

ABSTRACT BOOK



The First International Orchid Symposium

National Museum of Natural Science
Taichung, Taiwan

January 12-15, 2010

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Welcome to Taiwan

I sincerely welcome all of you to the International Orchid Symposium under the auspices of the International Society for Horticultural Science (ISHS) held on January 12-15, 2010. With the fine climate, Taiwan is famous for the breeding and mass production of *Phalaenopsis* and other orchid genera, such as *Oncidium*, *Cymbidium* and *Paphiopedilum*. The National Museum of Natural Science is located in downtown of Taichung City; the hotels, restaurants, shopping stores, parks, etc. are within easy walking distance.

The main themes of this symposium contain orchid breeding and genetics, ecology, biotechnology, propagation, production, post-harvest, and marketing. The symposium consists of three-day sessions including the invited lectures by renowned orchid scientists and oral/poster presentations from the world, and one-day technical tour. The submissions in this symposium will be given the opportunity to publish papers in *Acta Horticulturae*. During the technical tour, the members will visit the commercial orchid production in the central and southern Taiwan.

We appreciate the great efforts of the members of Scientific Committee and Organizing Committee. We are also grateful for the financial supports given by the Council of Agriculture, the National Science Council, and the National Museum of Natural Science. The dedication and efforts of all committee members are extremely appreciated.

We hope all of you will enjoy the fruitful symposium and your stay in Taiwan.

A handwritten signature in black ink that reads "Tsai-mu Shen". The signature is written in a cursive, flowing style.

Tsai-Mu Shen
Chair of the Organizing Committee

Dear Colleagues,

Welcome to Taiwan! The conveners and members of the organizing committee are proud to present the first International Orchid Symposium under the auspices of the International Society for Horticultural Science (ISHS). Taiwan is home to large-scale commercial production of orchids, particularly *Phalaenopsis* and related genera, as well as numerous scientists focused on orchid research.

The symposium is being held at the National Museum of Natural Science in Taichung from January 12 to 15, 2010. We're excited to have three days of oral and poster presentations and a one-day technical tour. Many of the presenters have submitted manuscripts for subsequent publication in a volume of *Acta Horticulturae*. We appreciate the efforts of the members of the scientific committee to help ensure scientific rigor in the publications, as well as clear communication through proper use of the English language.

This symposium covers a wide range of orchid research topics including (but not limited to): orchid breeding and genetics, ecology, biotechnology, propagation, production, post-harvest, and marketing. This program book includes all of the abstracts that were submitted and accepted and summary information about the symposium's activities.

We sincerely appreciate Taiwanese financial support provided by the Council of Agriculture, the National Science Council, and the National Museum of Natural Science. In addition, members of the Taiwan Society for Horticultural Science and the Taiwan Seed Improvement and Propagation Station have been of great assistance in organizing the symposium. Finally, we would like to acknowledge Mr. Norman Fang and the American Orchid Society, who will provide honorariums for the best presentations.

We hope you have a productive and informative symposium and enjoy your stay in Taiwan.



Yung-I Lee
Co-Convenor



Erik Runkle
Co-Convenor

Organization

The symposium is organized by
International Society for Horticultural Science (ISHS)
Section Ornamental Plants
Orchid Working Group

Taiwan Society for Horticultural Science
National Museum of Natural Science
Taiwan Seed Improvement and Propagation Station



The symposium is sponsored and supported by
Council of Agriculture, Taiwan, ROC
National Science Council, Taiwan, ROC
Department of Horticulture, National Chiayi University
Department of Horticulture, National Chung Hsing University
Department of Horticulture, National Taiwan University
Department of Plant Industry, National Pingtung University of Science and Technology
Department of Horticulture, National Ilan University
Department of Life Science, National Cheng Kung University
Department of Life Science, National University of Kaohsiung
Agricultural Biotechnology Research Center, Academia Sinica
Agricultural Research Institute
Taiwan Orchid Growers Association
Taiwan Orchid Breeders Association
American Orchid Society

Co-conveners

Yung-I Lee (National Museum of Natural Science, Taiwan)
Erik Runkle (Michigan State University, USA)

Scientific Committee

Joseph Arditti (University of California, USA)
Choy-Sin Hew (Nanyang Technological University, Singapore)
Syoichi Ichihashi (Aichi University of Education, Japan)
Yung-I Lee (National Museum of Natural Science, Taiwan)
Kee-Youep Paek (Chungbuk National University, Korea)
Erik Runkle (Michigan State University, USA)
Wagner Vendrame (University of Florida, USA)
Yin-Tung Wang (Texas A&M University, USA)
Edward Yeung (University of Calgary, Canada)
Tomohisa Yukawa (National Museum of Nature and Science, Japan)

Organizing Committee

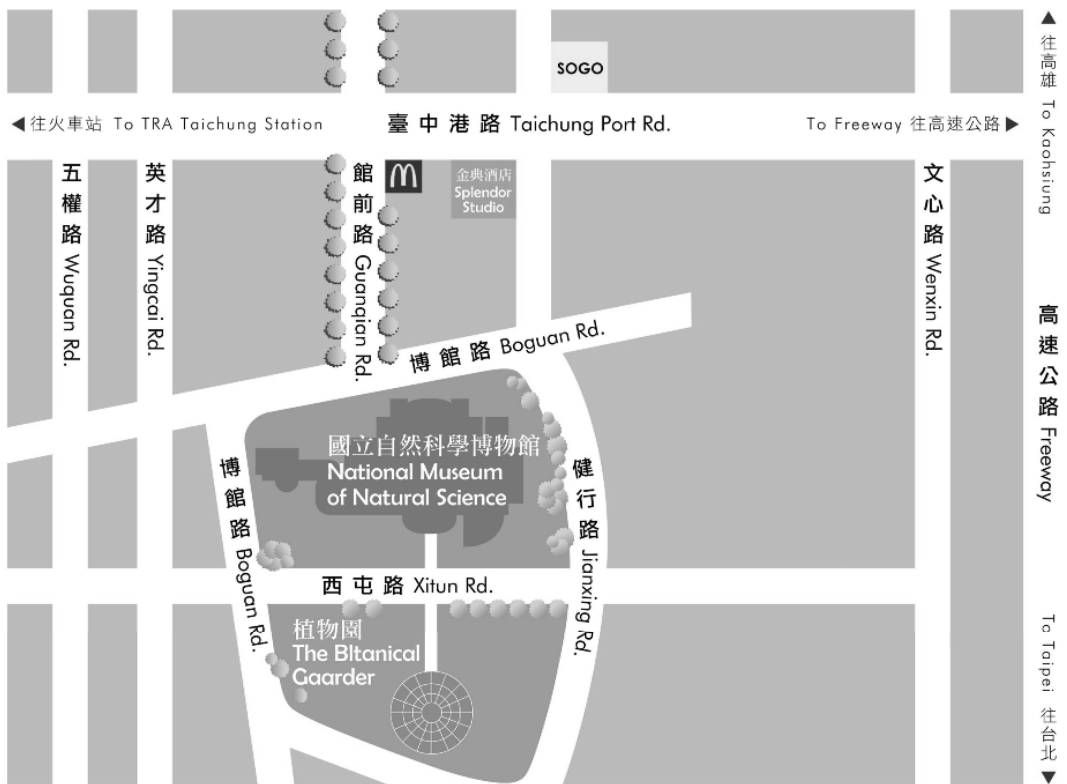
Tsai-Mu Shen (National Chiayi University, Taiwan) Chair
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Tzong-Shyan Lin (National Taiwan University, Taiwan) Vice Chair
Kuo-Hsiung Chen (Taiwan Seed Improvement and Propagation Station, Taiwan)
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Hong-Hwa Chen (National Cheng Kung University, Taiwan)
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Shui-Ho Cheng (Taoyuan District Agricultural Research and Extension Station, Taiwan)
Ming-Che Shih (Agricultural Biotechnology Research Center, Academia Sinica)
Ming-Tsair Chan (Agricultural Biotechnology Research Center, Academia Sinica)
Tsang-Yu Lee (Taiwan Orchid Growers Association)
Tin-Hsung Lee (Taiwan Orchid Breeder Association)

**I International Orchid Symposium
Program at a Glance**

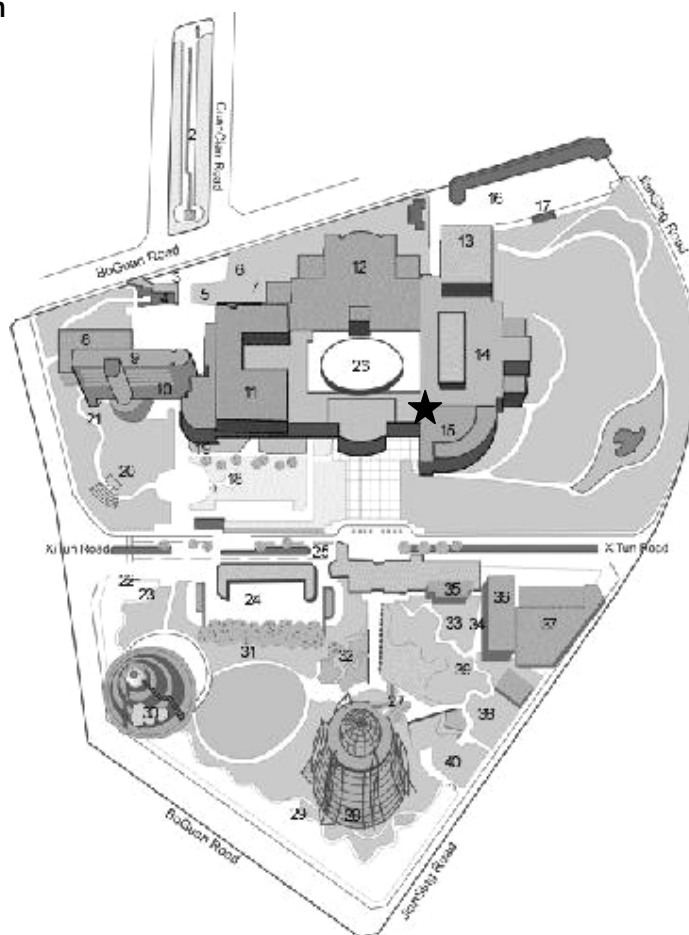
Date	Morning	Lunch	Afternoon	Evening
11 Jan (Mon)			Registration (16:00 – 20:00)	
12 Jan (Tue)	Registration (08:00 – 12:00) Opening remarks (08:30 – 09:00) Oral session I (09:00 – 10:30) Break, group photo (10:30 – 10:50) Oral session II (10:50 – 12:20)	12:20 – 13:20	Oral session III (13:20 – 14:40) Poster session I (14:20 – 15:20) Oral session IV (15:20 – 16:20)	Welcome reception (18:30 – 20:30)
13 Jan (Wed)	Oral session V (08:30 – 10:20) Break (10:20 – 10:40) Oral session VI (10:40 – 12:20)	12:20 – 13:20	Oral session VII (13:20 – 15:00) Poster session II (15:00 – 16:00) Orchid working group business meeting (16:00 – 16:30)	
14 Jan (Thu)	Oral session VIII (08:40 – 10:30) Break (10:30 – 10:50) Oral session IX (10:50 – 12:20)	12:20 – 13:20	Oral session X (13:20 -15:00) Closing Remarks (15:00 – 15:30)	Farewell party (17:00 – 19:00)
15 Jan (Fri)	Technical tour (08:00 – 18:00)			

Map

Location of National Museum of Natural Science



Aerial View of the Museum



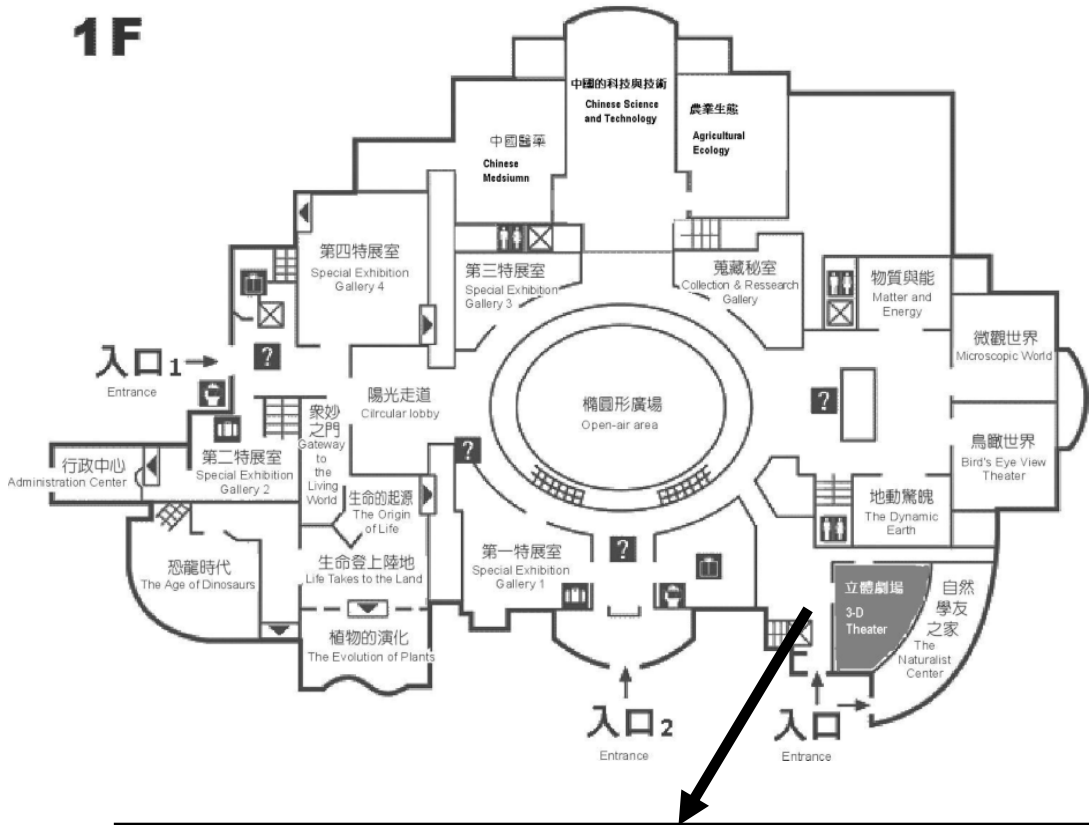
- 01. Windmill
- 02. The Path of Evolution
- 03. Sundial
- 04. Luoh-Shu
- 05. Ho-Twu
- 06. Canadian Red Deer
- 07. Restrooms
- 08. Space Theater
- 09. Science Center Ecological Area
- 10. Administration Building
- 11. Life Science Hall
- 12. Human Cultures Hall
- 13. Information Building
- 14. Global Environment Hall
- ★ 15. 3-D Theater
- 16. Parking Lot
- 17. Restrooms
- 18. Reflecting Scope
- 19. Prehistoric Garden
- 20. DNA Double Helix

- 風車
- 演化史步道
- 日晷
- 洛書
- 河圖
- 加拿大紅鹿
- 廁所
- 太空劇場
- 科學中心
- 行政中心
- 生命科學廳
- 人類文化廳
- 資訊大樓
- 地球環境廳
- 立體劇場
- 停車場
- 廁所
- 視覺反射筒
- 暴龍與五角龍
- DNA 雙股螺旋

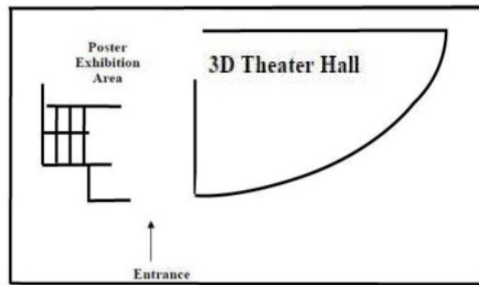
- 21. Paradox
- 22. Restrooms
- 23. Bus Drivers' Lounge
- 24. Parking
- 25. Water Clock
- 26. Oval Plaza
- 27. Birdwing Butterfly
- 28. Tropical Rainforest Greenhouse
- 29. Northern Lowlands
- 30. Central Lowlands Ecological Area
- 31. Southern Lowlands Ecological Area
- 32. Monsoon Forest Ecological Area
- 33. Coral Atoll Ecological Area
- 34. Native Lianas and Legumes
- 35. Special Exhibition Room
- 36. Research and Education Center
- 37. Nursery
- 38. Orchid Island Ecological Area
- 39. Littoral Forest Ecological Area
- 40. Taitung Cycads

- 不規則中的規則
- 廁所
- 司機休息室
- 停車場
- 水鐘
- 數與形
- 珠光鳳蝶
- 熱帶雨林溫室
- 北部低海拔區
- 中部低海拔區
- 南部低海拔區
- 季風雨林區
- 隆起珊瑚礁區
- 藤蔓展示區
- 特展室
- 研究教育中心
- 苗圃區
- 蘭嶼區
- 海岸林區
- 台東蘇鐵區

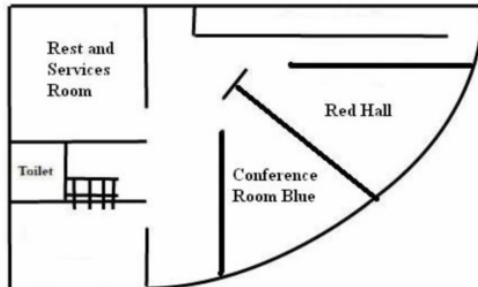
1F



**The 3-D Theater Building,
1F**



**The 3-D Theater Building,
B1 floor
Seminar Room
Conference Room Blue**



Registration

The registration and information desk is located at **the B1 floor of 3-D Theater Building**. Participants who have not paid prior to coming to the Congress will be required to do so at the registration. Cash payments can only be accepted in New Taiwan Dollars or US Dollars, Credit card payments are accepted. The desk will be opened during the following hours:

Monday, January 11 16:00-20:00

Tuesday, January 12 8:00-12:00

For security purposes, all participants must wear their name badge in all social functions and for entrance to the congress venue and attend the scientific sessions.

Presentation Guidelines

Oral Presentation: For oral presenters, please submit the presentations at the audio-visual desk at your earliest convenience. There will be facilities for you to check your presentations before hand. Power point presentation is encouraged. The computers are set for Microsoft Windows system. Submitted oral presentations are a maximum of 15 minutes in length with 5 minutes for questions and discussion. Invited speakers are provided with 25 minutes for their presentation and 5 minutes for questions and discussion. The chairperson of each session will work with the timekeeper to ensure we stay on time.

Poster Presentation: The poster board size is 90 x 120 cm. All posters must be displayed on the designated poster boards in the poster hall before 12:00 on Tuesday, January 12. Poster presenters are asked to stand next to their poster during the last 30 minutes of their session on Tuesday, January 12 (14:40-15:40) or Wednesday, January 13 (15:00-16:00). All posters must be removed by 15:30 on Thursday, January 14.

Social Events

Welcoming Reception: The reception will be the traditional Chinese style to be held at Banquet Hall, 13F, the Splendor Hotel on Monday, January 11 from 18:30 – 20:30.

Farewell Party: The dinner will be buffet style to be held at the Tropical Rainforest Greenhouse on Thursday, January 14 from 17:00 – 19:00. All participants are invited to attend the Welcoming Reception.

Technical Tour

The full day technical tour is to visit the commercial orchid growers and the floriculture research center in the central and southern Taiwan. Lunch is included. Tour buses will depart from the Splendor Studio.

Friday, January 15

- 8:00 Departure from the Splendor Studio.
- 8:30 Arrival at In-Charm Orchids Laboratory which is a specialized *Paphiopedilum* Grower in Taiwan. (Taichung County). In In-Charm Orchids Laboratory, participants will visit the breeding works and the production of slipper orchids.
- 10:00 Departure from In-Charm Orchids Laboratory.
- 11:30 Arrival at Ching Hua Orchids. In Ching Hua Orchids, a large variety of orchids is produced and grown in the greenhouses, including *Phalaenopsis*, *Cattleya*, *Paphiopedilum*, *Dendrobium*, *Angraecoids*, and *Bulbophyllum*. (Tainan County)
- 12:30 Lunch (-13:30) at the restaurant of Taiwan Orchid Plantation (Tainan County).
- 13:30 Visit the greenhouses at Taiwan Orchid Plantation (TOP). The establishment of TOP combines industrial basis and biological science and technology resources. In TOP, participants will visit the modern system of *Phalaenopsis* plants cultivation and the cut flower production.
- 15:00 Departure from Taiwan Orchid Plantation.
- 16:00 Arrival at Floriculture Research Center, ARI, COA (Yunlin County). This center has been founded for breeding and environmental control researches for oriental plants. In this center, participants will visit the breeding and cultivation works of orchids.
- 17:00 Departure from Floriculture Research Center.
- 18:00 Return to the Splendor Studio.

ISHS International Orchid Symposium
January 11-15, 2010

Symposium Program

Oral presentations

Submitted oral presentations are a maximum of 15 minutes in length with 5 minutes for questions and discussion. Invited speakers are provided with 25 minutes for their presentation and 5 minutes for questions and discussion. The chairperson of each session will work with the timekeeper to ensure we stay on time. Thank you for your cooperation.

Poster presentations

Poster presenters are asked to stand next to their poster during the last 30 minutes of their session.

<u>Monday, 11 January 2010</u>		
16:00 - 20:00	Registration	
<u>Tuesday, 12 January 2010</u>		
8:00 - 12:00	Registration	
8:30 - 9:00	Welcome and Opening Remarks	
Oral session I: Orchid propagation, breeding, and genetics		
Chairpersons: Fure-Chyi Chen and Mei-Chu Chung		
9:00 - 9:30 12-V-1	From Goats and Thrushes to Test Tubes: Development of Sexual and Clonal Propagation Methods for Orchids (Invited)	Joseph Arditti
9:30 - 10:00 12-V-2	Polyploidy and Variety Improvement of <i>Phalaenopsis</i> Orchids (Invited)	Wen-Huei Chen
10:00 - 10:30 12-V-3	Chromosome Research for Orchid Breeding (Invited)	Mikio Aoyama
10:30 - 10:50	Break; group photo	
Oral session II: Orchid breeding and genetics		
Chairpersons: Wen-Huei Chen and Ming-Che Shih		
10:50 - 11:20 12-V-4	Pollen Meiotic Behavior in Relation to <i>Phalaenopsis</i> Breeding (Invited)	Fure-Chyi Chen
11:20 - 11:40 12-O-1	Breeding Barriers in Red <i>Phalaenopsis</i> Orchids	Tsai-Mu Shen
11:40 - 12:00 12-O-2	Phylogenetic Analysis in the Genera <i>Phaius</i> and <i>Cephalantheropsis</i> Using <i>rpl32-trnL</i> Marker	Shui-Ho Cheng

12:00 - 12:20 12-O-3	Chloroplast DNA Barcoding in <i>Spathoglottis</i> Species for Genetic Conservation	Florence C. Ginibun
12:20 - 13:20	Lunch	
Oral session III: Orchid ecology		
Chairpersons: Tsai-Mu Shen and Yin-Tung Wang		
3:20 - 13:50 12-V-5	Ecophysiological Traits of <i>Paphiopedilum</i> and <i>Cypripedium</i> (Invited)	Hong Hu
13:50 - 14:20 12-V-6	<i>Ex situ</i> Orchid Conservation – a Case Study from the Singapore Botanic Gardens (Invited)	Tim Wing Yam
14:20 - 15:20	Poster session I (odd numbers)	
Oral session IV: Orchid ecology		
Chairpersons: Shui-Ho Cheng and Tim Wing Yam		
15:20 - 15:40 12-O-4	Mechanisms for Recruitment and Population Growth in <i>Nervilia nipponica</i> , an Endangered Orchid in Japan	Stephan Gale
15:40 - 16:00 12-O-5	Mycorrhiza Colonization Patterns in <i>Bletia</i> species in a Natural Forest Reserve in Michoacán, Mexico	María de los Ángeles Beltrán-Nambo
16:00 - 16:20 12-O-6	Evaluation of Growth and Flowering of Terrestrial Native Chilean orchids: <i>Bipinnula volkmannii</i> Kraenzl. , <i>Gavilea glandulifera</i> Poepp. , <i>Chloraea magellanica</i> Hook. f. , and <i>C. philippii</i> Reichb. F.	Ximena Calderón Baltierra
18:30 - 20:30	Welcome reception	
Wednesday, 13 January 2010		
Oral session V: Orchid production		
Chairpersons: Chien-Young Chu and Kee Yoeup Paek		
8:30 - 9:00 13-V-1	Environmental and Hormonal Regulation of Flowering in <i>Phalaenopsis</i> Orchids (Invited)	Erik Runkle
9:00 - 9:30 13-V-2	Control of Spiking in <i>Phalaenopsis</i> by Application of NH ₄ -N and Plant Growth Regulators (Invited)	Syoichi Ichihashi
9:30 - 10:00 13-V-3	<i>Phalaenopsis</i> Mineral Nutrition (Invited)	Yin-Tung Wang
10:00 - 10:20 13-O-1	Benzyladenine Induced Flowering of Potted Noble <i>Dendrobium</i> Red Emperor 'Prince' Orchid Plants	William Sakai
10:20 - 10:40	Break	

Oral session VI: Orchid production		
Chairpersons: Pung-Ling Huang and Syoichi Ichihashi		
10:40 - 11:00 13-O-2	Effect of Bulb Size and Gibberellin Application on the Bulb Growth of <i>Pleione formosana</i> Hayata	Mei-Chun Lu
11:00 - 11:20 13-O-3	Effects of Emerging Shoot Size, Temperature, and Benzyladenine on Growth and Flowering of <i>Zygopetalum</i> Redvale 'Fire Kiss' orchids	Matthew Blanchard
11:20 - 11:40 13-O-4	Evaluation of Growing Media Components for <i>Phalaenopsis</i>	Suzanne Amberger-Ochsenbauer
11:40 - 12:00 13-O-5	Growth and Flowering of <i>Oncidium</i> in an Artificial Textile Fiber Substrate	Ting-En Dai
12:00 - 12:20 13-O-6	Improvement of Inflorescence Quality by Calcium Supplement Nutriculture on <i>Oncidium</i> Gower Ramsey	Jung-Hua Hsu
12:20 - 13:20	Lunch	
Oral session VII: Orchid production, postharvest, and marketing		
Chairpersons: Erik Runkle and Ruey-Song Lin		
13:20 - 13:40 13-O-7	Crassulacean Acid Metabolism in <i>Phalaenopsis aphrodite</i> subsp. <i>formosana</i> during Different Developmental Stages	Wendy Yang
13:40 - 14:00 13-O-8	Photosynthetic Midday Depression in <i>Oncidium</i> alliance	Yao-Chien Alex Chang
14:00 - 14:20 13-O-9	Process Management and Marketing for Orchid Firms	Ching-Chun Shih
14:20 - 14:40 13-O-10	Purchasing and Non-purchasing Decisions in Orchid Consumption in the Taiwan Market	Nai-Hau Chen
14:40 - 15:00 13-O-11	Value Chain Dynamics in the Taiwan Orchid Industry	Sherrie Wei
15:00 - 16:00	Poster session II (even numbers)	
	Dinner on own	
Thursday, 14 January 2010		
Oral session VIII: Orchid micropropagation and seed germination		
Chairpersons: Sek-Man Wong and Yao-Chien Alex Chang		
8:40 - 9:10 14-V-1	Establishment of Large-scale Micropropagation System of <i>Anoectochilus formosanus</i> in Bioreactors (Invited)	Kee Yoeup Paek

9:10 - 9:30 14-O-1	The Development of Orchid Production in Israel	Duby Wolfson
9:30 - 9:50 14-O-2	Pollination, Seed Development and <i>In Vitro</i> Germination of <i>Cymbidium sinensis</i> 'Huang Dao'	Chen Chang
9:50 - 10:10 14-O-3	Effect of Banana Extracts on PLBs Proliferation and Growth of Different Orchid Hybrids	Maziah Mahmood
10:10 - 10:30 14-O-4	Micropropagation of a Rare Orchid <i>Dendrobium gratiosissimum</i> Rchb.f. Using Thin Cell Layers	Niramol Rangsayatorn
10:30 - 10:50	Break	
Oral session IX: Orchid biotechnology		
Chairpersons: Joseph Arditti and Hong-Hwa Chen		
10:50 - 11:20 14-V-2	Genetic Transformation of Orchids (Invited)	Masahiro Mii
11:20 - 11:50 14-V-3	Studies on Synergism and Complementation of Long Distance Movement of <i>Cymbidium Mosaic Virus</i> RNA by <i>Odontoglossum Ringspot Virus</i> Coat Protein (Invited)	Sek-Man Wong
11:50 - 12:20 14-V-4	Decontamination of <i>Odontoglossum Ringspot Virus</i> and <i>Cymbidium Mosaic Virus</i> on Tools and Cultivation Environment of Orchids Using Culture Filtrate of <i>Streptomyces</i> spp. (Invited)	Chin-An Chang
12:20 - 13:20	Lunch	
Oral session X: Orchid biotechnology		
Chairpersons: Masahiro Mii and Chin-An Chang		
13:20 - 13:40 14-O-5	Regeneration and <i>Agrobacterium</i> -mediated Transformation of <i>Oncidium</i> and <i>Odontoglossum</i> with Ethylene Receptor Mutant Gene ETR1-1	Barbara Raffener
13:40 - 14:00 14-O-6	A Novel Functional Homodimer Geranyl Diphosphate Synthase for Scent Production in <i>Phalaenopsis bellina</i> (Orchidaceae) Flower	Yu-Yun Hsiao
14:00 - 14:20 14-O-7	Modification of <i>Phalaenopsis</i> Metabolism Through Genetic Engineering	Ying-Chun Chen
14:20 - 14:40 14-O-8	Molecular Mechanisms Underlying Orchid Floral Morphogenesis	Wen-Chieh Tsai
14:40 - 15:00 14-O-9	Molecular and Morphological Diversity in <i>Vanda tessellata</i> , an Epiphytic Orchid from the Eastern Ghats of India	Shaik Khasim
15:00 - 15:30	Closing Remarks	
17:00 - 19:00	Farewell party	

Friday, 15 January 2010	
8:00 - 18:00	Technical tour. Bus departs Spondor Studio hotel promptly at 8:45am
	Dinner on own

Poster sessions

12 (Tue) 14:40 – 15:40

- 12-P-1 Optimum Conditions for Seed Germination of *Phalaenopsis* 'Silky Moon'
A. Thongpukdee, C. Thepsithar and T. Rojanawong
- 12-P-2 Simple Media for Orchid Seed Germination
B. Ngampanya and W. Homla-aor
- 12-P-3 Effect of Flower Stalk Stage, Lateral Bud Position and 6-Benzyladenine Concentration on Micropropagation of *Phalaenopsis* Cultivars
Chin Yi Tsao, Uei Chern Chen, Min Tze Wu and Chi Ni Hsia
- 12-P-4 Effect of 6-Benzyladenine Concentration and Explant Types Derived from Flower Stalk on Micropropagation of *Phalaenopsis* Cultivar
C. Y. Tsao, U. C. Chen, M. T. Wu and C. N. Hsia
- 12-P-5 Effects of Organic Supplements in Local Fertilizer Medium on Growth and Development of *Phalaenopsis* 'Silky Moon' Protocorms
C. Thepsithar, A. Thongpukdee and N. Thongdeelert
- 12-P-6 Multiple Shoot Induction from Seed Derived Shoots of *Paphiopedilum* Hybrid
E. Nisayan, C. Thepsithar and A. Thongpukdee
- 12-P-7 Effect of Culture Vessel Type on *In Vitro* Propagation of *Aerides falcata* Lindl.
K. Kaewchum and N. Rangsayatorn
- 12-P-8 *In Vitro* Conservation of *Dendrobium draconis* Rchb. f., Under Minimal Growth Conditions.
N. Rangsayatorn and C. Jaiphet
- 12-P-9 Preliminary Study on Seed Germination of *Sirindhornia* (Orchidaceae) Endangered Orchid from Thailand
K. Srimuang and P. D. Eungwanichayapant, S. Watthana and N. Rangsayatorn
- 12-P-10 Micropropagation of Three Cultivars of *Cattleya* Hybrids through *In Vitro* Culture
Pinaki Sinha and M. Firoz Alam
- 12-P-11 Micropropagation of *Rhynchostylis retusa* (Lin.) Blume through *In Vitro* Culture and their Establishment in the Nursery
Shyamal K. Roy
- 12-P-12 *In Vitro* Asymbiotic Germination and Development of Chilean Native Terrestrial Orchids
Baltierra Ximena Calderón, Claudia Alonso and Manuel Monsálvez

- 12-P-13 Rapid Mass Propagation through Multiple Shoots Induction from Rhizome of Chinese Cymbidiums
Hui-Fang Chiang, Jia-Rong Lin, Kong-Shen Liu, Chien-Yuan Kao and Yu Chu
- 12-P-14 Chitosan Stimulates Growth of Micropropagated *Dendrobium* Plantlets
Piyada Tantasawat, Anakkaorn Wannajindaporn, Chowarit Chantawaree, Chutiporn Wangpunga and Athitaya Somtip
- 12-P-15 Effects of Culture Media and Capsule Maturity on Seed Germination of Intraspecific and Interspecific Crosses in the Genus *Calanthe*.
Shu-Jen Lee, Fang-shin Liao and Shui-Ho Cheng
- 12-P-16 Germination and Seedling Development of Some *Phalaenopsis* Species
Yung-I Lee, Ming-Chuan Chen and Chia-Ying Huang
- 12-P-17 A New Hybrid Genus *Chenara* (Orchidaceae) from the Cross between *Doritaenopsis* and *Paraphalaenopsis*
Chi-Chu Tsai and Hua-Ting Chuang
- 12-P-18 A New Hybrid Genus *Chouara* (Orchidaceae) from the Cross between *Angraecum* and *Doritaenopsis*
Chi-Chu Tsai and Hua-Ting Chuang
- 12-P-19 Comparison of Chloroplast Genomes from Two Native *Phalaenopsis* Orchid Species in Taiwan
Cheng-Fong Jheng, Ching-Chun Chang, Wen-Luan Wu and Tien-Chih Chen
- 12-P-20 Variation in DNA Content in the Cultivated *Lycaste* and Related Species
J. Kato, H. Shiota, T. Oguri, M. Abou and S. Ichihashi
- 12-P-21 Karyomorphological Observation on Some *Paphiopedilum* Hybrids
Y. I. Lee and M. C. Chung
- 12-P-22 Plant Regeneration from Callus Culture of an Endangered Orchid, *Geodorum densiflorum* (Lam.) Schltr.
Mei-Chun Lu
- 12-P-23 Sheath and Root Rot of *Phalaenopsis* Caused by *Fusarium solani* in Taiwan
J. F. Su, Y. C. Lee, C. W. Chen, T. F. Hsieh and J. H. Huang
- 12-P-24 The Role of Pollen Germination on Hybridization of *Oncidium*
Tung-Ming Tsai, Rung-Yi Wu, Keng-Chan Chuang and Ting-Fang Hsieh
- 12-P-25 Collection, Conservation and Utilization of Taiwan Native Orchid Germplasm
Rung-Yi Wu, Ting-Fang Hsieh, Ting-En Dai and Yen-Hsu Lai

- 12-P-26 Molecular Identification and DNA Fingerprint of *Phalaenopsis* Varieties with Plant Breeders Rights (PBR) in Taiwan Using Microsatellite Markers
Yi-Tzu Kuo, Yu-Ling Lee, and Wen-Luan Wu
- 12-P-27 Selection of Highly Informative Microsatellites (SSRs) for Genotyping of *Phalaenopsis* Cultivars
Yu-Ling Lee, Yi-Tzu Kuo and Wen-Luan Wu
- 12-P-28 Classification of *Paphiopedilum* Genus into Three Subgenera and Parental Identification of *P. delenatii* in Hybrids by ITS Molecular Marker
Yung-Wei Sun, Yu-Ju Liao, Yih-Horng Chang, Kuo-Shiung Chen and Fang-Sheng Wu
- 12-P-29 The Embryo Development and Seed Germination *In Vitro* of *Bulbophyllum fascinator*
Y. I. Lee and E. C. Yeung

13 (Wed) 15:00 – 16:00

- 13-P-1 The Effect of Packaging on Shelf-life of Cut *Dendrobium* Big White Jumbo
A. Uthairatanakij, S. Manuwong, Jitareerat and K. Obsuwan
- 13-P-2 Physiological Changes of Cut *Mokara* Hybrids Inflorescences
J. Sartpech, P. Jitareerat, A. Uthairatanakij and K. Obsuwan
- 13-P-3 Non-Persistent Virus Transmission in Orchids by *Periplaneta australasiae*
Carol Allen
- 13-P-4 Identification and Characterization of UDP-Glucosyltransferases for Major Flavonoid and Anthocyanin Synthesis in *Phalaenopsis* Orchids
Ching-Fen Wu, Hao-Yun Cheng, Wen-Chieh Tsai, Ping-Chung Kuo, Wen-Huei Chen and Hong-Hwa Chen
- 13-P-5 A First Survey of the Orchid (*Phalaenopsis equestris*) Genome Composition through BAC end Sequence Analysis
Yu-Lin Chung, Yi-Tzu Kuo, Yu-Ling Lee, Tien-Chih Chen, Chia-Chi Hsu, Wen-Chieh Tsai, Hong-Hwa Chen and Wen-Luan Wu
- 13-P-6 High-efficiency *Agrobacterium*-mediated Transformation of *Dendrobium* by Targeting Protocorms
D. P. Chin, W. Phlaetita and M. Mii
- 13-P-7 The Growth of *Oncidium* and *Phalaenopsis* is Influenced by CymMV and ORSV Inoculation
Han-Tsu Shen, Yung-Wei Sun, Yu-Ju Liao, Tso-Chi Yang and Kuo-Hsiung Chen

- 13-P-8 Physiology of the Flowering in Commercial Varieties of Chilean Orchid of Cold Climates
J. Delatorre, X. Calderón, P. Espinoza, S. Espinoza, P. Salinas, A. Villacorta and X. Calderón
- 13-P-9 Influence of Foliar Chitosan Sprays on Growth of *Mokara* and *Phalaenopsis* Seedlings
K. Obsuwan, K. Sawangsri and A. Uthairatanakij
- 13-P-10 The Application of Chitosan for Induce *In Vitro* Growth of *Rhynchosstylis gigantea* Protocorms
K. Obsuwan, S. Yudee and A. Uthairatanakij
- 13-P-11 Effect of Chitosan Concentration on *In Vitro* Growth of *Dendrobium* Hybrid Seedlings
K. Obsuwan, K. Sawangsri, S. Ukong and A. Uthairatanakij
- 13-P-12 Gamma Irradiation Affecting Deterioration of *Dendrobium* Sonia Bom#17
M. Buanong and S. Kanlayanarat
- 13-P-13 Preservative Solution Delays Deterioration of *Dendrobium* Sonia Bom#17 after Gamma Irradiation
M. Buanong and S. Kanlayanarat
- 13-P-14 Phenylalanine Ammonia –Lyase (PAL) Activity of Selected *Dendrobium* Orchids
N. Nisha and M. Maziah
- 13-P-15 High-Performance Liquid Chromatography Profiling of Pigments from *Phalaenopsis* Hybrids and Their Contribution to Antioxidant and Antityrosinase Activities
P. C. Kuo, G. F. Chen, M. L. Yang and T. S. Wu
- 13-P-16 Effects of Fertilizer Formulae on the Flowering of *Doritaenopsis* I-Hsin Madame in the Gradational Nutrition Management
K. H. Chang, R. Y. Wu, and T. F. Hsieh
- 13-P-17 Induction of Plant Disease Defence and Growth of *Dendrobium* 'Eia Sakul' by Chitosan Treatments
S. Sakornyen, A. Uthairatanakij and P. Jitareerat
- 13-P-18 Cloning and Functional Characterizations of Sesquiterpene Synthase in *Phalaenopsis equestris* S82-159
Ya-Ping Yang, Yu-Yun Hsiao, Wen-Chieh Tsai, Chia-Ying Li, Tian-Shung Wu, Wen-Hui Chen, Mei-Fen Jeng and Hong-Hwa Chen
- 13-P-19 Study of MYB Factors Involved in the Regulating Fragrance Biosynthesis in *Phalaenopsis bellina*
Yu-Chen Chuang, Yu-Yun Hsiao, Wen-Chieh Tsai and Hong-Hwa Chen

- 13-P-20 Characterization of *AGL6*-like Genes Involved in Perianth Development of Orchids
Yun-Ru Luo, Chih-Chin Cheng and Hong-Hwa Chen
- 13-P-21 Identification and Characterization of *SEP*-like Genes in *Phalaenopsis*
Zhao-Jun Pan, Wen-Chieh Tsai, Wen-Huei Chen and Hong-Hwa Chen
- 13-P-22 Postharvest Performance of White crane Orchid (*Calanthe triplicate*) Cut Flower in Taiwan
Tsutsuen Wang, Jiayu Tsai, Shujen Lee, Fangshin Liao and Shuiho Cheng
- 13-P-23 A New Technical System with Higher Energy Efficiency, Higher Facility Utility and Automatic Operation for *Phalaenopsis* Spiking Seedling Production
Y.H. Chen, S.S. Wang, D.L. Lin, J.J. Cheng, Y.T. Chang and C.S. Han
- 13-P-24 A Preliminary Study of the Effect of Ultraviolet Radiation on Visible Flower Coloration in Two Orchids
W.S. Sakai, E. Band and J. Edward
- 13-P-25 Analysis of the Mitochondrial Genome from *Phalaenopsis equestris*, a Native Species in Taiwan
Yu-Jhang Liu, Wen-Luan Wu, Yu-Lin Chung, Yueh-Long Chang and Ching-Chun Chang

Invited Lectures

12-V-1

From Goats and Thrushes to Test Tubes: Development of Sexual and Clonal Propagation Methods for Orchids

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Keywords: asymbiotic germination, explant, *in vitro* culture, orchid seeds, symbiotic germination, tissue culture

Abstract

Many far-fetched theories were proposed for the origin of orchids because their seeds were not seen or recognized until about 500 years ago. Nearly four centuries passed from the time orchid seeds were first seen and the development of a practical asymbiotic method for their germination. Tissue culture based micropropagation methods were developed not long after that. Prof. Georges Morel is generally and erroneously credited as being the discoverer of orchid micropropagation, but the first to propagate orchids *in vitro* orchid propagation were Dr. Gavino Rotor in 1949 and the nurserman Hans Thomale in 1956.

12-V-2

Ploidy and Variety Improvement of *Phalaenopsis* Orchids

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Keywords: *Phalaenopsis* orchids, polyploidy, chromosome doubling, variety improvement, breeding, nuclear DNA content, germplasm development

Abstract

In the breeding history of *Phalaenopsis* orchids, polyploidy played an important role in the variety development of the orchid. The polyploids, such as *P. amabilis*, *P. Be Tris*, *P. Doris* etc. were the ancestors of many popular hybrids. The 'Big White Flower' hybrids of *Phalaenopsis* common in the market are tetraploids. However, analysis of the pedigree of this group of orchids indicated that the genetic base of these hybrids was quite narrow. On the other hand, the native species of *Phalaenopsis* are diploids. Therefore, it is difficult to transfer their favorable genes to the advanced commercial tetraploid hybrids because of the difference in the ploidy levels. In addition, it was reported that the chromosome sizes varied tremendously among different species of *Phalaenopsis*. Hybrids developed from the crosses between these species were usually sterile due to the problem of genome homology. Chromosome doubling is the method used to restore the fertility among the progeny of these hybrids. Due to the above reasons, a research project was initiated since 2004 to develop simple and effective methods to detect the nuclear DNA content by flow-cytometry and to double the chromosome of *Phalaenopsis* species and varieties by the use of tissue culture without application of anti-microtubule agents. A survey of the nuclear DNA content of the native species and some of the related hybrids of *Phalaenopsis* was carried out by employing flow-cytometry in several germplasm banks and orchid nurseries in Taiwan. Based on this information, *Phalaenopsis* species were grouped into low, medium and high nuclear DNA content which reflects the sizes of chromosome of the species. This will provide useful information to orchid breeders for the selection of parental varieties for hybridization. Through chromosome doubling techniques, a conversion program to develop a series of tetraploid native species of *Phalaenopsis* is in progress. This project will provide a wide range of tetraploid germplasm matching the ploidy level of the commercial hybrids in order to accelerate the breeding of novel varieties of *Phalaenopsis* for the market. With the upgrading of the breeding program to the polyploid level in full-scale, the production of a wide range of new hybrids of *Phalaenopsis* is expected to create a significant impact on the orchid industry in the coming years.

12-V-3

Chromosome Research for Orchid Breeding

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Keywords: orchid, cytogenetics, chromosome, polyploidy, breeding

Abstract

The documented chromosome numbers and karyotypes made by detail observations of mitotic chromosomes clarify the interspecific and intergenetic relationships in orchid taxonomy. The polyploidy investigations and genome analysis made by observations of chromosome behavior in meiosis contribute to efficient breeding. In this paper, some cytogenetical researches on orchid breeding are summarized as follows: 1. Cytogenetical evidence of intergeneric hybrids involving *Cymbidium*. 2. Relationship between microspore morphology and sectional taxonomy in *Phalaenopsis* F₁ hybrids. 3. Chromosome counting in *Phalaenopsis* hybrids and producing the artificial polyploid. 4. Chromosome counting in *Lycaste* hybrids. 5. Observations of higher polyploidy and relationship between chromosome number and DNA content in *Epidendrum* hybrids.

12-V-4

Pollen Meiotic Behavior in Relation to *Phalaenopsis* Breeding

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Keywords: *Phalaenopsis* spp., *Doritaenopsis* spp., tetrad analysis, mitotic inhibitors

Abstract

The breeding of *Phalaenopsis* orchids involves the cross-pollination of two selected parental plants. Parents with regular meiotic behavior can often produce seeds successfully. Currently most *Phalaenopsis* hybrids are obtained after interspecific hybridization, unfortunately breeding barriers may occur during the meiotic process thereby affecting fertilization events and reducing seed set. Through pollen tetrad analysis, a correlation between successful seed set and a high tetrad percentage was observed in both *Phalaenopsis* and *Doritaenopsis* orchids. To improve the breeding of hybrid polyploid cultivars, mitotic inhibitors were applied to young flower buds before pollen meiosis in order to monitor changes in the production of tetrads. When compared to the control, a higher number of dyads were observed after treatment with mitotic inhibitors. Treated pollen grains were then attached to the stigma of female parents for self and cross -pollination, resulting in successful fruit set in several cases. We will continue to study the use of mitotic inhibitors for the breeding of polyploidy.

12-V-5

Ecophysiological Traits of *Paphiopedilum* and *Cypripedium*

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Keywords: *Paphiopedilum*, *Cypripedium*, photosynthesis, leaf traits, water use strategy, adaptation, habitat

Abstract

The leaf structures and physiological functions of *Paphiopedilum* and *Cypripedium* reflect the adaptation to their habitats, especially the leaf morphological and physiological evolution of *Paphiopedilum* is related to water-conserving traits in the karst habitat. Our results provide evidence of divergent evolution of congeneric orchids under natural selection. The results would be beneficial for the development of conservation and cultivation strategy of *Paphiopedilum* and *Cypripedium*.

12-V-6

Ex Situ Orchid Conservation – a Case Study from the Singapore Botanic Gardens

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Abstract

Singapore is located near the equator, off the southern tip of the Malay Peninsula. The whole country consists mostly of lowland. It has many interesting types of natural habitats such as primary rain forest, freshwater swamp forest, mangroves, secondary forests, shrub, grasslands, and urban parks and fields. The climate is equatorial with relatively uniform temperature and high humidity.

Some 226 species of native orchids have been recorded in Singapore. Of these, 178 are considered to be extinct, 40 are critically endangered, one is endangered (*Bulbophyllum vaginatum*), two are vulnerable (*Vanilla griffithii*, *Bulbophyllum trifolium*), and only five are considered to be common (*Arundina graminifolia*, *Bromheadia finlaysonianum*, *Dendrobium crumenatum*, *Eulophia graminea*, *Spathoglottis plicata*). The orchid conservation program at the Singapore Botanic Gardens aims to monitor existing species, explore ways to conserve their germplasm, and increase their number for subsequent re-introduction into appropriate habitats, including managed parks and roadsides. Thus far, we have successfully re-introduced *Grammatophyllum speciosum*, *Bulbophyllum vaginatum*, *Bulbophyllum membranaceum*, *Cymbidium finlaysonianum* and *Cymbidium bicolor*. A special designated area at the National Orchid Garden has been set up to showcase the rich diversity of native species. The Orchid Cryo-Seed Bank, began several years ago, is showing some promising result. We have successfully stored seeds of several native species including *Cymbidium finlaysonianum*, *Cymbidium bicolor*, *Grammatophyllum speciosum*, *Dendrobium crumenatum*, *Spathoglottis plicata*, *Bulbophyllum vaginatum*, and *Dendrobium anosmun*.

13-V-1

Environmental and Hormonal Regulation of Flowering in *Phalaenopsis* Orchids: A Mini-Review

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Keywords: benzyladenine, cytokinins, gibberellic acid, light, potted plants, temperature

Abstract

Phalaenopsis, *Doritaenopsis*, and their related hybrids are the most common commercially produced flowering potted orchids. Temperature regulates flower initiation and development of *Phalaenopsis*; inflorescence initiation (sometimes referred to as spiking) occurs in mature plants after at least 2 to 3 weeks of temperatures below 26°C under otherwise favorable conditions. Flowering of many hybrids is increasingly delayed as the daily duration at $\geq 28^{\circ}\text{C}$ increases, and can be completely suppressed with extended high-temperature exposure. Once an inflorescence has developed, time to open flower is a function of temperature and variety. Exogenous application of gibberellic acid (GA) can be used to increase inflorescence length, but more commonly, chemicals that inhibit the biosynthesis of active GA are used to suppress inflorescence elongation. In addition, cytokinins such as benzyladenine can increase inflorescence number of *Phalaenopsis* when applied near the onset of exposure to inductive temperatures. However, in some hybrids, inflorescences can develop abnormally if cytokinins are applied at an excessive rate, after flower initiation, or both. Collectively, commercial producers of *Phalaenopsis* and their related hybrids can manipulate flowering by controlling temperature and applying chemicals that influence endogenous hormone levels to produce architecturally desirable, floriferous plants for predetermined market dates.

Control of Spiking in *Phalaenopsis* by Application of NH₄-N and Plant Growth Regulators

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Keywords: *Phalaenopsis*, spiking control, plant growth regulator, ammonium

Abstract

In *Phalaenopsis* production, all the year around production is available by temperature control. However, the cost of heating and cooling is high and development of the energy saving method is the major concern in *Phalaenopsis* growers. In this experiment, effects of nitrogen fertilizer and plant growth regulators (PGRs) on spiking were investigated to develop cost saving methods of flowering control in *Phalaenopsis* production. Nutrient solutions containing 26.2 ppm NH₄-N and 82.7 ppm NO₃-N (control), control + 140 ppm of NH₄-N, and control + 140 ppm of Urea-N were applied weekly for 5 to 14 times. Application of excess NH₄-N delayed spiking, inflorescence growth and flowering. Flowering was delayed about two weeks by application of excess NH₄-N. Urea-N showed no more clearer effects than NH₄-N. Plant growth regulators (PGRs) were another resources to control spiking and flowering of *Phalaenopsis*. Naphthalene acetic acid sodium salt (NAA·Na), Benzyl adenine (BA), Thidiazuron (TDZ), Gibberellin A3 (GA3) and s-Abcisic acid (s-ABA) were applied to investigate the effects on spiking. Among PGRs tested, weekly application of 1000 ppm NAA·Na delayed the spiking of *Phalaenopsis* depending on the frequency of NAA application. s-ABA application also delayed the spiking. GA3 and BA showed no clear effects. TDZ stimulated lateral bud growth markedly. Combination of excess NH₄-N and PGRs affected spiking. Both application of NH₄-N and NAA·Na showed clear additional inhibitory effect on spiking. Combined application of NH₄-N and s-ABA affects conversely both inhibitory and stimulatory depending on the s-ABA concentrations. To elucidate efficient and practical method to delay spiking by excess NH₄-N and NAA·Na, the method of NAA·Na application was investigated. Application by a finn pipette, spray by a sprayer, alginic acid or Gelrite gel pellets containing NAA·Na were experimented. Among these methods, application by a finn pipette was the most effective followed by spray of NAA·Na solution. Application of NAA·Na by alginic acid gel showed slight effects but Gelrite gel showed no inhibitory effects.

13-V-3

***Phalaenopsis* Mineral Nutrition**

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Keywords: nitrogen, phosphorus, potassium, moth orchid, fertilizer, root substrate, water relations

Abstract

Phalaenopsis is the single most important potted orchid around the world. It has a long juvenile period. High fertility in general accelerates vegetative growth and fast accumulation of the total leaf area to have plants mature quickly. In most bark mixes, fertilizers providing 200 mg/L nitrogen promote growth over lower concentrations. High fertility ensures increased flower count, but not always increases flower size. *Phalaenopsis* needs at least 50% of the nitrogen in the nitrate form for improved growth and flowering, regardless of being planted in a bark mix or in sphagnum moss. Nitrogen concentration close to 300 mg/L, particularly in the ammonium form, has been reported to defer spiking and flowering to a certain extent. *Phalaenopsis* does not appear to require very high phosphorus levels for optimum growth. Phosphorus at 25 to 50 mg/L may be adequate. Phosphorus concentration in the nutrient solution does not affect flower size. Starting to apply high levels of phosphorus one month before spiking does not further enhance flowering. Fertilization may be terminated completely when the inflorescence has reached 10 cm in length or at first flower opening without affecting flower count. Fast-growing larger plants in a bark/peat mix have lower phosphorus concentration in their leaves than the slow-growing smaller plants in pure bark, but the individual plants in both media have similar total amounts of phosphorus. When using high nitrogen level, potassium must be at 200 mg/L or higher for obtaining healthy foliage, increased flower count, and larger flowers than lower concentrations. Plants under low potassium appear healthy when vegetative. As soon as being induced to enter the reproductive phase, yellowing and abscission of the lower leaves becomes apparent. Foliar feed alone does not provide adequate amounts of nutrients for *Phalaenopsis*. Moisture tension of the tightly packed sphagnum moss remains at or below -20 kPa until 90% of the water is lost, compare with 55% water loss in a peat/diatomite mix; thereby, providing higher amount of available water to roots. The pH of the sphagnum moss in growing containers is often between 3.0 and 3.5. Yet, this low pH does not seem to negatively affect plant growth or cause apparent toxicity from the accumulation of certain micronutrients in plants.

14-V-1

Establishment of Large-scale Micropropagation System of *Anoectochilus formosanus* in Bioreactors

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Keywords: *Anoectochilus formosanus*, Bioreactor culture, shoot proliferation, pigments, metabolites

Abstract

An efficient protocol was developed to optimize culture conditions for large-scale micropropagation of *Anoectochilus formosanus* using bioreactor technology relation to the growth, pigments, metabolite content and antioxidant properties. The proliferation response was significantly influenced by aeration volume in bioreactor culture. The highest shoot multiplication and shoot height were observed at 0.06 vvm aeration volume as well as higher fresh and dry weight. Number of shoots and shoot height were reduced by increasing aeration volume (from 0.09 to 0.12 vvm). Comparative studies between solid and liquid (bioreactor) cultures revealed that shoot multiplication and growth were more efficient at 0.06 vvm air volume and 90 explants as initial inoculum size. Chlorophyll and carotenoid contents were increased with increasing inoculum density from 30 to 90 explants/culture vessel. In contrast, the lowest value was observed at inoculum density of 120 explants. The highest contents of total phenol, flavonoid and DPPH radical scavenging activity were also obtained at inoculum density of 90 explants. These results suggested that bioreactor technology can be successfully applied for the large-scale micropropagation of *A. formosanus* in biotechnological approach.

14-V-2

Genetic Transformation of Orchids

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Abstract

Genetic transformation technology of plants is one of the most powerful tools for cultivar improvement as well as for studying gene function in plants. In the past two decades, genetic transformation procedures for most of the commercially important orchids have been established. In our laboratory, we have concentrated our efforts to develop the genetic transformation methods for various ornamental plants, especially for commercially important orchids such *Phalaenopsis*, *Cymbidium*, *Dendrobium* and *Cattleya*. Furthermore, we generated a series of transgenic plants in these orchid genera, which harbor the useful genes for disease and stress resistances, flower color, flower shape, plant height, etc. This review will describe the summarized results of these orchid studies and some related topics on other horticultural plants.

14-V-3

Studies on Synergism and Complementation of Long Distance Movement of *Cymbidium Mosaic Virus* RNA by *Odontoglossum Ringspot Virus* Coat Protein

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Abstract

There are two common orchid viruses, namely *Cymbidium mosaic virus* (CymMV) and *Odontoglossum ringspot virus* (ORSV). Complementation of movement and coat proteins of these two orchid viruses was studied. We produced four transgenic lines, each harbouring one of the movement protein (MP) or coat protein (CP) genes of CymMV or ORSV. The MP of CymMV consists of three overlapping open reading frames (ORFs) called the triple gene block (TGB). We also generated CymMV and ORSV mutants each carrying an inactivated MP or CP. Complementation was studied by infecting transgenic plants with *in vitro* transcripts generated from these mutants. The cell-to-cell movement of a movement-deficient CymMV could be restored in transgenic plants carrying the ORSV MP transgene. Similarly, CymMV TGB123 transgenic plants were able to rescue the cell-to-cell movement of a movement-deficient ORSV mutant. ORSV CP transgenic plants were able to support systemic movement of a CymMV CP-deficient mutant. *Trans*-encapsidation was not observed. The complementation of MPs and CPs of CymMV and ORSV facilitates movement of the two viruses in plants, with an exception in long distance movement of ORSV RNA by CymMV CP. The synergism between CymMV and ORSV was examined using viral RNA and CP accumulation in single and double infections.

14-V-4

Decontamination of *Odontoglossum Ringspot Virus* and *Cymbidium Mosaic Virus* on Tools and Cultivation Environment of Orchids using Culture Filtrate of *Streptomyces* spp.

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Keywords: orchid, viruses, ORSV, CymMV, decontamination, sanitation

Abstract

A novel sanitation approach by using liquid culture filtrate (CF) of a *Streptomyces* isolate (CA5) that can readily disinfect coat proteins (CP) of ORSV and CymMV and eliminate their infectivities is demonstrated. By treating only 30 sec with a preparation of CA5 CF on the materials or tools commonly used in orchid nurseries including razor blade, plastic clip, labels, and gloves coated previously with ORSV and CymMV infected plant sap, it is shown that no ELISA signals and infectivity will be recovered on the treated materials. Similarly, virus contaminated human nails and surface of *Phalaenopsis* leaf tissue can also be treated without detecting any leftover ELISA signal and virus infectivity. We also show that CA5 CF has the potential to be used to eliminate virus contamination on the surface of orchid seeds leading to the production of clean orchid seedlings. The virus degradation activity of CA5 culture filtrate is heat labile to temperature treatment higher than 60 C indicating the activity is possibly due to a proteinaceous substance. Further experiments of dialyzing CA5 culture filtrate using different molecular cutoff membranes shows that the molecular mass of the substance should be larger than 100 kDa.

Oral Presentations

12-O-1

Breeding Barriers in Red Phalaenopsis Orchids

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Abstract

Breeding barriers of producing hybrids by intergeneric, intrer- and intra-specific hybridization in phalaenopsis have been found. In this study, four cultivars of red phalaenopsis, including *Doritaenopsis* Queen Beer (designated as QB), *Dtps.* Ever Spring Princes (ESP), *Dtps.* Taida Salu (TS) and *Phalaenopsis* Brother Gril (BG), which are difficult in hybridization, were collected to investigate the phenomenon of crossing barriers. The root tip squash technique was used to determine the chromosome number, and the results showed that these selected four cultivars all belonged to the triploid group. To test crossing behaviors, these four cultivars were self-crossed, or were used as the pistillate parent during reciprocal crossing with other normal cultivars, *Dtps.* Tai Lin Redangel 'V31', *Phal.* Brother Irene 'Feng Fong' and *Phal.* Sogo Yukidian 'V3'. The results showed that closure of the stigma cavities occurred in TS and BG, and stigma closure was never observed when QB or ESP was used as the pollen parents in crosses. However, hybrid progenies were only obtained from combinations that ESP or TS was used as the pistillate parent crossing with normal cultivars tested. These results might suggest that crossing difficulties of these selected four cultivars in this study might be causing by chromosome number, male or female parent factor.

12-O-2

Phylogenetic Analysis in the Genera *Phaius* and *Cephalantheropsis* Using *rpl32-trnL* Marker

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Keywords: *Cephalantheropsis*, *Phaius*, *Calanthe*, *rpl32-trnL* marker, dendrogram

Abstract

The taxonomic status of *Cephalantheropsis gracilis* and *Cephalantheropsis calanthoides* remained uncertain before 1970, either pertained to the genus *Phaius* or *Calanthe*. Four species in the genus *Phaius*, two species in the genus *Cephalantheropsis*, and three species in the genus *Calanthe* endemic to Taiwan, were analyzed with *rpl32-trnL* marker to assess their phylogenetic relationship. A dendrogram based on sequence analysis of *rpl32-trnL* marker showed that *Phaius tankervilleae*, *Phaius flavus*, *Phaius takeoi*, and *Phaius mishmensis* were clustered together and *Cephalantheropsis gracilis* was grouped with *Cephalantheropsis calanthoides*. *Calanthe alismifolia*, *Calanthe sieboldii*, and *Calanthe triplicata* were clustered together as the outgroup. The present results strongly support the traditional taxonomy as described by Hu (1972) and Liu & Su (1978). The *rpl32-trnL* marker which is a non-coding sequence of chloroplast DNA is a useful genetic marker for phylogenetic analysis between species.

Chloroplast DNA Barcoding in *Spathoglottis* Species for Genetic Conservation

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Keywords: *Spathoglottis*, chloroplast DNA, DNA Barcoding

Abstract

Spathoglottis is one of the most popular terrestrial orchids because it is attractive, easy to cultivate and continuously blooms in the garden. *Spathoglottis* is native to Malaysia and listed as endangered species. Therefore, these species need to be conserved for commercial and environmental purpose. DNA barcoding is a technique for species identification, using a short DNA sequence from a standard and agreed-upon position in the genome. It is playing an important role in biodiversity conservation for animals but is not yet well established in plants. Chloroplast DNA was chosen for the study to build a DNA barcoding in *Spathoglottis* species. Among the *Spathoglottis* species about seven (7) species were used to carry out the study. They are *Spathoglottis plicata*, *Spathoglottis gracilis*, *Spathoglottis aurea*, *Spathoglottis plicata alba*, *Spathoglottis unguiculata*, *Spathoglottis kimbaliana* and *Spathoglottis* species consider as a hybrids. Ten (10) regions (*accD*, *matK*, *ndhJ*, *rpoB*, *rpoC1*, *ycf5*, *rbcl-a*, *trnH-psbA*, *ITS1* and *ITS2*) in the chloroplast genome were selected and screen to define a universal barcoding region across all the seven selected species. The study found that four (4) chloroplast regions (*matK*, *rbcl-a*, *rpoB* and *rpoC1*) were successfully amplified from all the species tested. DNA sequencing from each chloroplast region was compared within and between the species and analyzed to differentiate intra and inter genetic variation. The analysis of chloroplast regions were done for single regions and as a combination of the four regions. The haplotype for multiple analysis showed sufficiently high resolution to enable differentiation between the selected *Spathoglottis* species. This study showed that the chloroplast DNA regions has high potential to be developed for DNA barcoding in the *Spathoglottis* species. This is the first step towards the development of universal DNA barcoding technique for all native orchids in Malaysia.

12-O-4

Mechanisms for Recruitment and Population Growth in *Nervilia nipponica*, an Endangered Orchid in Japan

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Keywords: autogamy, clone, conservation, spatial genetic structuring, *Nervilia nipponica*, recruitment cycle, seed viability

Abstract

Elements of the recruitment cycle of *Nervilia nipponica*, an endangered terrestrial orchid endemic to Japan, were examined towards the development of a conservation strategy. Field studies and bagging experiments indicated that the species is capable of outbreeding, but is habitually autogamous and therefore not pollinator-limited for seed set under natural conditions. Though most seeds harvested from open-pollinated capsules consistently exhibited close to 100% germination when cultured in vitro, germination of seeds from the same capsules was variable and averaged just 1.3% following incubation in the soil in seed packets at the source populations. A demographic study of marked tubers indicated a strong capacity for annual recruitment via vegetative propagation, with increased investment in flowering one year limiting genet growth the next, possibly indicative of resource limitation. The affects of these processes on spatial genetic structuring were examined using AFLPs. Limited diversity was found within the species as a whole, and one intensively sampled population was found to harbour just two distinct, possibly clonal genotypes. Despite iterative inbreeding, seed is viable and may disperse over wide geographic distances. Subsequent population growth appears to occur primarily through vegetative propagation. Rarity in *N. nipponica* may be associated with limited recruitment from seed resulting from heterogeneity in soil microsite conditions. Conservation is most likely to be effective if targeted at habitat management for genet persistence.

12-O-5

Mycorrhiza Colonization Patterns in *Bletia* Species in a Natural Forest Reserve in Michoacán, Mexico

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Keywords: *Bletia*, orchid mycorrhiza, pelotons, quality of a site

Abstract

México is the diversification center of the subtribe Bletinae and therefore, a great richness of species of this gender are found in its territory. In spite of the fact that the country possesses a great taxonomic knowledge of its species, little is known about the interactions of the Mexican orchids with their mycorrhizal fungi, which are important for these plants because of their contribution in the absorption of nutrients. The present work had as aim to generate an initial diagnosis study about the degree of natural mycorrhization that have different kinds of orchids of the genus *Bletia*; the in situ characterization of the mycorrhiza colonization in mature roots of three terrestrial orchids, *Bletia roezlii*, *B. punctata* and *B. campanulata* was performed; located in a gradient of disturbance in the Natural Reserve Gully of the Cupatitzio, Michoacán, Mexico. For this, we determinate the intensity, extension and phenology of the mycorrhiza colonization, that was evaluated in the flowering season (July - November, 2008). This one appeared along the whole root in *B. punctata* and *B. roezlii*, whereas in *B. campanulata*, the intensity and extension of the colonization was high and with a major proportion of digested pelotons in the segments of the end apical ($P \geq 0.01$) diminishing in the base close to the pseudobulb. The intensity of colonization increased at the end of November that corresponds to the end of the rainy season when plants were beginning to lose leaves. *B. roezlii* showing the highest colonization in the disturbed areas. It is possible that this may partly explain why these species presented a broad distribution and abundance in the reserve. Studies of patterns of mycorrhizal colonization in these species related to the quality of a site associated with forest and its conservation status may partly explain their persistence in terms of disturbance and therefore their vulnerability.

12-O-6

Evaluation of Growth and Flowering of *Bipinnula volkmannii* Kraenzl., *Gavilea glandulifera* Poepp., *Chloraea magellanica* Hook. f., and *C. philippii* Reichb. F. Part I

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Keywords: Native Chilean Orchids/morphogenic development, Geophite, vegetative growing, phenologic state

Abstract

We present a study of different genera and species of terrestrial native Chilean orchids mainly threatened by human activities, which provides enough reasons to preserve them through genetic bank.

Plant growth control has been done in the Xth region at Puerto Mont City, Chile. The geographical location of the evaluated plant is 41° 28' S and 72° 56' W and experiments will be finished in January 2010. The main goal is to measure, analyse and compare the growth and flowering of 4 native orchids' species from the south of Chile. These species have been growing in a simple greenhouse. Every 15 days, over 5 plants per species, were measured according to different parameters: length and width of leaves and roots; lateral bud; leaves area and weight of rhizome. The flowering stage will be evaluated by measuring number of leaves, anthesis, the period between first and last flower development, number of inflorescence by plant, flower size, floral rod height and senescence initiation. Correlations between the obtained data are statistically analysed in a completely random system. From the start to the present, we have observed that the species *C. philippii* presents a significantly greater lateral buds development with respect to the rest of the analysed species. *C. magellanica* presents a lower foliar growth with respect to the other species in the same abiotic conditions of temperature, humidity and fertilization.

13-O-1

N⁶-Benzyladenine Induced Flowering of Potted Nobile *Dendrobium* Red Emperor 'Prince' Orchid Plants

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Keywords: cytokinins, flower induction, orchids

Abstract

Hybrid nobile dendrobium orchids, mostly derived from the species *Dendrobium nobile* Lindl., normally flower in the late winter and early spring following a period of cooler temperatures. Commercially, flowering size plants are grown in full sun, to increase photosynthesis, using fertilizers with a low percentage of nitrogen, to decrease vegetative growth and increase food storage in the mature pseudobulb. In the study reported here 450, 900 and 4500 ppm solutions of N⁶-benzyladenine (BA) induced flowering without cool temperature treatment. Control, non-treated plants did not flower. Treated plants were in full flower 10 weeks after treatment.

13-O-2

Effect of Bulb Size and Gibberellin Application on the Bulb Growth of *Pleione formosana* Hayata

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Keywords: terrestrial orchid, flowering, scape length, spraying, proliferation rate, fresh weight

Abstract

Pleione formosana Hayata, known as the one-foliate orchid, is a native terrestrial orchid in Taiwan. In this study, we investigated the effect of mother bulb size on flowering and daughter bulb formation, the application of gibberellic acid on the bulb growth in Nan-Chung village. The result showed that the bulb size was in proportion to the flowering percentage, floret number, diameter, scape number and length. However, the shelf life was not influenced by bulb size. The number and weight of daughter bulb after harvest were in proportion to the size of mother bulbs. Different concentrations of gibberellin (0, 25, 50, 75 and 125 mg/L) was applied to bulbs by spraying during growth or immersion before planting, and the daughter bulb characteristics were investigated after harvest. The result indicated that GA₁₋₄ spraying during growth significantly increased the average fresh weight, diameter and height of daughter bulbs. It might suggest that the application of GA₁₋₄ during growth promoted the bulb growth and decreased the duration for bulb cultivation.

13-O-3

Effects of Emerging Shoot Size, Temperature, and Benzyladenine on Growth and Flowering of *Zygopetalum* Redvale 'Fire Kiss' Orchids

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Keywords: flower induction, plant growth regulator, vernalization

Abstract

Zygopetalum is an epiphytic and terrestrial orchid genus that originates from mountainous regions of South America and has exotic, fragrant flowers. We performed experiments to determine how emerging shoot length, temperature, and application of benzyladenine (BA) influence growth and flowering of *Zygopetalum* Redvale 'Fire Kiss'. In the first experiment, the length of one immature vegetative shoot was measured on each plant at week 0 and plants were assigned to groups based on emerging shoot length: 1.0 to 2.0 cm, 3.0 to 4.0 cm, 6.0 to 7.0 cm, and 9.0 to 10.0 cm. Plants were subsequently transferred to environmental chambers with constant temperatures of 12.5, 15.0, 20.0, and 25.0°C and a 9-h photoperiod and grown for 8 weeks. Plants were then grown in a greenhouse at a constant temperature of 23°C with a 16-h photoperiod. After 73 d, 63% or 75% of plants had developed a visible inflorescence (VI) if emerging shoots between 3.0 and 7.0 cm in length were exposed to 12.5 or 15.0°C. No plants developed a VI when grown at 20 or 25°C, regardless of shoot length. In the second experiment, vegetative 5.5-year old plants were grown in a greenhouse with a constant temperature of 14 or 20°C and a 16-h photoperiod. Plants received a foliar spray application (volume of 0.2 L/m²) of BA at 200 or 400 mg/L at week 0 and 4 or week 4 and 8. Control plants did not receive a BA application. At week 8, plants were transferred to a greenhouse at a constant temperature of 20°C with a 16-photoperiod. After 4 weeks, plants that were treated with BA had developed a mean of 7.2 to 10.7 new immature shoots per plant, while untreated plants had only 0.4 to 0.8 new shoots. Thus, application of BA could be used to stimulate shoot development to increase vegetative growth of *Zygopetalum*.

13-O-4

Evaluation of Growing Media Components for *Phalaenopsis*

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Keywords: moth orchid, pine bark, peat fibres, wood fibres, sphagnum moss, coconut husk chips

Abstract

Pine bark, originating from South-France or Portugal, is a commonly used growing medium for the cultivation of *Phalaenopsis* in Europe. During the last years this major component of orchid growing media has become scarce and expensive because of the great demand by large-scale commercial orchid production in several countries. To evaluate possible substitutes for pine bark, various proportions of peat fibres, coarse wood fibres, sphagnum moss and coconut husk chips were tested against a standard mix of 70 % pine bark and 30 % coarse weekly decomposed peat.

Young plants of *Phalaenopsis* 'Pauline' were potted in transparent 12-cm pots and cultivated to full bloom. The eight different growing media were supplied with 0.5 g/L of a water soluble fertilizer ($N+P_2O_5+K_2O+MgO = 15+10+15+2$) as well as additional microelements and adjusted to a pH ($CaCl_2$) of 5.5. During the cultivation period, all plants weekly were fertilized with 50 ml of the same nutrient solution (mg/L): 105 N, 15 P, 88 K, 9 Mg⁺ micronutrients. Additional nitrogen supply was given in all variants, except for 100 % sphagnum moss, at a rate of 120 mg N per plant, divided into seven parts during the first quarter of the cultivation period.

With all tested growing media plants in good or adequate quality could be produced. There was no effect of the growing medium on plant size except for mixes with coco chips, in which the plants remained significantly smaller. A clear influence of the growing medium occurred referring to flowering traits. Plants cultivated in mixes containing coarse wood fibres developed an equal high number of flower spikes and flowers like plants cultivated in the standard mixture. Mixes with coco chips resulted in significant less spikes and flowers and media containing sphagnum moss showed equal high numbers of flowers, but less spikes than plants in the standard mixture. Mixes containing wood fibres or sphagnum moss resulted in very well rooting and superior root quality.

The inferior results with coco chips may be caused by too high salt contents, especially sodium and chloride. The good quality of the roots in media with coarse wood fibres is due to the high air capacity which is added by this material. As with most organic residues used in growing media, nitrogen immobilization can occur and should be controlled and counteracted by additional nitrogen supply, if necessary.

13-O-5

Growth and Flowering of *Oncidium* in an Artificial Textile Fiber Substrate

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Keywords: Sweet Ears 'Pacific Gold', polyamide, polyethylene terephthalate, sphagnum moss, bark

Abstract

Most *Oncidium* cultivation in Taiwan is under shade structure without rain protection. Currently, the major medium used for *Oncidium* cultivation is a mix consisting of bark and coconut husk chips. It is not easy to control the fertigation for high quality productions. The *Phalaenopsis* industry is well developed in Taiwan and can be used as a model for improved *Oncidium* culture.

The sphagnum mosses used for *Phalaenopsis* cultivation in Taiwan are imported from Chile, China and New Zealand at about 1100 tons annually. In recent years, the rising cost and unstable quality of mosses influence the production and quality of *Phalaenopsis* in Taiwan. Artificial textile fiber, a waste product of the textile industry, is made of polyamide (PA) and polyethylene terephthalate (PET), manufactured by the technology of spinning cotton yarn. The artificial textile fiber is clean and neutral. Therefore, it could be a promising alternative medium for orchid cultivation. The effect of artificial textile fiber and bark mix on the growth and performance of *Oncidium* Sweet Ears 'Pacific Gold' was studied. When evaluated on the growth of pseudobulbs, numbers of new shoots, time of anthesis and flower count of plants grown in the artificial textile fiber substrate appear to be better than or equal to those grown in the bark medium. More research is needed to set up a cultural system and to determine fertilization and water requirements.

13-O-6

Improvement of Inflorescence Quality by Calcium Supplement Nutriculture on *Oncidium* Gower Ramsey

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Keywords: *Oncidium*, cultural medium, flower quality, rockwool, calcium

Abstract

The research was to investigate the growth status of *Oncidium* plants grown in various medium, which were rockwool, sphagnum moss and mixed medium containing stone, bark, and charcoal. Beside, various sources of calcium were added to the nutriculture system, and the other experiment was *Oncidium* plants treated with calcium and kinetin. Then flower quality of *Oncidium* during the reproductive stage was investigated. The experiment results indicated that the growth of pseudobulb of *Oncidium* had been promoted and had accumulated storage of starch within pseudobulb when grown in rockwool. Compared with other treatments, *Oncidium* plants grown in rockwool resulted in thicker pseudobulb with a fresh weight at 31.0 g, a TSS content of 45.1% and starch content 35.5%, the contrast treatment that plants grown in mixed media had pseudobulb with a fresh weight 23.3 g, a TSS content of 11.3% and a starch content of 19.4%, respectively. Moreover, calcium nitrate applied to culture solution resulted in an improvement in quality of *Oncidium* flower. Calcium nitrate at a rate of 300 ppm had flower stalks at 117.07 cm, branch number at 9.5 and floret number at 108. Calcium applied with kinetin sprayed made inflorescence qualities improve. The treatment of calcium at a rate of 300 ppm with kinetin at a rate of 100 ppm had more branch and floret number of inflorescence than others.

13-O-7

Crassulacean Acid Metabolism in *Phalaenopsis aphrodite* subsp. *formosana* during Different Developmental Stages

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Keywords: CAM daily rhythm, photosynthesis pathway transition, protocorm, malate, phosphoenolpyruvate carboxylase, *PPC*

Abstract

Mature *Phalaenopsis* is a typical CAM plant; whereas, no remarkable day/night titratable acidity rhythm was detected at protocorm stage. The observation suggested that *Phalaenopsis* at its youth may undergo C3 photosynthesis pathway and lead to our hypothesis that there exists a photosynthetic transition, transits from C3 to CAM during plant developing. *Phalaenopsis aphrodite* var. *formosa*, the most important indigenous specie in Taiwan, was used to analyze PEPC activity and gene expression related to such photosynthesis transition. There was no significant daily change in malate and PEPC activity from stage 0 to 2. Malate and PEPC activity showed daily rhythm since stage 3. At stage 4, which the protocorm developed the first leaf, the typical CAM daily pattern of malate and PEPC activity was observed. Hence, stage 4 may be the photosynthesis transition period after leaf differentiation. However, the expression of *PPC*, encoded for PEPC, revealed a constitutional pattern in all developmental stages without day/night difference. The results suggested that *PPC* expression is not the key factor controlling the transformation of photosynthesis type, and genes responsible for activation of PEPC are our next targets for studying photosynthesis transition.

13-O-8

Photosynthetic Midday Depression in *Oncidium* Alliance

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Keywords: *Oncidium* alliance, *Colmanara*, gas exchange, chlorophyll fluorescence, Fv/Fm, temperature response curve, light response curve

Abstract

Oncidium is an important orchid for cut flower and potted plant production. The photosynthesis of *Oncidium* in hot summer days exhibited a pattern of midday depression (MD), which gas exchange rate decreased around noon time and recovered to some extent in the afternoon. The Fv/Fm value, reflecting quantum efficiency of PSII, also dropped to its minimum in the midday hours. These phenomena were observed in *Onc. Gower Ramsey* 'Sunkist', *Onc. Gower Ramsey* 'Volcano Queen', and *Colmanara* Wildcat. Further experiments were then conducted to investigate the relationship between environmental factors and MD and methods to relief MD. In growth chamber studies, the net photosynthesis (Pn) of *Onc. Gower Ramsey* decreased when photosynthetically active radiation (PAR) exceeded 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ or when temperature exceeded a critical value, which was 24 in April and 30°C in November. In multiple sites studies, the average Pn of *Onc. Gower Ramsey* grown in a rain shelter was lower than that in a phytotron or in a growth chamber. The plants had the highest Pn and the least MD when grown in a growth chamber, in which PAR was well controlled at 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and temperature at 25°C. In field studies, the Pn of *Onc. Gower Ramsey* was more sever in a sunny day than that in a sunny day with afternoon shower. In phytotron studies, the Pn of *Onc. Gower Ramsey* decreased dramatically when plants were moved from 25°C to 35°C but increased *vice versa*. Data revealed that high temperature was the main factor increased the MD in *Oncidium*. The Pn of plants received hourly mist was higher than that of controls. These results confirmed the above conclusion and offered a practical method for reducing MD in *Oncidium* alliance.

13-O-9

Process Management and Marketing for Orchid Firms

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Keywords: process management, orchid, Taiwan, case study, marketing

Abstract

The concept of marketing as processes is well documented in the service literature, but is equally relevant for marketing physical products, including orchids where there are service components due to their perishability and quality requirements in general. Marketing as processes emphasizes relationship building by offering a holistic package of solution to customers, rather than simply selling bare products and leaving customers to figure out accompanying issues during the process. As well noted in literature and successful firms, process management is a 'perspective', not a set of tools, of doing business that requires conscious handling of every firm-customer interface throughout the business relationship. The marketing process perspective originated from Nordic countries where an 'experience economy', as apposed to an 'ordinary economy' was first and greatly appreciated and made explicit as a research area. The spread of experience economy is very uneven across countries, with more outcomes among developed countries. In less developed countries, it might be more difficult for firms to adopt a marketing process perspective where firm resources were often depleted for short-term survival. This study discusses the management and marketing by several orchid firms in Taiwan. Marketing practices by Taiwan firms are useful to look at because Taiwan had remarkable economic performances, and yet could be considered between an experience economy and an ordinary economy. This study uses four very different cases in Taiwan to elucidate how the management and marketing of orchid should be viewed as a holistic process to create values for customers. In-depth interviews were conducted with owners or managers for all the firms involved in this study. Depending on whether value is created for customers, there are both positive and negative lessons learned from the cases presented in this study. Results from the case studies also provide elaboration for firms wishing to maintain long-term sustainability in the orchid industry, particularly in developing and newly developed economies.

13-O-10

Purchasing and Non-purchasing Decisions on Orchid Consumption in the Taiwan Market

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Keywords: Taiwan consumers, orchid, purchasing decision, Bayesian network

Abstract

Taiwan is a relatively better-off economy and has earned a reputation in the orchid industry. However, the general public consume very modestly for flowers, including orchid which Taiwan is well known for. The purpose of this study is to learn about the factors that influence consumer decision making sequence in purchasing and non-purchasing of orchid pot plants and cut flowers. Data were collected through intercepting visitors at two orchid shows, several flower markets and traditional markets. Bayesian network analysis was used to analyze the connectedness between those factors that influence consumer purchasing and non-purchasing. Results from 139 valid cases revealed the relative importance of factors determining purchasing and non-purchasing. Among purchasers, the first factor in the decision making sequence for purchasing was reasonable price. Once the price was acceptable, customers considered the following factors jointly, offering caring instructions, longer blossom period, and minimal chemical residues. Among non-purchasers, the first main factor entering the decision making sequence was 'don't know how to look after orchids', which was associated with six other factors. Improvement in these factors might increase the incentives for non-purchasers to buy orchids: longer blossom period, price reduction, minimal chemical residues, offering quality pots, offering caring instructions, and market information. The implications for improving domestic consumption for flowers were also discussed.

13-O-11

Value Chain Dynamics in the Taiwan Orchid Industry

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Keywords: value chain, Taiwan, orchid, supply chain, dynamics

Abstract

Taiwan is a small island about the size of Holland, but is well known for its orchid culture industry, particularly *Phalaenopsis*. While Taiwan has some subtropical climatic advantages in growing orchid, the availability of advanced modern cultural techniques under greenhouse conditions, has made the advantage substitutable to a large extent. In fact, without such climatic advantage, Holland has emerged rapidly as one seeming market leader in the orchid culture industry. This study offers a critical review of the Taiwan orchid industry infrastructure, the sources of competitive advantage and long-term sustainability of the industry. Through in-depth interviews with various stakeholders in the industry, this study argues that the thrust of past success in the orchid culture industry could be attributed to the characteristics of its supply chains, or called value chains in this study where value appropriation is the focus. The industry is characterized by heterogeneity of value chains with a large number of micro-, small-, and medium-sized firms which enter and exit the market relatively easily. This is made possible by cultural practices courses held frequently by government extension officers. These firms compete and yet complement with each other, frequently providing new release of varieties and lower to medium high range products in the world market. To date, the dynamics of the orchid supply chains has provided firms with competitive advantages which have appropriated rather continuous flow of short-term profits. Finally, in discussing industry sustainability, some prescriptive suggestions were made to orchid culture supply chains in relation to changing public attitudes toward environmental conservation and quality warranty for end users.

14-O-1

The Development of Orchid Production in Israel

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Abstract

Israel is a small country with variable climatic conditions. The climate is characterized by a long hot dry summer with high solar radiation. The rainy season is during the winter; however winter solar radiation is high. These climatic conditions are far from ideal for tropical orchids well-known worldwide. Several species of wild terrestrial orchids, which flower in early spring and vanish in the summer, are found in Israel.

Modern agriculture in the area started at the end of the 19th century, and flower production began in the 1950's. Flower production was based on traditional crops, with a simultaneous search for new crops. By the 1970's, research and development of plants of the Orchidaceae family had commenced at the Faculty of Agriculture, focusing mainly on the physiological aspects of *Phalaenopsis*. At the same time, private entrepreneurs were studying acclimatization and breeding of *Cymbidium*, as a cut flower. *Cymbidium* requires low temperatures for flowering, and for that reason plants were grown in the hill areas (600-800 m. above sea level). *Phalaenopsis* and other warm temperature orchids were produced in warmer areas of Israel. Attempts by the growers to export those products yielded low prices, so production during those years focused on cut flowers and plants to the local market.

In recent years, more growers have gradually joined orchid production, growing mainly *Phalaenopsis*. Those growers import young plants from Holland. The plants are grown in Israel for 12-24 months, depending on the original size and desired end product size, and then sold on the local market. Those nurseries grow mainly *Phalaenopsis* and other Orchidaceae, such as *Dendrobium*, *Oncidium*, *Phaphiopedilum* and *Cattleya*. Moreover there are enthusiastic orchid lovers who belong to a non-profit organization, the Israeli Orchid Society.

Since 2000, Solo-Shtil – a nursery with many years of experience in production of house plants, roses, and more – has started to grow orchids. Solo-Shtil produces *Dendrobium bigibbum* (also referred to as *Dendrobium phalaenopsis*) for export. The beginning of the process involved selection from many different prototypes grown from seedlings originating in Thailand. Some 15 prototypes were chosen for tissue culture propagation and further selection. Out of those prototypes 4 were chosen as varieties with good production potential. Those 4 all have pink-colored flowers but vary in flower size, pseudobulb length, number of flowers in the inflorescence and the main difference – flowering time. By selecting types with different flowering times, the nursery is able to supply flowering plants almost year-round, and this is its specialty. Moreover, high winter light intensity in Israel increases the number of flowering pseudobulbs per plant exported to Holland. Interestingly, it was found that production time can be extended by selecting different types/varieties that have similar flowers but vary in flowering time. However more work must be done to widen the color range of *Dendrobium bigibbum* to supply the changing demands of the world market.

14-O-2

Pollination, Seed Development and *In Vitro* Germination of *Cymbidium sinensis* 'Huang Dao'

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Abstract

The flowers of *Cymbidium sinensis* 'Huang Dao' were handed pollination within 1-7 days after flowering (DAF), there were 74-97 % fruit set and no difference on seeds size, embryo size and embryo percentage at 150 days after pollination (DAP) with DAF treatments. The seeds were observed clearly at 12 weeks after pollination (WAP), and then seeds developed with 1178 μm in length at 24 WAP. Meanwhile, the embryo length increased from 20 WAP, reaching max with 206 μm at 32 WAP. The seeds did not germinate before 16 WAP, globular embryo formation was observed at 20 WAP, and the seed water content began to reduce. Seed germination percentage was higher at 28 WAP than other time. However, the highest germination percentage was 7.9 % of 32 WAP seeds at 24 weeks culture. Furthermore, rhizome formation percentage was higher for 22 WAP seeds, at around 3.4 %. The germination percentages of 1 day \times 1 day and 3 day \times 3 day (stigma \times pollen) at 150 DAP were 4.6 and 5.2 %, respectively. This percentage exceeded that for other combinations, but no effect was observed for 224 DAP seeds. In conclusion, similar tendencies were observed in experiments involving maturity and pollination. Younger seeds germinated slowly in early culture, but increased rapidly and formed more rhizomes in later culture. In contrast, older seeds germinated quickly during early culture, but formed less rhizomes during later culture.

14-O-3

Effect of Banana Extracts on PLBs Proliferation and Growth of Different Orchid Hybrids

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Keywords: *in vitro*, seedlings, culture media, chlorophyll, glucose content

Abstract

The addition of organic additives to orchid culture medium is a common practice with the objective to improve the growth of orchid protocorm-like bodies (PLBs) and plantlets. In addition, it reduces the use of plant hormones and may help in preventing the occurrence of undesirable somaclonal variants. Extracts of four different types of banana cultivars (Berangan, Emas, Rastali, and Tanduk) were chosen to examine their effectiveness on proliferation of PLBs of *Phalaenopsis bellina*. The half-strength MS medium supplemented with 10 % (w/v) of banana homogenate was used in this study. The result obtained showed that medium supplemented with 10 % (w/v) of Berangan-extract was the best banana additive giving higher growth and proliferation rate when compared to other banana types, Emas, Rastali and Tanduk. The PLBs cultured in Berangan-containing medium has the highest dried weight followed by that of Emas- Rastali- and Tanduk-added media. Similarly PLBs cultured in medium containing Berangan extract had the highest amount of glucose (37.54 ug/g DW) followed by that of Tanduk medium (135.20 ug/g DW), Rastali medium (134.03 ug/g DW) and Emas medium (133.45 ug/g DW). The chlorophyll content of the proliferated PLBs showed no significant different, although it appeared slightly higher in Berangan-containing media. However, in *Vanda Mimi Palmer* 5.5 % of commercial banana powder exhibited maximum plantlet growth while 3.5 % of commercial banana powder was sufficient to enhance root development of *V. Mimi Palmer in vitro* seedlings. In this study the type of banana cultivar suitable for orchid tissue culture medium is identified. Hence, a simple and efficient medium for improved proliferation of PLBs is, therefore, established.

14-O-4

Micropropagation of a Rare Orchid *Dendrobium gratiosissimum* Rchb.f. , Using Thin Cell Layers.

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Keywords: *Dendrobium gratiosissimum*, thin cell layers, micropropagation

Abstract

Dendrobium gratiosissimum Rchb.f. , is known as a very graceful *Dendrobium*. It is a wild orchid of Thailand which is a rare species. Establishment of an efficient method for micropropagation is urgently needed because of its conservation and commercial requirement. An efficient method of a rapid and mass propagation of *D. gratiosissimum* using thin cell layers (TCLs) had been developed. Four-week old protocorms were segmented into two halves and each half was considered as a TCLs explant. The shoots, each with 1-2 node were cut into approximately 0.3-0.5 mm thickness from the base to the shoot tip. These thin sections were used as TCLs explants for plant regeneration. The TCLs explants from both protocorms and young stems were cultured on MS (Murashige and Skoog, 1962) medium supplemented with different combinations of N⁶-benzyladenine (BA), kinetin (Kn), 1-naphthaleneacetic acid (NAA), and 20 mg/L sucrose. MS medium without growth regulators failed to induce protocorm-like bodies (PLBs) formation from stem explants. The TCLs explants from both protocorm and stem developed PLBs within 3-4 weeks on the MS containing growth regulators. Of the two different TCLs explants types used, the highest percentage of PLBs formation (83 %) and highest number of PLBs (18) per explants were observed on the protocorm sections, whereas only 66 % of stem sections were able to produce PLBs with an average 9 PLBs per explants. MS containing 2 mg/L Kn was found to be the optimum concentration for PLBs development from thin protocorm section. In contrast, the highest PLBs production of stem sections was obtained from explants on 5 mg/L Kn and 1.0 mg/L NAA. Plantlet conversion from PLBs was successfully achieved on regulator-free growth medium.

14-O-5

Regeneration and *Agrobacterium*-mediated Transformation of *Oncidium* and *Odontoglossum* with Ethylene Receptor Mutant Gene *ETR1-1*

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Keywords: genetic transformation, in vitro, orchids, somatic embryogenesis, regeneration, thidiazuron

Abstract

Oncidium and *Odontoglossum* are ornamental orchids with a wide spectrum of flower colours and patterns and also with increasing economic importance. Because of their sensitivity towards ethylene, their senescence process is initiated very fast. Traditional breeding is a time-consuming process in these species. Therefore, the aim of this study was to develop an efficient protocol for *in vitro* regeneration via somatic embryogenesis and *Agrobacterium*-mediated transformation, to shorten the breeding cycle.

Starting from *in vitro* shoot cultures of the cultivars *Oncidium* 'Sweet Sugar' and 'Sharry Baby', as well as the cultivars of *Odontoglossum* hybrids 'Stirling Tiger', 'Hansueli Isler' and Cambria 'Plush' we established a reliable protocol for regeneration of somatic embryos. The most suitable type of explants were leaf tips, on which the formation of protocorm like bodies, which resemble somatic embryos in the Orchidaceae family, was observed in frequencies of 60 % after 12 weeks. Most efficient regeneration was obtained on thidiazuron containing media and in complete darkness.

The objective of our project was to transform *Oncidium* and *Odontoglossum* with the ethylene receptor mutant gene *etr1-1* from *Arabidopsis* under control of a flower-specific promoter to achieve a prolonged shelf life. Applying our regeneration protocol we tested different parameters for *Agrobacterium*-mediated gene transfer of PLBs in liquid culture and on solid medium. So far three transgenic lines were proven by Southern analysis to be successfully transformed with *etr1-1*.

14-O-6

A Novel Functional Homodimer Geranyl Diphosphate Synthase for Scent Production in *Phalaenopsis bellina* (Orchidaceae) Flower

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Keywords: Orchidaceae, *Phalaenopsis bellina*, flower fragrance, geranyl diphosphate synthase

Abstract

Geranyl diphosphate (Nogues et al.) is the precursor to monoterpenes, the major floral scent in *Phalaenopsis bellina*. The cDNA of *P. bellina* GDP synthase (*PbGDPS*) was cloned, and its sequence contained a glutamate-rich motif (Glu-rich) corresponding to the second aspartate-rich motif (SARM) but no any aspartate-rich motifs (Asp-rich). The recombinant *PbGDPS* enzyme revealed dual prenyltransferase activities by the production of both GDP and farnesyl diphosphate (FDP) and yeast two-hybrid assay revealed that *PbGDPS* was able to form a homodimer. Spatial and temporal expression analyses illustrated that the expression of *PbGDPS* was flower specific and concomitant with maximal emission of monoterpenes on day 5 post-anthesis. In addition, the expression was strongly related to the intensity of the emitted scent among different plant varieties. This is the first report of a functional GDPS without an Asp-rich motif functioning as a homodimer in a plant (*P. bellina*, Orchidaceae, monocot).

14-O-7

Modification of *Phalaenopsis* Metabolism by Genetic Engineering

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Keywords: *Phalaenopsis*, Transgenic orchid, *vhb* gene, pollen tube mediated pathway, malic acid, *Vitreoscilla* hemoglobin

Abstract

Phalaenopsis is one of the most important flower crops in Taiwan. However, the long growth period hampers its commercial production. In an attempt to increase the metabolic rate and the growth of *Phalaenopsis*, the orchids were transformed with the *Vitreoscilla* hemoglobin (VHb) gene via pollen tube-mediated delivery. Putative *vhb*-transformants were analyzed by PCR, southern blotting, and western blotting, to verify stable integration of the *vhb* gene. The transgenic orchids were further characterized by measuring their leaf and flower size, photosynthetic rate, chlorophyll content, malic acid accumulation, and reduction activity of root tip. The results indicated *vhb* has modified the metabolism of *Phalaenopsis*.

14-O-8

Molecular Mechanisms Underlying Orchid Floral Morphogenesis

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Keywords: orchids, floral morphology, MADS-box genes, ABC model, labellum, gynostemium

Abstract

Orchids are known for both their floral diversity and ecological strategies. The versatility and specialization in orchid floral morphology, structure and physiological properties have fascinated botanists for centuries. In floral studies, MADS-box genes contributing to the now famous ABC model of floral organ identity control have dominated conceptual thinking. The sophisticated orchid floral organization, such as labellum and gynostemium, offers an opportunity to discover new variant genes and different levels of complexity to the ABC model. In our studies, we suggest duplication and differential expression of B and C class genes may contribute the specificity of orchid floral morphology. We also proposed a molecular model to explain development of orchid sterile and fertile floral organ. Knowledge about MADS-box genes encoding ABC functions in orchids will give insights into the highly evolved floral morphogenetic networks and the diversification of orchids.

14-O-9

Molecular and Morphological Studies in *Vanda tessellata*, an Epiphytic Orchid from the Eastern Ghats of India

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Keywords: *Vanda tessellata*, Eastern Ghats, India, SDS-PAGE, RAPD analysis, morphological studies.

Abstract

Morphological and molecular analysis of genetic variability has been investigated by using SDS-PAGE and RAPD markers in *Vanda tessellata* (Roxb.) Hook ex G. Don., an epiphytic orchid from eastern ghats of Andhra Pradesh, India. Plants collected from two different geographical areas in eastern ghats show variation in their morphological and molecular characters. The stomata are paracytic type and hypostomatic in distribution. A low stomatal frequency and an extensive lignification on the inner walls of the innermost layer of velamen and on the outer walls of the exodermal cells were recorded in populations located in Nallamalai forests as compared to those of Srikakulam area in Andhra Pradesh. It is interpreted to be associated with conservation of water. RAPD and protein profile data indicate the inter population diversity in between these two sites. This can be attributed to the ecological and climatic conditions prevailed in these two reference sites of eastern ghats of India.

Poster Presentations

12-P-1

Optimum Conditions for Seed Germination of *Phalaenopsis* 'Silky Moon'

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Keywords: *Phalaenopsis*, seed germination

Abstract

A study on the optimum capsule-age and the suitable medium for seed germination of *Phalaenopsis* 'Silky Moon' was determined. Seeds from 2–4 month-old capsules were cultured on liquid and semi-solid Murashige and Shoog (MS), Vacin and Went (VW) Hyponex (H) medium including modification of the three media. It was found that seeds from a 3-month-old capsule provided the highest percentage of germination at 98.65% on modified liquid Hyponex medium supplemented with 2 g/L peptone, 100 g/L potato juice and 1 g/L activated charcoal. However, seeds from a 4-month-old capsule showed the highest numbers of plantlet regeneration on the same medium.

12-P-2

Simple Media for Orchid Seed Germination

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Keywords: simple media, orchid, protocorm, fertilizer

Abstract

The simple media which composed of different commercial fertilizers available in market and natural substances were formulated in order to reduce medium cost and time consuming of orchid seed germination. The 15 modified MS media were tested for germinating *Dendrobium* orchid seed compared with MS medium. The development of orchid seed to protocorm was observed within one months on the W1 medium which composed of 1.35 g/L of Weethong fertilizer (20-20-20), MS micronutrients and 30 g/L of sugar while the germination of seed on the P1 and Z1 media which composed of 2 g/L of Pokon (21-21-21) and 2.5 g/L of Zingnong fertilizer (15-15-10) in respectively were not found. Additionally, the supplement of coconut water and/or banana in those of such modified MS media can increase more complete development of orchid seed. Interestingly, the Zingnong fertilizer can replace MS macro and micronutrients to germinate orchid seed even longer time was required. The results obtained here indicated at least 7 modified MS media (W3, P2, P3, P4, W1, W2 and W4) can use as simple media for germination of orchid seed.

12-P-3

Effect of Flower Stalk Stage, Lateral Bud Position and 6-Benzyladenine Concentration on Micropropagation of *Phalaenopsis* Cultivars

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Abstract

Using lateral buds on flower stalk for *in vitro* shoot proliferation has become important in *Phalaenopsis* industry therefore factors influence the production efficiency of flower stalk needed to be clarified more. Lateral buds taken from various developmental stage of flower stalk (stage 1-visible of flower stalk, stage 2-visible of first flower, stage 3-opening of first flower, stage 4-visible of last flower, stage 5-opening of last flower, stage 6-senescence of first flower, stage 7-senescence of last flower) of *Phalaenopsis* Sunrise Spring 'SRM153' were cultured on a medium containing VW basal salts, B5 vitamins, 20% coconut milk, 5 mg/L BA and 0.2% active charcoal for shoot induction. Four types of differentiation including vegetative shoot, reproductive shoot, protocorm like body and shoot remaining dormant were observed for the shoot induction. Among seven stages of flower stalk, lateral buds from the stage 2 had the highest (90-100%) shoot induction rate along with better shoot growth quality. Effect of lateral bud position at flower stalk was further investigated using the stage 2 flower stalk of *Phal.* JAP042Y 'T343'. Explants were indicated as shoot tip of inflorescence and the numbed lateral bud beneath the first visible flower. Explants were cultured on a medium containing 1/2 MS basal salts, 0.1 mg/L NAA in combination with various BA concentrations (1.25, 2.5, 5, 10 and 15 mg/L). Results showed that both BA concentration and position of lateral bud at flower stalk had significant influences on shoot induction. Explants of shoot tip could only induce a single small shoot with short stem whereas bud closing to differentiated flower tended to grow into a reproductive shoot before its turning to a vegetative shoot. Shoots induction increased along with BA concentrations and no dormant bud was found at 10 mg/L BA concentration for lateral buds taken from any positions.

12-P-4

Effect of 6-Benzyladenine Concentration and Explant Types Derived from Flower Stalk on Micropropagation of *Phalaenopsis* Cultivar

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Keywords: *Phalaenopsis*, flower stalk, micropropagation

Abstract

Four types of explants were initiated from lateral buds of *Phalaenopsis* (Taisuco Snow x Wataboushi) 'T343' flower stalk at stage of invisible the first flower bud. It included adventitious shoots, leaves or roots from adventitious shoots, and stem explant with 3 mm in length after the adventitious shoot was cut out. Derived explants were tested on a medium containing 1/2 MS basal salt, 0.1 mg/L NAA in combination with BA concentrations (1.25, 2.5, 5.0, 10 and 15 mg/L) for its consecutive shoot proliferation capability. Results showed that BA concentration as well as the derived explants had significant effects on shoot production. Short stem section without leaf derived from adventitious shoots was found with high rooting rate (50-70%) at 0.0-1.25 mg/L BA whereas more shoots produced after BA concentrations increased. Leaf and root explants was found no response at lower BA treatment nevertheless PLB produced in 20 and 33%, respectively when BA concentration up to 10 mg/L. Explants with the short stem section were found having surviving rate between 62.5-100% and grown into four types of regenerants (single or tufted vegetative shoot, shoot with protocorm like body (PLB) or shoot with callus-like tissue) among various BA treatments. Although shoot production increased along with BA concentration with the highest 4.8 shoots/explant at 10 mg/L BA, shoot with callus-like tissue and tufted shoot increased to 41% and 50%, respectively at the highest level of 15.0 mg/L BA.

12-P-5

Effects of Organic Supplements in Local Fertilizer Medium on Growth and Development of *Phalaenopsis* 'Silky Moon' Protocorms

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Keywords: *Phalaenopsis*, organic compound, fertilizer, micropropagation

Abstract

Seed-derived protocorms of *Phalaenopsis* 'Silky Moon', approx 0.005 g, were used as explants for protocorm-like bodies (PLBs) and plantlets induction. Basal culture media contained 2.75 g/L Viking Ship (10N-20P-30K), local commercial fertilizer, supplemented with 22.2 μ M N⁶-Benzyladenine (BA), 20 g/L sucrose, 1.0 g/L activated charcoal and 2.0 g/L Phytigel. Effects of organic supplements on growth and development of explants were investigated by adding 100 g/L boiled potato juice, 2 g/L peptone, 10 g/L polyethylene glycol, 10 mg/L adenine sulphate, 6 g/L malt extract agar or 150 mg/L glutamine in basal medium with different combinations. All explants were cultured for 18 weeks. The basal medium containing glutamine with peptone and that containing glutamine, peptone with adenine sulfate provided high number of plantlets (5.1 - 6.2) with significant higher total fresh weight (FW) (1.064–0.943 g), shoot FW (0.598 and 0.518 g) and root FW (0.466 and 0.425 g) than other media did. The shoot FW and root FW ratios obtained from the both media were 1.22 and 1.28, respectively. Although the highest number of plantlets (6.0 and 9.7) and number of protocorm-like bodies (PLBs) (6.4 and 5.0) were found from the basal medium containing boiled potato juice with peptone and that containing boiled potato juice, malt extract agar, adenine sulphate with polyethylene glycol (PEG), respectively, the shoot FW and root FW ratios were very high at 4.05 and 2.06, respectively. Effects of other organic supplements on PLBs and plantlets formation were discussed.

12-P-6

Multiple Shoot Induction from Seed Derived Shoots of *Paphiopedilum* Hybrid

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Keywords: *Paphiopedilum*, multiple shoot induction, micropropagation

Abstract

Shoots, derived from seeds, of *Paphiopedilum* 'Delrosi' were used as explants for multiple shoot induction. Modified Hyponex medium was supplemented with N⁶-benzyladenine (BA), thidiazuron (TDZ) or kinetin (Kn) alone and in combinations with 2,4-dichlorophenoxyacetic acid (2,4-D) or adenine sulphate. All explants were cultured for 15 weeks. It was found that TDZ alone at the concentration of 0.45 μ M and 4.52 μ M 2,4-D in combinations with 0.45 μ M TDZ or 4.44 μ M BA or 4.65 μ M Kn promoted multiple shoots. High shoot sprouting efficiencies (76.92, 84.62 and 76.92%) and new shoot numbers (1.69, 1.46 and 1.38) were obtained from medium containing 0.45 μ M TDZ, 4.65 μ M Kn with 4.52 μ M 2,4-D and 4.44 μ M BA with 4.52 μ M 2,4-D, respectively. Fresh weight, height, numbers of leaf and root of initial plants and new shoots were discussed.

12-P-7

Effect of Culture Vessel Type on *In Vitro* Propagation of *Aerides falcata* Lindl.

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Keywords: *Aerides falcate*, vessel culture, micropropagation

Abstract

The *in vitro* propagation of *Aerides falcata* Lindl. in semi-solid medium was compared in various size of test tubes (10x75 mm, 13x100 mm, 16x150 mm and 25x150 mm) and glass culture bottle (4 x 6 cm). Individual shoots, six months old were cultured on MS medium in difference type of vessels. After 2 months of culture, the mean height of shoots in the test tube (2-3 cm height) was significantly greater than that of shoots in glass culture bottles (0.5-1 cm height). The highest plantlets were archived using 25x150 mm test tube. Thus the type of vessel used influenced the *in vitro* plantlet growth of *A. falcata* in semi-solid culture.

12-P-8

***In Vitro* Conservation of *Dendrobium draconis* Rchb. f., Under Minimal Growth Conditions**

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Keywords: *Dendrobium draconis*, *in vitro* conservation, minimal growth

Abstract

A protocol for *in vitro* storage of *Dendrobium draconis* Rchb. f., under minimal growth conditions was established for conservation of the genetic diversity of orchid. The response to different modified Murashige and Skoog (MS) medium was examined using *D. draconis* collected from northern Thailand. Seeds were cultured on MS medium supplemented with 20 g/L sucrose. Three-month old protocorms were transferred to minimal growth medium. *In vitro* culture could effectively be conserved at 25 ± 2 °C for 12 month on 1/4 strength MS medium without an intervening subculture. The shoots on this medium exhibited very slow growth and rarely developed another shoot. The culture was also free of contamination. The conserved shoots were subsequently recovered on MS medium and multiplied normally in MS medium with 1.0 mg/L BA. The rooted shoots were successfully transferred to the nursery.

12-P-9

Preliminary Study on Seed Germination of *Sirindhornia* (Orchidaceae) Endangered Orchid from Thailand

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Keywords: *Sirindhornia*, seed germination, terrestrial orchid

Abstract

The objective of this study was to find an appropriate method for seed germination of *Sirindhornia mirabilis* H. A. Pedersen & Suksathan and *S. monophylla* (Collett & Hemsl.) H. A. Pedersen & Suksathan. To determine stages of seeds suitable for *in vitro* germination, we divided the capsules into two groups; immature and mature. It was found that while *S. mirabilis* mature capsules did not germinate *in vitro*, the immature capsules could develop to protocorms within 3 months in VW medium. For *S. monophylla*, the protocorms were obtained from the mature capsules in 18 months but not from the immature ones. The germination of these orchids was also observed in their natural habitats for 1.5, 3, 6, 9, 12, 15 and 18 months. We were able to conduct a study of orchid germination *in situ* and observe seasonal growth and mortality of the seedlings. It was found that only *S. mirabilis* seeds could develop to the whole plants within 18 months of sowing. This technique could be useful for the study of terrestrial orchid ecology.

Micropropagation of Three Cultivars of *Cattleya* Hybrids through *In Vitro* Culture

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Abstract

Three cultivars of *Cattleya* hybrids were taken for the study and from each cultivar leaf base and TCL explants were used. For PLB induction both the explants responded similarly and the best medium for large number of PLBs induction was MS + 2% (w/v) sucrose + 1.5 mg/L BA + 0.5 mg/L NAA + 10% (v/v) CW + 2 g/L peptone + 1 g/L activated charcoal + 2.2 g/L gelrite, on which 8.0-9.5 and 8.5-9.5 PLBs were induced from leaf base and TCL explants, respectively, within 12 weeks. Subculture of PLBs for 8 weeks on the same nutrient medium enhanced the number of PLBs up to 45. For proliferation of PLBs, development of PLBs into shoots as well as formation of secondary PLBs, the best medium as determined was MS + 2% (w/v) sucrose + 10% (v/v) CW + 2 g/L peptone + 1 g/L activated charcoal + 150 mg/L L-glutamine, on which during the first 8 weeks PLBs were proliferated up to 214.6-220.0 and 212.5-220.5 in leaf base and TCL derived cultures, respectively. Keeping the cultures on the same medium for further 4 weeks, the PLBs were developed into leafy shoots and huge number of secondary PLBs were induced. For plantlet formation the best medium was 1/2 MS + 2% (w/v) sucrose + 10% (v/v) CW + 2 g/L peptone + 1 g/L activated charcoal + 50 g/L banana pulp, on which cent percent shoots rooted within 8 weeks. The pH of all the categories of cultures were maintained at 5.6 before autoclaving and the cultures were incubated at 2000-3000 lux light intensity for 16/ 8 hour light/dark period at $24 \pm 2^\circ\text{C}$. For acclimatization of regenerated plantlets 6-hour intervals of misting for 30 days was optimal for highest percentage (80.8-86.6%) of survival of plantlets. For rearing and establishment of plantlets the best fertilizers were 30N-10P-10K in 3 g/L at 10-day inter intervals for the first 12 months followed by 20N-20P-20K (3 g/L) solution spraying at 10-day intervals. Regenerated plants blossomed in three years.

12-P-11

Micropropagation of *Rhynchosytilis retusa* (Lin.) Blume through *In Vitro* Culture and their Establishment in the Nursery

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Abstract

For high frequency regeneration of *Rhynchosytilis retusa* leaf base segment explant was found to be the most suitable. Half strength MS + 3% sucrose + 1.5 mg/L BA + 0.5 mg/L NAA + 2 g/L peptone + 15% (v/v) coconut water (CW) + 0.5 g/L activated charcoal (AC) was the best, on which 20.5, 16.2, and 21.1 micro shoots-protocorm like bodies (PLBs) were induced from leaf base, nodal segments and thin cell layer (TCL) explants, respectively, within 12 weeks. Subculture of micro shoots-PLBs for 8 weeks on the same nutrient medium enhanced the number of micro shoot-PLBs up to 150, 100 and 80, respectively, from leaf base, nodal segments and TCL derived cultures. For proliferation of micro shoot-PLBs, their development into shoots as well as formation of secondary micro shoots and PLBs, the best medium determined was 1/2 MS + 3% sucrose + 2 g/L peptone + 15% (v/v) CW + 0.5 g/L AC + 150 mg/L L-glutamine, on which, during 8 weeks, micro shoots elongated to shoots, PLBs proliferated and developed into shoots and new micro shoots-PLBs were induced from the base of the old ones. For plantlet formation with induction of roots the best medium was 1/2 MS + 3% sucrose + 2 g/L peptone + 15% (v/v) CW + 0.5 g/L AC + 50 g/L banana pulp, on which 93-94% shoots rooted within 8 weeks. The pH of all the categories of cultures were maintained at 5.6 before adding 2.2 g/L gelrite and autoclaving, and the cultures were incubated at 2000-3000 lux light intensity for 16/8 hour light/dark period at $24 \pm 2^\circ\text{C}$. Regeneration of plantlets was continued by repeated subculture of micro shoots-PLBs. For acclimatization of regenerated plantlets, 6-hour intervals of misting for 30 days was optimal for highest percentage (93-94%) of survival of plantlets. For rearing and establishment of plantlets the best fertilizers were 30N-10P-10K in 3 g/L at 10-day intervals for the first 12 months followed by 20N-20P-20K in 3 g/L spraying at 5-day intervals. Regenerated plants blossomed in three years. Within the first 36 weeks after initiation of culture 1500 plantlets were obtained from a single explant of leaf base segment. Repeating the subculture of micro shoot-PLBs on the proliferating medium and subsequently on the rooting medium, 13500 plantlets could be produced next every 24 weeks, which would not only be applicable in commercial aspect but also conservation of this important indigenous orchid species.

12-P-12

***In Vitro* Asymbiotic Germination and Development of Chilean Native Terrestrial Orchids**

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Abstract

Seeds of terrestrial Chilean native orchids were collected in a natural stand, surface disinfected and grown *in vitro* on a gellified growth medium in absence of symbiotic fungi. Here we present different genera and species of Chilean Terrestrial Native Orchids growing from seeds under *in vitro* and asymbiotic conditions. We compare both the effects of different Culture Media, like light conditions. Immature capsules with immature seeds were superficially sterilized with Clorox 30% solution plus Tween 20 during 18 minutes, and to mature seed a Clorox 10% solution plus Tween 20 during 4 minutes was applied, then they were washed several times with double distilled water. We compare Knudson, Gamborg and NLN media with the same hormones, amino acid and vitamins concentration. We left one half in dark conditions and the other half in a photoperiod 16/8 and 20°C/19°C ±2.

C. lutea is one of the more difficult species to growth under the same *in vitro* conditions. They need one year to produce plantlets. One month under the same *in vitro* conditions, immature seeds of *Gavilea glanduliphera* formed the first protocorm-like bodies. And we observed they have the biggest protocorm in respect to the other species.

We measured both size of immature and mature seeds. Same time we measure both length and wide of protocorm. Immature seeds measurements were done each month, and mature one each 15 days. Same time samples of different states of germination were analyzed under optic microscopic with the aim of comparing morphological changes between these species.

Mature seeds from eleven species different were collected from VIII – XI regions in the south of Chile. We measured both length and width of seminal cover and protocorm-like bodies.

By comparing size of the same species of *G. odoratissima* coming from different two geographical points 38° 46' S 72° 38' W 38° 46'S (IXth Region) and 36° 47' s, 73° 7' W (VIIIth Region), they show a significant different size.

C. reflexa embryos grew to form adventitious protocorm, irrespective of seed treatment, followed by plantlet formation. Plantlets were successfully acclimatized and transferred to soil conditions. Asymbiotic *in vitro* culture is therefore shown as a potential tool to produce viable plantlets for use in natural site restoration.

12-P-13

Rapid Mass Propagation through Multiple Shoots Induction from Rhizome of Chinese Cymbidiums

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Abstract

In vitro plantlets propagated usually from the rhizomes in the Chinese cymbidium cultures. For establishment of multiple shoot proliferation and plantlet regeneration, we cultured rhizomes of Chinese cymbidiums including *C. sinense*, *C. formosanum* and *C. faberi* in liquid media. We used MS basal medium with cytokines or auxin at different concentrations to compare the number and the rate of shoot regenerated on the rhizome of these. The shoot formations were not observed in *C. formosanum* and *C. faberi*. In *C. faberi*, nodular-like structures were observed on the differentiated into shoots for prolong culture. In *C. sinense*, shoots can differentiate from rhizome sections in liquid medium containing BA and/or TDZ, while enlarged rhizome in the medium containing NAA alone. Shoots differentiation was enhanced supplemented with TDZ in contrast to BA addition; whereas the growth of shoot is better in medium with BA. The multiplication of rhizome is better when the culture medium containing NAA. This research offers a new path of *in vitro* mass proliferation from rhizome in Chinese cymbidiums.

12-P-14

Chitosan Stimulates Growth of Micropropagated *Dendrobium* Plantlets

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Keywords: *Dendrobium*, chitosan, triacontanol, melissyl alcohol, tissue culture, growth, micropropagation

Abstract

Chitosan, derived from the cell walls of fungi, crustacean exoskeletons, cuticles of insects and some algae, has been proposed to have growth promoting activities in several plants. The effect of chitosan on growth of *in vitro* cultured *Dendrobium* plantlets was evaluated at various concentrations, 50, 250 and 1,250 mg/L, in modified VW medium. It was found that at low concentration of 50 mg/L, chitosan significantly enhanced the plantlet growth. The number of shoots, leaves and roots of plantlets increased 2.3-, 3.6- and 1.8-fold, respectively, compared to control (without chitosan) at 12 weeks of culture. However, no stimulatory effect of chitosan was observed at higher concentrations. Supplementation of triacontanol (TRIA) at the concentration of 2 mg/L to the medium containing 50 mg/L chitosan led to the reduction in fresh weight, shoot diameter and leaf width, but the increase in leaf length of plantlets in comparison with the addition of chitosan alone. These results substantiate the stimulatory effect of chitosan on growth of *in vitro* cultured *Dendrobium* which could be beneficial for commercial micropropagation.

12-P-15

Effects of Culture Media and Capsule Maturity on Seed Germination of Intraspecific and Interspecific Crosses in the Genus *Calanthe*

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Keywords: *Calanthe triplicata*, *Calanthe masuca*, seed germination, capsule maturity, intraspecific cross, interspecific cross

Abstract

In order to overcome the low percentage of seed germination in hybridization within and between species in the genus *Calanthe*, intraspecific crosses (sib crosses) of *Cal. triplicata* and *Cal. masuca*, and interspecific crosses of *Cal. triplicata* x *Cal. masuca*, seeds at different capsule maturity were harvested and cultured in Hyponex #1 basal medium and basal medium supplemented with 50 g/L banana, 150 ml/L coconut water, 50 g/L potato, 2 g/L tryptone, 2 g/L peptone, 1 ppm NAA and 1 ppm kinetin, respectively. Capsules of sib crosses were harvested on 144, 155, 167, 173, 179, and 188 days after pollination for *Cal. triplicate*, and 91, 112, 129, 149, and 180 days for *Cal. masuca*. Capsules of interspecific crosses were harvested on 91, 130, 151, and 155 days after pollination for *Cal. triplicata* x *Cal. masuca*, and 94, 139, 160, and 175 days after pollination for the reciprocal. In intraspecific crosses, the highest percentage of seed germination in *Cal. triplicata* was 25.7 % for seeds from capsule harvested on 188 days after pollination and cultured on basal medium, while that in *Cal. masuca* was 28.5% for seeds from capsules harvested 91 days after pollination and cultured on basal medium + 1 ppm kinetin. In interspecific crosses, the highest percentage of seed germination was 9.3% and 10.9% for seeds from capsules harvested on 151 and 94 days after pollination and cultured on basal medium + 2 g/L peptone in *Cal. triplicata* x *Cal. masuca* and its reciprocal, respectively.

12-P-16

Germination and Seedling Development of Some *Phalaenopsis* Species

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Keywords: asymbiotic germination, medium, moth orchid, protocorm

Abstract

Phalaenopsis has become one of the most important potted plants in the world. In order to create novel flower colors and types, various species have been introduced in the breeding programs. For the conservation and sustainable utilization of the germplasm, here, we investigate the effect of medium composition on the asymbiotic germination and seedling development of five *Phalaenopsis* species. Seeds of these five species germinated well on 1/10, 1/4 and 1/2 MS basal media. However, the survival percentage of young protocorm is different depending on the species and the concentration of MS basal media. The optimum protocorm survival for *P. aphrodite* var. *formosa* and *P. philippinensis* was found in 1/10 MS medium; but the protocorms became necrosis seriously in 1/2 and 1/4 MS media. For *P. appendiculata*, the protocorm survival was significant higher in 1/2 MS medium than the others. For *P. lindenii* and *P. viridis*, the optimum protocorm survival was found in 1/4 and 1/10 MS media. From the results of this study, the requirements of asymbiotic seed germination and development *in vitro* are dissimilar with the modern hybrids. The various responses to the basal salt concentrations among these species may be related to the diversity of their natural habitats.

12-P-17

A New Hybrid Genus *Chenara* (Orchidaceae) from the Cross between *Doritaenopsis* and *Paraphalaenopsis*

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Abstract

Doritaenopsis is an artificial hybrid genus between *Phalaenopsis* and *Doritis*. Both *Phalaenopsis* and *Doritaenopsis* are important economic orchids in the world. To date, thousands of *Doritaenopsis* varieties have been bred and commercialized. In order to introduce the unique characteristic of the flower color in brown of *Paraphalaenopsis labukensis* into *Doritaenopsis* germplasm, intergeneric hybridization between *Doritaenopsis* Ruey Lih Beauty (♀) and *Paraphalaenopsis labukensis* (♂) were conducted by artificial pollination. After four months later, the capsules were harvested. The immature embryos with placenta were manipulated and transplanted into germination medium. Approximate 500 intergeneric embryos for each capsule were rescued. After subculturing for one year in the culture medium and two-week hardiness, intergeneric hybrids were cultivated in greenhouse. After one and half year cultivation, the first flower of the hybrid was bloomed. The new hybrid was named as *Chenara* Coffee Red and registered at the Royal Horticulture Society. This is a new hybrid genus belonging to a trigeneric hybrid, including *Paraphalaenopsis*, *Doritis* and *Phalaenopsis*, and named for professor Dhing-Hsia Chen in Taiwan. The morphology in leaves of the hybrid is lanceolate, coriaceous and purplish dark green. The first flower color of the hybrid is red with light brown.

12-P-18

A New Hybrid Genus *Chouara* (Orchidaceae) from the Cross between *Angraecum* and *Doritaenopsis*

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Abstract

Doritaenopsis is a hybrid genus between *Phalaenopsis* and *Doritis* based on artificial pollination. Both *Phalaenopsis* and *Doritaenopsis* are important economic orchids in the world. To date, thousands of *Doritaenopsis* varieties have been bred and commercialized. In order to introduce unique characteristics of the spur structure from the flower of *Angraecum* into *Doritaenopsis* germplasm, intergeneric hybridization between *Angraecum sesquipedale* (♀) and *Doritaenopsis* Ruey Lih Beauty (♂) were conducted by artificial pollination. After three to four months later, the capsules were harvested. The immature embryos with placenta were manipulated and transplanted into the germination medium. Approximate 200 intergeneric embryos for each capsule were rescued. After subculturing for one and half years and hardiness, the intergeneric hybrids were cultivated in greenhouse. After two-year cultivation, the first flower of the hybrid was bloomed. The new hybrid was named as *Chouara* Kaohsiung Dream and registered at the Royal Horticulture Society. This is a new hybrid genus belonging to a trigeneric hybrid, including *Angraecum*, *Doritis* and *Phalaenopsis*, and named for professor Chang-Hung Chou in Taiwan. The morphology in leaves of the hybrid is lanceolate and greenish. The first flower of the hybrid is red and bears a short spur on the back of the lip.

12-P-19

Comparison of Chloroplast Genomes from Two Native *Phalaenopsis* Orchid Species in Taiwan

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Keywords: *Phalaenopsis aphrodite* subsp. *formosana*, *Phalaenopsis equestris*, chloroplast genome

Abstract

There are two native *Phalaenopsis* orchid species, *Phalaenopsis aphrodite* subsp. *formosana* and *Phalaenopsis equestris* in Taiwan. Previously, we have determined the complete nucleotide sequence of the chloroplast genome from *Phalaenopsis aphrodite* subsp. *formosana*. To further determine the chloroplast genome sequence from *Phalaenopsis equestris*, in this study, we have screened the Bacterial Artificial Chromosome (BAC) libraries of *Phalaenopsis equestris* with mixed probes from three plastid-coding genes *ndhB*, *psbA* and *rbcL*. Twenty out of forty BAC clones which potentially contain plastid genomic DNA was further hybridized with individual probe from dispersed plastid-coding genes of *Phalaenopsis aphrodite*. One BAC clone which can hybridize to most probes was identified and subsequently sequenced by Roche 454-FLX pyrosequencing. Eleven contigs which sequences are highly homology to plastid genomic DNA of *Phalaenopsis aphrodite* was obtained. The gaps among contigs have been filled by PCR method. The total length of chloroplast genome in *P. equestris* is 148,960 bp. The genes in chloroplast genome are currently under annotation. Comparative chloroplast genomes between two native Taiwan moth orchids will be performed in near future.

12-P-20

Variation in DNA Content in the Cultivated *Lycaste* and Related Species

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Keywords: DAPI, *Lycaste*, flow cytometry, ploidy level

Abstract

Estimation of ploidy level is one of the important factors for plant breeding. When ploidy level of parental somatic cell is even number, normal reduced gamete are mainly produced by meiosis; when ploidy level of parental somatic cell is odd number, aneuploid gamete are often formed and that is one of the causal factors to obtain many sterile seeds or unexpected morphologic plants. Variations in DNA content between species or between cultivars within an ornamental crop are known and can be caused by differences in ploidy level or, in C-value among species, by taxonomic differences. Flow cytometric analysis is useful methods to measure DNA content. However, estimation of ploidy level from DNA contents is sometimes difficult in orchids because interspecific or intergeneric crossing using species with different C-value as parents have been performed to produce novel cultivar.

In breeding of *Lycaste*, interspecific or intergeneric crosses, the crosses between hybrids and the backcrosses with major species, e.g. *Lyc. skinneri*, used as a recurrent parent are also performed to produce novel cultivars. The estimation of ploidy level is essential for efficient further breeding of *Lycaste*. In flow cytometric analysis, a small variation in DNA content between diploid species of *Lycaste* or related genus was observed, up to a difference of 9.2% in DNA content between *Ida fulvescens* and *Lyc. dennigiana*. Seven cultivars in *Lycaste skinneri* showed 1.5 times or 2 times larger than diploid and these were estimated as triploid or tetraploid, respectively. According to small variation of C-value among species, estimation of ploidy level was possible by flow cytometric analysis. Variation of ploidy level was also found between cultivars. Ploidy level of cultivars was consisted of diploid, triploid, tetraploid, octaploid and aneuploid by flow cytometric estimation. Therefore the flow cytometric analysis is useful to estimate ploidy level in cultivars of *Lycaste*.

12-P-21

Karyomorphological Observation on Some *Paphiopedilum* Hybrids

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Keywords: chromosome, lady's slipper orchid, karyotype, *Paphiopedilum*

Abstract

Paphiopedilum is known as lady's slipper orchid that is valuable in horticultural market. Numerous interspecific hybridizations have been used for breeding novel cultivars in *Paphiopedilum* for more than one hundred years. The chromosome constitution of *Paphiopedilum* species varies markedly ($2n = 26-42$) which makes the karyotype of the hybrids more complicated. Understanding the karyomorphological information of the hybrids is useful for the subsequent breeding programs. In this study, we investigated the primary hybrids of *P. glaucophyllum* x *P. spicerianum* ($2n = 33$), *P. Leanum* ($2n = 28$), *P. Woluwense* ($2n = 26$), *P. Vanda M. Pearman* ($2n = 26$), and *P. Vangard* ($2n = 30$); the Maudiae-type hybrids of *P. Alma Gavaert* ($2n = 32$), *P. Starr War 'Eureka'* ($2n = 34$) and *P. Laser* ($2n = 34$); the multiflora hybrid of *P. Lebeau* x *P. rothschildianum* ($2n = 41$) the complex hybrid of *P. Formosa Lily* ($2n = 26$).

12-P-22

Plant Regeneration from Callus Culture of an Endangered Orchid, *Geodorum densiflorum* (Lam.) Schltr.

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Keywords: protocorm, embryogenic callus, protocorm-like body (PLB), 2,4-dichlorophenoxyacetic acid (2,4-D), 1-phenyl-3-(1,2,3-thiadiazol-5-yl) urea (TDZ)

Abstract

An *in vitro* method was developed to regenerate plantlets from protocorm-derived callus of *Geodorum densiflorum* (Lam.) Schltr. cultured on half-strength Murashige-Skoog medium with 2,4-dichlorophenoxyacetic acid (2,4-D) 3-5 mg/L and 1-phenyl-3-(1,2,3-thiadiazol-5-yl) urea (TDZ) 0.1-0.5 mg/L in the dark. The proliferation rate of the pale-yellow embryogenic callus was 3.9 to 4.2 fold in mediums containing 2,4-D 3 mg/L and TDZ 0.1-0.5 mg/L after one month of culture in the dark. The callus was regenerated to plantlets via protocorm-like bodies (PLBs) on medium containing TDZ 0.5 mg/L after 6 months of culture, and 644 PLBs and 56 shoot buds per 0.1 g callus were obtained after 12 months of culture. The well-rooted plantlets with pseudobulbs were transfer to sphagnum-containing pots and acclimatized in the greenhouse. The embryogenic callus of the endangered orchid, *Geodorum densiflorum* (Lam.) Schltr. has been established.

12-P-23

Sheath and Root Rot of *Phalaenopsis* Caused by *Fusarium solani* in Taiwan

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Keywords: *Nectria haematococca*, perithecia, disease survey, pathogenicity

Abstract

This study described that *Fusarium solani* (teleomorph *Nectria haematococca*) caused sheath and root rot of *Phalaenopsis* in Taiwan. *Phalaenopsis* is the one of the most popular flower crops in Taiwan as well as in the world. Recently, a new fungal disease widely occurred in orchid gardens of *Phalaenopsis* and caused serious economic loss to the industry. The disease incidence of some susceptible varieties of *Phalaenopsis* was as high as 30%-60% in certain gardens. The diseased plants showed symptoms firstly on lower leaves which turned yellowing with sheath black rot, and the diseased leaves defoliated after a short period of time, and eventually the infected orchid plants died. Usually, several small red granules (perithecia) with white mycelia were observed on the discolored sheath surface. In some cases, the disease lesions extended to pseudostems and roots which displayed brown to black rot with perithecia on surface. *Fusarium solani* was isolated from 80% of the diseased tissues. Disease symptoms induced by an isolate FST1 of the suspected candidate fungus in greenhouse were identical to those in the fields. Meanwhile, *F. solani* was recovered from the artificial inoculated diseased tissues. The perithecia of *N. haematococca* (sexual stage of *F. solani*) with salmon color were formed when single spores of FST1 were cultured on PDA at 24 °C for 2-3 weeks. Up to date, only a few reports described the similar symptom caused by *Fusarium* spp. Except *F. solani*, *F. oxysporum* was also reported causing *Phalaenopsis* yellowing leaf in Taiwan. In addition, *F. solani*, *F. oxysporum* and *F. proliferatum* causing root and/or basal stem rot of moth orchid have been reported in Korea and *N. haematococca* inducing *Phalaenopsis* necrotic blight in Japan. However, the symptoms of root rot and perithecia produced on discolored root surface in Taiwan were different from those in Korea and Japan.

12-P-24

The Role of Pollen Germination on Hybridization of *Oncidium*

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Keywords: Oncidiinae, pollen viability, cross pollination, capsule, breeding

Abstract

Some factors are proved to affect the pollination of orchid plants. Pollen germination is one of important factors to decide the success of seed production. The germination of male pollen derived from 10 parents of commercial *Oncidium* varieties was measured by microscope one to ten days after incubation at 24 °C. Data showed that the percentage of pollen germination of 5 varieties such as *Oncidium* Hawaiian Sunset, *Oncidium* Cleo's Pride 'Hama', *Oncidium lanceanum*, *Odontonia* Lorraine's Fourteenth, and *Degarmoara* Winter Wonderland 'White Fairy' was over 70%; the percentage of pollen germination of *Brassia* Datacosa 'C.C.', *Oncidium* Sweet Sugar, and *Colmanara* Jungle Monarch 'Everglades' was 60-70%; the percentage of pollen germination of *Odontoglossum*. Wild Cat was only 25%; and the pollen of *Oncidium* Gower Ramsey did not germinate during trial period. This experiment suggested that correct selection of male parents for cross-pollination is an important thing to make breeding success. If *Odm.* Wild Cat or *Onc.* Gower Ramsey is contributed as a male parent on *Oncidium* breeding, it won't be successful. On the other hand, the male pollen derived from *Onc.* Hawaiian Sunset and *Onc. lanceanum* with higher germination percentage can be taken as the reasonable breeding materials to obtain a relatively higher success-crossing.

12-P-25

Collection, Conservation and Utilization of Taiwan Native Orchid Germplasm

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Abstract

Taiwan located in the subtropical rainy area with its special geography and diverse ecosystem resulted in abundant ecological resources comprising more than 300 orchid species according to the investigation. Many species are worth to be developed for further utilization such as ornamental and herb uses. We have put our efforts to positively collect the native orchids from middle mountain area in Taiwan. Up to date, more than 60 species of orchids were collected and the database for the characteristics of most collected orchid was readily established. Some collected native orchids can be for Chinese herb. The intermediate stage goal for this research is to find out the unique utilization of collected orchid species and to develop a method for the long-term germplasm preservation. The long-term goal is to propagate important germplasm and breed some new varieties via inter-species or inter-genera cross. Hopefully, the precious germplasm in Taiwan can be inherited and the resources of local precious native orchids enable to be well preserved through sustainable management.

12-P-26

Molecular Identification and DNA Fingerprint of *Phalaenopsis* Varieties with Plant Breeders Rights (PBR) in Taiwan Using Microsatellite Markers

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Abstract

Phalaenopsis orchid is one of the most popular ornamental plants owing to its long florescence duration and elegant floral morphology. The criteria for registration of new varieties and for obtaining Plant Breeders Rights (PBR) include the passing of a so-called distinctness-uniformity-stability (DUS) testing. It is difficult to distinguish different *Phalaenopsis* varieties based solely on morphological characteristics. Therefore, the establishment of a fast and cost-effective identification system of commercial valuable varieties of *Phalaenopsis* orchid is imperative. Microsatellites, or simple sequence repeats (SSRs), are tandemly repeated motif of 1-6 bases found in all eukaryotic genomes and are preferred powerful molecular markers based on their codominance, high level of polymorphism, high reproducibility, largely unaffected by environment, ease of detection by PCR with flanking primers and being amenable for automation and high-throughput analysis. In this study, we have collected and extracted genomic DNA of 21 *Phalaenopsis* or *Doritaenopsis* varieties, including 20 varieties with PBR in Taiwan and a reference plant used in DUS testing. 15 highly polymorphic microsatellite markers were used to genotype the 21 varieties described above. Among these, only one marker, either PeCEMS004 or PeGBMS115, was enough to discriminate the 21 varieties. The polymorphic information content (PIC) values of these two microsatellite markers were 0.93 and 0.91, respectively, indicating that they have potential for genotyping other PBR orchid varieties. Furthermore, microsatellite markers can be applied in discriminating registered variety and its reference plants with similar morphology and also in identifying individuals of intra-variety. The results will provide the efficiency applicable information for the establishment of orchid variety identification system and benefit agricultural business for Taiwan.

12-P-27

Selection of Highly Informative Microsatellites (SSRs) for Genotyping of *Phalaenopsis* cultivars

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Abstract

Microsatellites, or simple sequence repeats (SSRs), are short (1-6 bp) tandemly repeated DNA sequences. Microsatellites are widely used as molecular markers in cultivar fingerprinting, genetic diversity assessment because they are codominant, multiallelic, highly polymorphic and easily detected by PCR. *Phalaenopsis* orchid is the most valued ornamentals and considered as an important floriculture industry in Taiwan. The development of very efficient SSR markers would be useful for orchid cultivar identification and served as a complementary tool in the system of DUS (distinctness, uniformity and stability) testing to enhance the plant breeders' rights (PBRs) protection. To reach the purposes described above, developing highly informative SSR markers for *Phalaenopsis* orchids is essential. In this study, over 300 SSR markers have been characterized from cDNA, small-insert, large-insert genomic libraries and chloroplast sequences of *P. aphrodite* subsp. *formosana* and *P. equestris*. About 100 SSR primer pairs were evaluated for amplification and genetic polymorphism in 12 *Phalaenopsis* species and a subset of these with high PIC (polymorphism information content) values were further exploited for the genotyping application. The SSR markers with higher distinguishing power among 32 commercial cultivars were applied to identify the cultivars having none or very few differences in morphological descriptors. Few SSR markers were needed to discriminate 4 cultivars with white flower-red lip and 3 cultivars with large white flowers. The results indicated the SSR markers developed from *Phalaenopsis* orchids could be effective for establishing the DNA molecular system for cultivar identification and the verification technology on variety infringement.

12-P-28

Classification of *Paphiopedilum* Genus into Three Subgenera and Parental Identification of *P. delenatii* in Hybrids by ITS Molecular Marker

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Abstract

The identification of varieties involving *Paphiopedilum* has been based exclusively on their morphological characteristics, particularly the shapes and colors of leaves and flowers. However these characteristics are rather variable and inconsistent under some circumstances. We investigated the use of internal transcribed spacers (ITS) of nuclear rDNA sequences as molecular markers for the phylogenetic analysis of *Paphiopedilum*. It was discovered that by a group of specific primer sequences of ITS in polymerase chain reaction, the genus of *Paphiopedilum* can be divided into three subgenera of *Parvisepalum*, *Paphiopedilum*, and *Brachypetalum*. The subgenus of *Paphiopedilum* can be further divided into five subgroups, similar to those described by Cribb (1997). Using these ITS markers, the parental lines of a hybrid can be traced. For example, if both parents were the subgenus of *Parvisepalum*, its hybrids would be classified as *Parvisepalum* by these markers. A hybrid between *Parvisepalum* and *Paphiopedilum* would show the presence of ITS markers specific for these two subgenera. We have also developed the other specific ITS markers for the identification of all hybrids known to possess the parental origin of *Paphiopedilum delenatii*. The molecular markers we have developed would help our understanding of the genomic relationships among these three subgenera and promote the conservation and efficient utilization in the genetic resources of *Paphiopedilum*.

12-P-29

The Embryo Development and Seed Germination *In Vitro* of *Bulbophyllum fascinator*

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Keywords: asymbiotic germination, *Bulbophyllum*, suspensor, testa

Abstract

Bulbophyllum fascinator is native to the lowlands of Malaya, Laos and Thailand that has a single and attractive flower. The embryo suspensor is represented by a single cell and this cell develops precociously and continues to elongate towards the micropyle as the embryo develops. Starch grains are prominent during the early stages of embryo development. However, the starch grains disappear as the embryo matures indicating that the suspensor may serve as a temporary food storage site for the developing embryo. In mature seeds, the walls of the testa are uneven in thickness. This wall feature may enable the embryo to take up water and the nutrients necessary for germination. The optimum germination was observed when the seeds were collected between 140 to 160 DAP. The desirable germination percentages (around 90%) were observed among three different concentrations of MS media, indicating that seed germination of this species can adapt to a wide range of inorganic salt concentrations.

13-P-1

The Effect of Packaging on Shelf-life of Cut *Dendrobium* Big White Jumbo

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Keywords: MAP, orchid, ethylene, active packaging

Abstract

Modified Atmosphere Packaging (MAP) is a capable providing the different permeability in order to maintain the optimum level of oxygen and carbon dioxide in the packaging. This experiment was aim to compare the effect of perforated polypropylene (PP) bag and high TOR bag (active packaging) on vase life of *Dendrobium* Big White Jumbo inflorescences. Inflorescences were harvested at commercial maturity. Stem-ends of individual inflorescences were re-cut and inserted into plastic tube containing distilled water, then packed in PP or active packaging bag with ethylene absorber (EA) and kept at 13°C for 5 days to stimulate air shipment and thereafter they were removed from the packaging and displayed at 25°C. The concentration of carbon dioxide, oxygen and ethylene and the relative humidity (RH) in the packages were determined daily during stimulated shipment. The results showed that active packaging bag had higher CO₂, C₂H₄ and RH than PP packaging. In contrast, oxygen concentration was lower in active packaging bag. There was not different in relative fresh weight. During display life found that inflorescences packed in PP bag had lower respiration and flower drop, but higher bud opening than active packaging bag. This is resulting in the shorter displayed life of *Dendrobium* Big White Jumbo inflorescences stored in active packaging (6 days) when compared to the inflorescences stored in PP packaging (10 days).

13-P-2

Physiological Changes of Cut *Mokara* Hybrids Inflorescences

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Keywords: orchids, vase life, water uptake, senescence, ethylene

Abstract

Mokara inflorescences are one of the most famous cut orchid hybrids for Thailand exporting. The objective of this research was to study the physiological changes of *Mokara* inflorescences. Inflorescences of *Mokara* Panee, *Mokara* Red, *Mokara* Nora 'Yellow', *Mokara* Chark Kuan 'Pink' and *Mokara* Nora 'Pink' were harvested from orchid nursery at commercial maturity stage and transported to the laboratory. The stem ends were re-cut in order to make the uniform size before placing in distilled water at 25°C. Ethylene production, respiration rate, fresh weight, water uptake and bud opening were measured daily interval. Vase life was determined as 50% of floret senescence. The results showed that *Mokara* Nora Pink showed the lowest of ethylene production, *Mokara* Panee had the highest respiration and bud opening, while *Mokara* Red had the highest fresh weight and water uptake. The vase life of *Mokara* Panee, *Mokara* Red, *Mokara* Nora 'Yellow', *Mokara* Chark Kuan 'Pink' and *Mokara* Nora 'Pink' was 13, 11.6, 12.4, 12.4 and 12.7 days, respectively. This result indicated that the vase life and physiological change of cut *Mokara* inflorescences depend on the cultivars.

13-P-3

Non-Persistent Virus Transmission in Orchids by *Periplaneta australasiae*

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Keywords: Australian Cockroach, *Periplaneta australasiae*, orchid virus, *Cymbidium Mosaic Virus*, CymMV, *Odontoglossum Ring Spot Virus*, ORSV

Abstract

The project goal was to demonstrate the possibility of orchid virus transmission by a chewing insect, the Australian Cockroach (*Periplaneta australasiae*) under controlled conditions. The experiments were housed in aluminum frame screen cages to best contain the cockroaches. In each cage were placed three plants: an orchid that tested positive for orchid virus, a young clone of *Oncidium* Sweet Sugar 'Kalender' that tested as virus free, and a virus indicator plant of *Nicotiana tobacum* cv Xanthi nc. Australian Cockroaches were introduced into four of the cages. Two cages were used as controls containing the above plant material, but no cockroaches. Approximately one third of the Australian Cockroaches used were 'wild' caught in a nearby conservatory. The rest of the cockroaches were purchased from a commercial supplier. The cockroaches were communally housed for a period of one week. Assuming any of the wild-caught cockroaches were carrying orchid virus, the cockroaches' behavior of mutual grooming would distribute the virus particles. The Australian Cockroaches were housed with the plant material until sufficient feeding damage was observed. At that time the orchid virus testing was repeated on the *Oncidium* Sweet Sugar 'Kalender'. Orchid samples were initially tested at a commercial laboratory and were subjected to an orchid virus screen that identifies nine viral agents known specifically to orchids.

13-P-4

Identification and Characterization of UDP-Glucosyltransferases for Major Flavonoid and Anthocyanin Synthesis in *Phalaenopsis* Orchids

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Abstract

Phalaenopsis, also called moth orchid, is a high-value cash crop, and their variant flower color has become an important factor for commercial orchid production. Anthocyanin belongs to a class of flavonoids that is the main pigment contributing to the red, violet, and blue color of flowers. In *Phalaenopsis*, one flavonoid glucoside, 6-glucose-apigenin-7-O-glucoside (saponarin), and an anthocyanin, cyanidin 3-O- β -D-glucopyranoside-7,3'-O-di-[6-O-(trans-sinapyl)- β -D-glucopyranoside], have been identified in flowers of *Phalaenopsis* hybrids with a substantial amount. Previously, full length cDNAs encoding putative major enzymes in the anthocyanin biosynthetic pathway have been identified from floral bud EST database of *P. equestris*. These included chalcone synthase (CHS), chalcone isomerase (CHI), anthocyanidin synthase (ANS), and UDP-glucose:flavonoid 3-O-glucosyltransferase (UFGT). Most enzymes involved in the anthocyanin biosynthesis showed similar transcriptional activity in both red and white color of *Phalaenopsis* cultivars, such as CHS, CHI, ANS, PeUFGT1 and PeUFGT2. In contrast, the *PeUFGT3* was highly expressed only in the red flower but not in the white flower. UFGT catalyzes the formation of the first stable colored pigment in anthocyanin pathway by transferring glucosyl moiety from UDP-glucose to the 3-hydroxyl group of anthocyanidin. To investigate the function of these three *PeUFGT* genes, we propose a hypothesis of the major flavonoid derivatives synthesis pathway in *Phalaenopsis*, and analyze the glucosyltransferase activity of these three PeUFGT enzymes. First, recombinant PeUFGT1~3 were ectopically expressed in *E. coli*. The proper expression systems for the optimal induction of recombinant proteins have been monitored. Functional analysis and biochemical characterization of these PeUFGT enzymes were carried out and compared to one another. Finally, molecular modeling of these three enzymes was performed to further elucidate the entity of their different enzyme activities.

13-P-5

A First Survey of the Orchid (*Phalaenopsis equestris*) Genome Composition through BAC end Sequence Analysis

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Abstract

Phalaenopsis orchid is the most valued floral crop and considered as an economically important floriculture industry in Taiwan. Because of its economic importance, genome sequencing and analysis of the orchid genes became the research object for orchid improvement. BAC end sequences (BESs) provide a first glimpse of the sequence composition of an unsequenced genome and yield molecular markers useful for genetic mapping and breeding. Two complementary BAC libraries (*Bam*HI and *Hind*III) have been constructed from *Phalaenopsis equestris* and used for this study. DNA sequence generated from 1,055 BAC clones was sequenced at both ends with a success rate of 93.8%. Of these BAC clones, 67.5% and 32.5% were from *Bam*HI and *Hind*III libraries, respectively. BAC end sequencing generated 1,980 reads representing 1,975,752 bp. The trimmed sequences ranged from 114 to 1292 bp with an average edited read length of 997.85 bp. These BESs were analyzed by sequence homology searches. 14.5% of them were predicted to be protein-coding regions while 58.0% contained repetitive DNA. The gene density from *Phalaenopsis* BESs was 5.2 kb per gene. The GC content of these BESs ranged from 11% to 77% with an average of 37.1%. From protein matching regions of *Phalaenopsis* BESs, it was determined that the GC content of coding regions was 40.5%. A total of 286 potential simple sequence repeats (SSRs) were discovered. The frequency of SSRs derived from these BESs was about one SSR per 6.9 kb of genomic sequence. Dinucleotides were the most abundant motifs, and AT/TA dimer repeats were the most abundant SSRs, representing 158 (55.2%) of all SSRs. However, *Phalaenopsis* orchid has large genome and repetitive DNA sequences approximately account for 50% of the genome. Development of strategies that focuses on targeted sequencing of gene-rich regions will provide an alternative to whole-genome sequencing. Therefore, we removed BAC clones containing heterochromatin by hybridizing BAC clones with Cot-100 DNA that contains the highly repetitive sequences. Comparing the BAC end sequences from randomly selected BAC clones and those of Cot-100 selected, the percentage of clones containing repetitive sequences was reduced from 58.5% to 57.4%, the proportion of genic hits being increased from 13.6% to 16.8%. Results from this study indicate that BES analysis provides useful information about the orchid genome with respect to the abundance of genes and repetitive DNA, and these BESs could serve as valuable resources for SSR marker development.

13-P-6

High-efficiency *Agrobacterium*-mediated Transformation of *Dendrobium* by Targeting Protocorms

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Abstract

A transformation protocol for dendrobium orchid was established by using immature protocorms as a target material for *Agrobacterium* inoculation. Seeds were obtained from the cross between two elite cultivars of *Dendrobium nobile*, 'Cinderella'×'True love', and germinated on New Dogashima medium containing 10 g/L sucrose without any plant growth regulators. Protocorms produced 3 weeks after sowing were subjected to co-cultivation for 3 days with *Agrobacterium tumefaciens* EHA101 containing plasmid pIG121-Hm that harbored genes for β -glucuronidase (*gus*), hygromycin phosphotransferase (*hpt*) and neomycin phosphotransferase II (*nptII*). Two months after selection of inoculated protocorms on medium containing 20 mg/L hygromycin, some protocorms were recovered and the transformation efficiency was over 33%. Integration of transgenes was confirmed by PCR analysis and Southern hybridization. Stable expression of *gus* gene was indicated by histochemical GUS assay in the leaves and roots of transgenic plants. The high efficiency of this transformation protocol makes it possible to obtain a large number (several hundreds) of transformants in a single experiment.

13-P-7

The Growth of *Oncidium* and *Phalaenopsis* is Influenced by CymMV and ORSV Inoculation

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Keywords : *Oncidium*, virus, tissue culture

Abstract

Tissue culture derived plantlets of virus-free *Oncidium* and *Phalaenopsis* (CK) were inoculated with either *Cymbidium mosaic virus* (CymMV) or *Odontoglossum ringspot virus* (ORSV), or both (C+O). Evaluation of their growth after the virus inoculation indicated that both multiplication rate and plantlet height of *Oncidium* PLB were the highest in plantlets inoculated with C+O, followed by CK, and ORSV, respectively. However, the differences are not great. After the plantlets were transplanted and grown in a greenhouse, CK has the highest numbers in both the leaf and new bud followed by CymMV, C+O, and ORSV. Meanwhile the seedling height also follows the same order.

The multiplication rate and plantlet height of *Phalaenopsis* PLB were the highest in CK, followed by those inoculated with CymMV and C+O, respectively. After the plantlets were transplanted and grown in a greenhouse, those inoculated with C+O has the highest leaf and bud numbers, followed by CK and CymMV, respectively. However, the CK has the highest leaf width and second leaf area followed by CymMV and C+O. Real-time PCR analysis using coat protein specific gene probes of CymMV and ORSV showed that the titer of CymMV is the lowest in the distal part of leaf and the highest in the distal part of roots. The titers of CymMV in the stem base, the root proximal, and the leaf's middle and tip areas are moderate. For ORSV, its titer is the lowest in stem base, followed in ascending order by the areas in root distal, root proximal, leaf distal, leaf tip, and leaf middle, which has the highest titer.

13-P-8

Physiology of the Flowering in Commercial Varieties of Chilean Orchid of Cold Climates

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Abstract

The orchids present a great variation respect of the necessary period to begin to madurez, specifically the beginning of flowering. The *Chloreae crispa* is a Chilean endemic orchid and growth in cold climate. It has a juvenile or vegetative period of five years before emitting a floral twig. The epoch of flowering is given between October and January in the south of Chile. The long period to reach the maturity impedes the production for flowers cutting.

The phase of flowering and vegetative period is regulated by diverse routes, between which they are: photoperiod, thermoperiod, nutrients and hormonal effects. Studies show that diverse sugars (saccharose, glucose), but especially saccharose, is involved in the sign posting that will lead to the flowering. Current studies suggest that there is a connection between the levels of starch and the time of flowering, for this reason it is postulated that more amount of starch in the roots will produce more liberation of saccharose, to level of the meristem, provoking the induction of the flowering in *Chloreae*.

The objectives of this study they were to characterize morfo - physiologically mature and juvenile orchids of cold climates. Determine the effect of the luminous intensity on the rate of assimilation of carbon dioxide, establishing the relation with the content of the soluble and insoluble sugar.

The plants were placed in conditions controlled of temperature and dampness in growth chambers. The morphologic parameters evaluated were height of plant (cm), number of leaves, number of rhizome, diameter of rhizome (mm), length of rhizome.

In addition there decided the content of starch and of saccharose by means of HPLC and the assimilation of carbon dioxide, the quantification of the acid maleic. Inside the results obtained in the morphologic part one concludes that you plant it of juvenile orchids they present a minor number of rhizomes that you mature. The mature plants lose great part of his reserves of starch during him process of flowering, while the content of saccharose increases. For the case of the glucose, juvenile and matures plants, lose an average of 2.5 mg of glucose for gram of dry matter when emitted the floral rod. The night accumulation of carbon dioxide reaches 2.99 μM of $\text{CO}_2 \text{ m}^2$.

13-P-9

Influence of Foliar Chitosan Sprays on Growth of *Mokara* and *Phalaenopsis* Seedlings

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Keywords: fresh weight, leaf area, dry matter, shoot/root ratio

Abstract

Chitosan is a natural compound which acts as plant elicitor. Therefore, this study was aimed to investigate the responses of *Mokara* and *Phalaenopsis* seedlings on chitosan application. Seedlings taken from *in vitro* propagation were soaked in chitosan solution at the concentrations of 0 (control), 10, 20 or 40 mg/L for 5 min and then transplanted in coconut fiber. Thereafter, seedlings were sprayed with chitosan combined with foliar fertilizer (21-21-21) weekly. The fresh weight, number of leaves and leaf area were measured monthly, whereas dry matter of shoot and root were weighed at month twelfth after transplant. It was found that cultivar and the concentration of chitosan had significant effect on fresh weight, number of leaves and leaf area. *Phalaenopsis* seedlings treated with chitosan at the concentration of 20 mg/L had the maximum leaf area, while *Mokara* seedlings treated with chitosan at the concentration of 40 mg/L had the maximum leaf area compared to the control. Fresh weight of *Phalaenopsis* seedlings treated with 10 and 20 mg/L chitosan were higher than that of seedlings treated 40 mg/L chitosan and the control. Fresh weight of *Mokara* seedlings treated with 20 mg/L chitosan was the highest, but there was not statistically different. After 12 month of transplant, chitosan spraying significantly affected the fresh and dry weight of both *Phalaenopsis* and *Mokara* seedlings. *Mokara* seedling had higher shoot to root ratio than *Phalaenopsis*. Shoot to root ratio of *Mokara* applied with foliar chitosan at 20 and 40 were the highest (2.30 and 2.38 g/DW, respectively). Whereas, *Phalaenopsis* sprayed with chitosan at 40 mg/L had the highest dry matter compared to other concentrations.

13-P-10

The Application of Chitosan for Induce *In Vitro* Growth of *Rhynchostylis gigantea* Protocorms

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Keywords: orchid, tissue culture, seedling, VW

Abstract

One month old protocorms of *Rhynchostylis gigantea* with approximately 0.2-0.3 cm in diameter were cultured on modified Vacin and Went (VW) medium supplemented with chitosan at the concentration of 0 (control), 10, 20, 40 and 60 mg/L for two months, thereafter only seedlings at stage 4, which the plant had one leaf with 1 or 2 roots, were selected and sub-cultured on to the same medium and sub-cultured every month for six months. Growth of protocorms was measured every two months. The results showed that seedlings cultured on VW supplemented with 60 mg/L of chitosan for two months had the highest number of leaves significantly. Whereas, the seedlings cultured on VW supplemented with chitosan at 40 mg/L had the highest number of roots significantly. After four months, seedlings cultured on VW supplemented with 40 mg/L of chitosan had the highest leaf length, leaf width and leaf area significantly. Six months after cultured, seedlings cultured on VW supplemented with 40 mg/L of chitosan had the highest fresh and dry weight of shoot and number of roots significantly. VW supplemented with 10 mg/L of chitosan increased the number of leaves, plant height and root dry weight whereas, seedling grown in VW supplemented with 20 mg/L of chitosan had the longest root length.

13-P-11

Effect of Chitosan Concentration on *In Vitro* Growth of *Dendrobium* Hybrid Seedlings

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Keywords: orchid, leaf area, plant height, fresh weight, root to shoot ratio

Abstract

Chitosan has been report as plant stimulation. The objective of this work was aimed to study the effect of chitosan on *in vitro* growth of *Dendrobium* hybrid seedling. Two months old seedlings of *Dendrobium* hybrid pansy type were sorted at the same size approximately 1.2-1.5 cm in height and were cultured on modified Vacin and Went (VW) medium supplemented with chitosan at the concentration of 10, 20, 40, 60 mg/L or without chitosan (control). Measurement of number of leaf, number of root and number of new shoots were observed weekly. After cultured for three months, chitosan at 20 mg/L increased root numbers. Seedlings grown on VW supplemented with chitosan at 10 and 20 mg/L had higher number of leaves compared to other treatments. The highest number of new shoots was found in VW media supplemented with chitosan at 20 mg/L. The effect of chitosan on morphological characteristic of leaf on adaxial side found that chitosan affected numbers of stomata, but not effect on number and size of chloroplast. On abaxial side, the concentration of chitosan did not affect chloroplast size, but chitosan reduced the number of stoma.

13-P-12

Gamma Irradiation Affecting Deterioration of *Dendrobium* Sonia Bom#17

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Keywords: *Dendrobium*, deterioration, gamma irradiation, physiological changes

Abstract

Effect of gamma irradiation on deterioration of *Dendrobium* Sonia Bom#17 was investigated. A 2 kGy gamma irradiation caused the significant increase in the rate of CO₂ output that eventually reached a peak respiration of *Dendrobium* flowers in day 4. The rise of CO₂ elevation coincided with ethylene production of flowers treated with 2 kGy gamma irradiation, which was reached the maximum of ethylene production in day 4 and 1.5 times as much as ethylene production in day 2 while untreated flowers (control) had constant ethylene production until flowers senescent. In addition, high dose irradiation had effect on the tissue of flowers. Both opening and bud flowers were damaged after 2 kGy gamma irradiation. The tissue of flowers seemed to collapse, shrank and turned dark as compared to the normal tissues (control) in both opening and bud flowers. Also, the fresh weight and water uptake of flowers treated with 2 kGy gamma irradiation significantly decreased as compared to the control. Longevity of flowers treated with 2 kGy gamma irradiation was the shortest and wilted within 5 d after irradiation. Moreover, no bud openings were found and there were the most of flower drop (37.82%) while untreated flowers had the longest vase life for 13 d and the highest average number of opening flowers.

13-P-13

Preservative Solution Delays Deterioration of *Dendrobium* Sonia Bom#17 after Gamma Irradiation

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Keywords: *Dendrobium*, deterioration, gamma irradiation, preservative

Abstract

Effect of different preservative solutions on delaying deterioration of *Dendrobium* Sonia Bom#17 after gamma irradiation under simulated harvesting, packing, treating and shipping condition was investigated. It was found that untreated flowers (control) had 6 times higher respiration rate and 12.5 times higher ethylene production than flowers treated with gamma irradiation, respectively. However, flowers treated with 2 kGy gamma irradiation would become insensitive to ethylene in the presence of preservative Sol 1. The flower opening of untreated flowers in the presence of preservative Sol 3 was 76.25% maximum while no flower drop was observed in flowers treated with 1 kGy gamma irradiation + Sol 1, 2 and 3 and with 2 kGy gamma irradiation + Sol 1. Flower treated with 2.0 kGy gamma irradiation + Sol 2 and 3 had no flower opening but the flower drop reached the maximum of 32.77 and 44.16%, respectively. Additionally, untreated flowers (control) were 17 days of vase life longer than other treatments while the vase life of flowers treated with 2 kGy gamma irradiation + Sol 2 and 3 was shortest (4 d). However, the treatment of 2 kGy gamma irradiation + Sol 1 significantly extended the vase life of flowers for 6 d.

13-P-14

Phenylalanine Ammonia – Lyase (PAL) Activity of Selected *Dendrobium* Orchids

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Keywords: flowering, stages, anthocyanin, protein, anthesis

Abstract

Dendrobium hybrids occupy the foremost position in floriculture trade especially in ornamental cut flower industry because of its capability of flowering continuously and a prolonged post-harvest life when compared with other orchid species. *Dendrobium* hybrids also produce flowers in a wide range of colours. Phenylalanine Ammonia - Lyase (PAL, EC. 4.3.1.5) is an important enzyme involved in the phenylpropanoid biosynthetic pathway that leads to the synthesis of many secondary products including pigments specifically anthocyanins. In addition, anthocyanins are major pigments found in many *Dendrobium* flower petals. This study was conducted to determine the PAL activity among commercially important *Dendrobium* orchids to investigate if there is any relationship between the flowering stages and the PAL activity. PAL activity was monitored at four various stages of flowering in petals of *D. Angel White* (DAW), *D. Dragon Eye* (DDE), *D. Aredang Blue* (DAB) and *D. Emerald Green* (DEG). Flowering stage 1 is equivalent to early bud formation with no colour pigments formed, stage 2 is when the colour pigments were first observed, and the flowers were slightly opened and fully bloomed for stages 3 and 4, respectively. PAL activity was found to be the highest in *D. Aredang Blue* followed by *D. Emerald Green*, *D. Dragon Eye* and *D. Angel White*. The activity of PAL was found to be high in stage 3 and 4 for all the hybrids mentioned above. This information will contribute to the understanding of anthocyanin synthesis in orchid petals.

13-P-15

High-Performance Liquid Chromatography Profiling of Pigments from *Phalaenopsis* Hybrids and Their Contribution to Antioxidant and Antityrosinase Activities

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Keywords: *Phalaenopsis* hybrids, pigments, HPLC, antioxidant, antityrosinase

Abstract

Phalaenopsis hybrids are economically one of the most important groups of orchids which are native in Asia with white, yellow, and red flowers. In the present study, the major pigments of various *Phalaenopsis* hybrid species with red color were identified and the quantitative analyses of the crude extracts were accomplished. The compositions of flavones and anthocyanin were simultaneously detected at 375 and 530 nm with the aid of high performance liquid chromatography/ultraviolet detector (HPLC/UV). The major pigments and co-pigments characterized in different *Phalaenopsis* species were (3',7-di-*O*-sinapylglucosyl)-3-glucosyl cyanidin (1), apigenin 6-*C*-ribosido-7-*O*-glucoside (2), and saponarin (3). The differences between various *Phalaenopsis* hybrids were only the contents of the pigments. In addition, the pigment constituents were also examined for their antioxidant properties using the scavenging of the, α,α -diphenyl- β -picrylhydrazyl (DPPH) free radical assay and the antityrosinase activities.

13-P-16

Effects of Fertilizer Formulae on the Flowering of *Doritaenopsis* I-Hsin Madame in the Gradational Nutrition Management

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Keywords: nitrogen, phosphorus, potassium, reproductive growth, spike induction

Abstract

Previous reports showed that an increase in P application did not stimulate spike induction and flowering of *Phalaenopsis* when K application was sufficient, whereas an increase in N application promoted flowering. However, it was obvious that the *Phalaenopsis* received excess of N took longer to anthesis. The objectives of this research were to determine the effects of the fertilizer formulae with gradational nutrition management on the flowering of *Dtps.* I-Hsin Madame. As comparing to the invariable nutrition management, the plants had higher spike emergence rate and faster growth rate of flowering spikes after gradational nutrition management, and the plants treated with 10-20-20 and 20-20-20 fertilizers had an earlier anthesis. In the same level of K application, higher N application increased the growth of flowering spikes and the total flower numbers. The plant analysis data suggested that the spike growth of *Dtps.* was depending on the N supply. However, the higher N application decreased the petal width of flower. The plant nutrient analysis suggested that the decrease in petal width might be due to the imbalance of N and K uptake or the insufficient amount of C accumulated in the flower.

13-P-17

Induction of Plant Disease Defence and Growth of *Dendrobium* 'Eia Sakul' by Chitosan Treatments

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Abstract

One most problems for growing *Dendrobium* orchids is disease infection resulting in losses of production and quality of inflorescence. At the present, the public has been concerning about chemical residues in environment, human health, and the increasing chemical resistance of pathogens. Therefore, the objective of this experiment was to study the effects of chitosan treatment on plant disease defence and *in vitro* growth of *Dendrobium* 'Eia Sakul'. The seedling of *Dendrobium* 'Eia Sakul' was cultured on Vacin and Went 1949 (VW) medium supplied with the chitosan at the concentrations of 0 (control), 10, 20, 40 and 80 mg/L at 25°C. Chitinase and beta-1,3-glucanase activities; the enzyme associated with plant disease defence, and the growth of *Dendrodium* were then determined after culturing for 4, 8, 12, 22, 24 and 26 weeks. The results revealed that the activities of chitinase and beta-1,3-glucanase dramatically increased during cultured, and the chitosan treated *Dendrobium* showed significantly the highest activities of both enzymes on week 22, 24 and 26 when compared with non-treated. However, chitosan treatments were not significant effects on the *Dendrobium* growth; plant height, number of leaves, length of leaves, width of leaves, number of root, length of root and the number of plant per cluster. Total chlorophyll content in leaves also measured. *Dendrobium* treated with chitosan at 10 and 20 mg/L was significant higher than that of control after culturing for 24 and 26 weeks.

13-P-18

Cloning and Functional Characterizations of Sesquiterpene Synthase in *Phalaenopsis equestris* S82-159

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Abstract

Orchid is an important floral industry in Taiwan, especially the *Phalaenopsis* orchid. However, productivity has always been limited by the phytopathogens with losses surging to economically damaging levels in the case of an outbreak. Terpenoids, including monoterpenes and sesquiterpenes, are often the most common and diverse group among phytopathogen or insect-induced plant volatile. To analyze the sesquiterpene synthase (STPS) in a native species *Phalaenopsis equestris* S82-159, a full-length cDNA of sesquiterpene synthase (PeSTPS) was isolated. Its length was 1,899 bp and encoded an open reading frame of 1,647 bp for a protein of 549 amino acids. Phylogenetic analysis of plant terpene synthase genes classified the PeSTPS to TPSa subfamily. We then analyzed the volatile components in the flowers of *P. equestris* S82-159 by using the dynamic headspace technique and analyzed by GC-MS. Qualitative and quantitative results showed to be terpenes (32.2 ng/flower/h), phenylpranoids (110.9 ng/flower/h), benzenoids (19.1 ng/flower/h) and fatty acid (397.8 ng/flower/h), respectively. For non-volatiles of *P. equestris* S82-159, on the surface of all floral organs on the blooming day were detected sesquiterpenes, including (R)-cuparene, α -longipinene, ylangene, and 1,5-diisopropenyl-2,3-dimethyl-cyclohexane on the surface of all floral organs and (R)-cuparene and thujopsene on the surface of lips and pedicles. Analysis of temporal expression of PeSTPS gene in different orchid species showed that PeSTPS transcript was expressed from the early stage of flower buds to day 3 post-anthesis with a maximal expression on blooming day. Interestingly, PeSTPS was expressed in vegetative tissues and all floral organs. Enzyme activity of the recombinant PeSTPS protein was revealed for the formation of major sesquiterpene and minor monoterpene by using radio-TLC analysis. This result suggests that the PeSTPS is a multifunctional sesquiterpene synthase.

13-P-19

Study of MYB Factors Involved in the Regulating Fragrance Biosynthesis in *Phalaenopsis bellina*

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Abstract

Floral fragrance is important for plant production because it is helpful for pollinator attraction. In *Phalaenopsis bellina*, monoterpenoids are the main components of the floral scents. Previously *P. bellina* geranyl diphosphate synthase (PbGDPS) was identified to be one of the key enzyme for supplying geranyl diphosphate for monoterpenes biosynthesis. To further study the regulation of *PbGDPS* expression, three MYB transcription factors, including PbMYB1, PbMYB2, and PbMYB6, were identified from the *P. bellina* floral EST database through datamining. PbMYB1 and PbMYB2 have typical R2R3 MYB DNA binding domain, while PbMYB6 is a MYB-CC (coil-coiled) type protein. Phylogenetic analysis results place PbMYB1 and PbMYB2 in the same group involved in regulation of phenylpropanoid biosynthesis. Study on the MYB-CC type transcription factors is mainly focused on controlling of phosphate metabolism in plants. Transcription level of *PbMYB1*, *PbMYB2* and *PbMYB6* all increased at D+5 (day 5 post-anthesis), consistent with both maximal emission of monoterpenes and expression of *PbGDPS*. To study the regulation of *PbGDPS* expression by these three MYB transcription factors, a 2515-bp promoter region of *PbGDPS* was isolated and consensus DNA-binding sites for MYB transcription factors were predicted. All three MYB transcription factors revealed the ability of driving the expression of both 2-kb and 1-kb *PbGDPS* upstream regulatory sequences in the dual-luciferase assay. Both promoters of *PeUFGT3* and *PeMADS6* were recruited as negative controls. The 1-kb upstream regulatory region was further dissected to three subfragments and analyzed by using yeast one-hybrid system. Preliminary results showed that both the nearest and the most upstream fragments were able to be bound by all three MYB proteins. Detail identification of the potential *cis*-elements on these two subfragments were mapped by comparing the promoter sequences among either scent or non-scent species. Site directed mutagenesis of these sites for confirming the significance of these sites was currently underway.

Characterization of AGL6-like Genes Involved in Perianth Development of OrchidsYun-Ru LuoInstitute of Biotechnology, National Cheng
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Phalaenopsis is a member of the Orchidaceae of monocots, its flower contain three sepals and two petals and a highly modified petal, the labellum or lip. Because of lip faces to column (a fusion of the male and female reproductive organs, with stamen on the column top), it is considered to be important for both pollination and evolution of orchids. Previously, we have identified five B-class MADS-box genes of *Phalaenopsis* involved in perianth development. Among them, *PeMADS3*, *PeMADS4* and *PeMADS6* are involved in lip development; while *PeMADS2*, *PeMADS3*, *PeMADS5* and *PeMADS6* are involved in petal development. In this study, three *AGL6*-like genes including *PeAGL6a*, *PeAGL6b* and *PeAGL6c* were identified and characterized. The *AGL6* genes of *Arabidopsis* are involved in ovary development. However, the *PeAGL6a*, *PeAGL6b* are specifically expressed in lip and petal, respectively, and *PeAGL6c* was expressed in pedicel that contains ovary. Furthermore, ectopic expression of *PeAGL6a* but not *PeAGL6b* was detected in the lip-like petal of peloric mutant of *Phalaenopsis*, suggesting the involvement of *PeAGL6a* gene for lip development. In the phylogenetic analysis, *PeAGL6c* was close to *AGL6*-like genes of monocots, while *PeAGL6a* and *PeAGL6b* were excluded from *AGL6*-like genes of angiosperm. These results suggest that *PeAGL6a* and *PeAGL6b* may have been highly modified and gained neo-/sub-function during evolution. In the yeast two-hybrid analysis, we found that *PeAGL6a* was able to interact with *PeMADS3*, *PeMADS4* and *PeMADS6*. In addition, *PeMADS6* can serve as a bridge protein for *PeMADS3* and *PeMADS4* as revealed in yeast three-hybrid analysis. Our results suggest that these four proteins may form a higher-order complex to dictate the orchid lip development. Furthermore, both virus-induced gene silencing of *PeAGL6a* in orchids and ectopic expression of *PeAGL6a* in *Arabidopsis* were also carried out for both loss-of-function and gain-of-function analyses.

13-P-21

Identification and Characterization of *SEP*-like Genes in *Phalaenopsis*

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Keywords: development, orchid, *Phalaenopsis*, *SEPALLATA*

Abstract

SEPALLATA (*SEP*) MADS-box genes play developmental roles in the regulation of floral meristem determinacy and the specification of floral organ identity. Some *SEP* genes appear to function redundantly, others have novel roles in fruit maturation, seed formation and plant architecture. The *SEP* homeotic proteins are thought to mediate MADS-box transcription factor complex formation during various plant developmental processes. Three *Phalaenopsis* *SEP*-like genes (*PeSEP1-3*) were identified and characterized. Southern blotting demonstrated that each *PeSEPs* had one copy in the *Phalaenopsis* genome. Sequence comparison and phylogenetic analysis indicated that *PeSEP1* and *PeSEP3* shared conserved C-terminal *SEP* I and *SEP* II motifs and belonged to *SEP3* clade, while *PeSEP2* had distinct *SEP* II motif and grouped in *LOFSEP* clade. The transcript of *PeSEP2* was detected in vegetative tissues but the signal of *PeSEP1* and *PeSEP3* were absent. The expression pattern of *PeSEPs* began to accumulate in the young floral buds and through all of flower development stages. Moreover, *PeSEPs* were expressed in the sepal, petal, lip, and column. Elsewhere in the pollinium, transcript was extremely low or was not detected. During developing seed, *PeSEPs* were expressed in early embryogenesis stages. Except for that, *PeSEP1* expression was not detected at later stages. During this period, signal of *PSEP2* was detected immediately after fertilization and diminished gradually at progressively later stages. *PeSEP3* was expressed strongly in the proembryo and weakly in the protocorm with crest after seed swing. Together, these data indicate that *PeSEP1* and *PeSEP3* might share similar expression pattern but *PeSEP2* varied in expression profiles. In yeast two-hybrid experiment, Furthermore, the protein–protein interaction studies suggested that complex formation between *PeSEPs* and B- and C-class protein homo- and/or heterodimers was shown for implying redundant and restricted biochemical function of *PeSEPs*.

13-P-22

Postharvest Performance of White Crane Orchid (*Calanthe triplicate*) Cut Flower in Taiwan

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Abstract

White crane orchid (*Calanthe triplicate*) is one of the terrestrial orchids native to Taiwan. It has an inflorescence up to 60-80 cm tall with a 10 cm long, densely many-flowered raceme. The floret is white with its lip deeply 4-lobed and widely spreading. There are red wart-like callus at the base of the lip and each floret looks like a little man stretching out both his hands and legs. This paper investigates the postharvest performance of white crane orchid inflorescence harvested at its full bloom stage in order to evaluate its potential as a cut flower for commercial production. When held in deionized water at 25°C, the fresh weight of the inflorescence increased at first due to absorption of water then declined. There were, in average, 21 fully-opened florets on the inflorescence at harvest; these florets started to senesce after 4 days with its lip gradually turning yellow and flaccid, and the column turning black. The average life of these florets was 5 days. On the other hand, there were over 20 buds on the inflorescence, and they started to open after 1 day with an average rate of 1 floret per day. These newly opened florets had an average life of 10 days. Therefore, there was an increase in the total number of opened florets on the inflorescence during the first 6 days in vase. Overall speaking, the vase life of the inflorescence was about 10 days. Treating the inflorescence with 1 ppm 1-MCP or 0.5 mM STS for 8 h after harvest delayed the senescence of opened florets and the overall vase life of the inflorescence could be extended to 18 day. The result of this study suggested that with proper postharvest treatments developed, there is high potential for cut flower production of white crane orchid in Taiwan.

13-P-23

A New Technical System with Higher Energy Efficiency, Higher Facility Utility and Automatic Operation for *Phalaenopsis* Spiking Seedling Production.

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Abstract

Being commercial benefit, *Phalaenopsis* spiking seedling with flower stalk about 5 cm in length has been a new target for exporting to foreign country in Taiwan. This product was used to produce in the green house with air-condition. A new technical system with higher energy efficiency, higher facility utility and automatic operation for the *Phalaenopsis* spiking seedling production was developed. The comparison among different light sources and light intensity had been achieved. The results showed that the fluorescent light with 3000 lux was available for the production of the spiking seedling. Being higher energy efficiency, *Phalaenopsis* plants were then moved to the artificial illuminating chamber instead of the green house with sun light. Multiple stages shelf was adopted in the illuminating chamber instead of one stage planting bed in the green house and the higher facility utility with several times than in the green house was achieved. For lowering labor cost, automatic operation system in the illuminating chamber was set up. The temperature required for this new production system in the illuminating chamber was similar to that in the green house. Relative researches concerned for this new production system were also conducted. The main works were as follow. The performance of this system would be enhanced by the higher light intensity. The study on the effect of different temperature on the new production system had been conducted. The results showed that there was obvious delay on the spiking date with lower and higher temperature treatments. The comparisons between different leaf numbers covered by aluminum sheet on the effect of the spiking date had also been conducted. Two or three leaves fully expended were needed for the flower spike emerged normally. Total of 35 varieties with different kinds of color and different kinds of source had been proved to be available for the new production system and the date for flower stalk with 5cm in length were ranged from 15 to 42 days. After these days, the spiking seedling with flower stalk about 5cm in length were moved back to the green house with air-condition for further flowering. The quality performance of the flower produced by the new production system was similar to that in the green house.

13-P-24

A Preliminary Study of the Effect of Ultraviolet Radiation on Visible Flower Coloration in Two Orchids

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Keywords: anthocyanins, carotenoids, *Burrageara*, *Odontocidium*

Abstract

Very little ultraviolet radiation is transmitted through polycarbonate-acrylic panels and in an earlier study red lettuce leaves grown under polycarbonate-acrylic (PC-A) panels were mostly green. However, red lettuce leaves grown under the UV-radiation transmitting, ethylene-tetrafluoroethylene copolymer film (F-Clean) were dark red in color. In the study reported here, orchid plants were grown under polycarbonate-acrylic panels and 30% and 100% UV-radiation transmitting F-Clean films to determine the effects of UV-radiation on flower color. The red-purple flowers of *Burrageara* Stefan Isler 'Lava Flow' grown with very little UV-radiation under the PC-A panels appeared to be only slightly lighter in coloration than those grown under 30% and 100% UV-radiation transmitting films. The yellow-bronze flowers of *Odontocidium* Tiger Crow 'Golden Girl' grown with very little UV-radiation under the PC-A panels, also appeared to be only slightly lighter in coloration than those grown under 30% and 100% UV-radiation transmitting films. Apparently, ultraviolet radiation in these orchid flowers is not as critical a factor in coloration as it is in leaves of red lettuce.

13-P-25

Analysis of the Mitochondrial Genome from *Phalaenopsis equestris*, a Native Species in Taiwan

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Keywords: *Phalaenopsis equestris*, mitochondrial genome

Abstract

Mitochondria are endosymbiotic from α -proteobacteria during evolution. They have independent transcription and translation system. Mammalian mitochondrial genomes are compact with approximately 15 kb in length, in contrast, plant mitochondrial genomes are diverse and range from 200 to 2,600 kb in size. Up-to-date, according to NCBI database, only 19 species of land plants, their mitochondrial genomes have been completely determined. There are two native *Phalaenopsis* orchid species in Taiwan, *Phalaenopsis aphrodite* subsp. *formosana* and *Phalaenopsis equestris*, respectively, which they have been commonly used in commercial breeding program. However, the mitochondria and their genome were less studied. In this study, we have screened the high density filters which contained about 41,472 Bacterial Artificial Chromosome (BAC) clones from *P. equestris* with seven mixed probes either from rice and/or tobacco mitochondria-encoding genes *atp1*, *atp6*, *ccmB*, *cox3*, *cob*, *nad6* and *rps3*. Sixty one BAC clones which potentially contain mitochondrial genomic DNA were selected and further hybridized with individual probe by Dot-blot. Thirty nine out of 61 BAC clones were identified which contain mitochondrial DNA fragments. Each BAC clone was restriction digested and separated by PFGE (pulsed-field gel electrophoresis) to determine its length. RFLP (restriction fragment length polymorphism) analyses were used to fingerprint BAC clones. In addition, each BAC clone was end-sequenced and BLAST into NCBI. These BAC clones will be further used to construct the physical map of mitochondrial genome. Selected BAC clones will be sequenced and mitochondrial genes will be annotated in the near future.

