.* 'nebula', direct somatic embryogenesis and subsequent secondary embryogenesis were induced from leaf explants. Factors including explant type, explant orientation, culture period, subculture period, light requirement, basal medium, medium composition and growth regulators for embryo induction are comprehensively described in this review.

Chi-Chu Tsai, Chang-Hung Chou (Taiwan) Molecular Phylogenetics of *Phalaenopsis* Taxa: An Updated Review (pp 44-50)

ABSTRACT

Invited Mini-Review: *Phalaenopsis* is one of the most popular and beautiful orchids, exhibiting an amazing flower morphology looking like moths. The *Phalaenopsis* species was first described by Linnaeus and was placed in the genus *Epidendrum* as *Epidendrum amabile* in 1753. Blume (1825) erected the genus *Phalaenopsis* and placed all of moth orchids into this genus. This genus was confused with its related genus, *Kingidium*, *Doritis*, *Polychilos*, for a long time. Sweet (1980) treated *Polychilos* as a synonym of *Phalaenospis*. Until recently, Christenson (2001) treated *Kingidium* and *Doritis* as synonym of *Phalaenopsis*, and divided into five subgenera, *Phalaenopsis*, *Polychilos*, *Parishianae*, *Proboscidioides* and *Aphyllae*. Molecular techniques are used to clarify the phylogeny of *Phalaenopsis* recently. First analyses of internal transcribed spacer (ITS) of ribosomal DNA supported that *Kingidium* and *Doritis*, *Polychilos* could be treated as a synonym of *Phalaenopsis* as suggested by Christenson (2001). However, the phylogeny of subgenera *Phalaenopsis*, *Polychilos*, *Aphyllae* was not supported nature groups. In addition, plastid DNAs, including *atp*B-*rbc*L intergenic spacer (IGS), *trn*L-F IGS, and *trn*L intron, were to clarify the molecular phylogeny of *Phalaenospis* since its maternal inheritance is separated from biparental inheritance of nuclear ITS of rDNA. Plastid DNAs also supported Christenson's treatment on generic level but not on subgeneric and sectional levels. Some incongruence between nuclear and plastid DNA are found. This is usually ascribed to a number of biological effects, such as hybridization, introgression, horizontal gene transfer, and lineage sorting. Furthermore, molecular data was also revealed the phylogeny of species complex and natural *Phalaenospis* hybrid as well as the identification of closely related *Phalaenopsis* cultivars.

Changmei Song, Jichuan Kang, Xiangbiao Ji, Chunhua Fu, Xiaopeng Wen, Lifen Yu, Guang Qiao (China) Efficient Plant Regeneration and Genetic Fidelity Assessment of *In Vitro*-derived Plants of *Dendrobium nobile* – an Endangered Medicinal and Ornamental Herb (pp 51-55)

ABSTRACT

Original Research Paper: *Dendrobium nobile* is of great interest because of its high value as a floricultural commodity and a kind of elite herbage plant in Chinese traditional medicine. An efficient *in vitro* propagation system via protocorm-like body (PLB) induction has been developed using one-year-old axillary node explants obtained from field-grown plants in the present research. The excised nodal buds began to sprout on Murashige and Skoog (MS) basal medium containing 4.0 μM 6-benzyladenine (BA) and 2.0 μM naphthalene acetic acid (NAA) after 20~25 days in culture. Axenic secondary explants were then transferred into new MS medium to which 4.0 μM NAA was added for callus induction. The highest callus differentiation index (CDI) was scored from MS medium containing 1.5 μM gibberellic acid (GA). Subsequently, the well-developed PLBs were transferred into a new MS medium containing 4.0 μM BA and 2.0 μM NAA, from which vigorous shoots were obtained on the 60th day after transfer. The regenerated shoots rooted well in ½MS basal medium when NAA (2.5~5.0 μM) and 3-indole butyric acid (IBA, 2.5~5.0 μM) were added. Genetic fidelity of callus-derived shoots was assessed by performing PCR using 38 arbitrary primers. It was observed that all the primers displayed the same profiles between the stock plant and the randomly-selected shoots regenerated from the callus that had been subcultured within 15 cycles in MS medium containing 4.0 μM NAA.

Doris C. N. Chang, L. C. Chou, K. C. Lee (Taiwan) New Cultivation Methods for *Anoectochilus formosanus* Hayata (pp 56-60)

ABSTRACT

Original Research Paper: *Anoectochilus formosanus* Hayata is a beautiful orchid plant with ornamental and medicinal use in Taiwan. Growth of *A. formosanus* Hayata seedlings *in vitro* or *ex vitro* could be highly enhanced by the inoculation of orchid mycorrhizal fungi R02 or R04. R02 is a binucleate *Ceratobasidium* sp. AG-A while R04 is known as multinucleate *Rhizoctonia solani*, AG-6. In this orchid, we observed a tolypophagy-type of mycorrhizal infection with hyphae coil and pelotons formed in the cortex cells of roots. This orchid was very susceptible to *Fusarium*, *Pythium* spp. and mites during *ex vitro* growth. So how to grow this orchid without applying pesticides and insecticides became our prime concern. We developed a plastic bag cultivation method (PBCM) to grow *A*. *formosanus* plants in a 5 inch plastic pot with fermented bark compost as the growth medium, and every two of the 5 in. pots were covered with an OPP transparency plastic bag (9.2 × 7.5 × 30.0 cm) for protection of water loss, disease and mite infection. Thereafter, plants with PBCM were placed under benches in greenhouses or trees, until 6-8 months later for harvesting. No more water or fertilizer was needed and minimum labor was required during cultivation. This PBCM had been proved to be a very effective and low cost cultivation method for producing fungicide- and insecticide-free *A*. *formosanus* for medicinal use.